ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 22 November 2019

Addressee: [redacted]

Decision number: CCH-D-2114488837-26-01/F
Substance name: Thiodiethylene bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate]
EC number: 255-392-8
CAS number: 41484-35-9
Registration number: [redacted]
Submission number: [redacted]
Submission date: 14/02/2018
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 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;**

2. **Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD 421/422) in rats, oral route with the registered substance;**

3. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;**

4. **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;**

5. **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 **29 November 2021**. You shall also update the chemical safety report, where relevant. The timeline 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

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.

Your registration dossier contains for multiple the properties adaptation arguments in form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual properties sections (In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.), Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.), Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.).

Grouping of substances and read-across approach

You have sought to adapt the information requirements listed above by applying a read-across approach in accordance with Annex XI, Section 1.5. According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances. This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis- (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds

have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

You consider to achieve compliance with the REACH information requirements for the registered substance Thiodiethylene bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate] (CAS 41484-35-9) (hereafter the 'target substance') using data of structurally similar substances [redacted] (EC no 253-039-2) (hereafter the 'source substance').

You have provided a read-across documentation as a separate attachment in the endpoint summary employing this approach. Furthermore you have provided within the read-across justification data matrices comparing the toxicological and physico-chemical properties of the target and source substances.

You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group:

"Based on Table 1 of ECHA's Read-Across Assessment Framework (RAAF) Document (Reference: ECHA-17-R-01-EN) from March 2017, scenario # 2 (analogue approach based on different compounds having qualitatively similar properties) has been chosen as the underlying basis for the current read across approach. The source and target substances are structurally very similar and the available reliable experimental data do not show relevant variations in properties among source and target substance."

"The analogue approach is based on the structural similarities and the similar toxicological properties of the two substances."

You further elaborate the arguments used to justify the read-across between the target and the source substance:

- Both compounds are diesters that share a closely related chemical structure. *"Differences in the structure are mainly limited to the nature of the glycol backbone connecting the two phenol moieties. For the target substance CAS 41484-35-9 this backbone is Thiodiglycol, whereas for the source chemical CAS 36443-68-2 the two phenol moieties are coupled via Triethylene Glycol."*
- As a result of the structural similarities, the physico-chemical properties of both chemicals are very similar." *Both target and source chemical characterized by low water solubility and they both have high log Pow values."*
- Both compounds have shown little acute toxicity, no irritation potential in rabbit's eye, no sensitization potential, no mutagenic activity in bacteria. In a subchronic feeding study and a subchronic gavage study, the source substance caused an adaptive enlargement of liver and thyroid upon repeated oral exposure to rats. The target substance did not cause secondary effects to the thyroids when given to rats, however liver enlargement was also observed in a dose dependent manner. You conclude that *"Apparently, the target chemical lacks the potency to induce liver enzymes or the potency for this mechanism is much lower."*
- No toxicokinetic investigations have been performed with the target compound. However, from the acute oral toxicity study *"No strong indications for systemic availability could be derived"* and *"together with the low water solubility and the high log Pow value suggests a poor absorption through the GI tract"*. *"In a 90-day oral toxicity study in rats increased liver weights were reported, suggesting a functional adaptation of the liver to increased systemic load indicating absorption and pre-*

systemic availability of the test substance. No indications are given for systemic circulation; therefore, the target compound and its metabolites might be excreted promptly after liver passage."

- *"For the source substance CAS 36443-68-2 several toxicokinetic and hydrolysis studies are available.[...] the source compound is rapidly absorbed in considerable amounts (50%) after ingestion. Absorption was rapid since the maximal blood/plasma values were reached one hour after administration. Radioactivity from plasma and blood was eliminated with half-lives ranging from 2.9 hours to 8.6 hours. Elimination with approximately equal ratio in urine and feces was basically complete within 72h. There is no indication of bioaccumulation of the substance."*
- You proposed a hydrolysis pathway for the target chemical. The mechanism based on the fact that the test article is a di-ester potentially subject to hydrolysis in the liver was also predicted by the liver simulator of OECD toolbox 4.0 followed by further modifications. These data were not provided in the dossier.
- For the source substance, hydrolysis studies performed in vivo and in vitro show that the *"source chemical was readily hydrolyzed to the corresponding carboxylic acid at the near physiological pH of 7.4 with hydrolysis half-times of 26.2 and 14.0 min with a test concentration of 200 µM by 0.5 and 1% rat serum, respectively. This rapid hydrolysis was confirmed in rats. Conjugates of the metabolite are eliminated with approximately equal ratio in urine and feces and elimination is basically complete within 72h."*
- You also provided a compilation of toxicological data for the predicted hydrolysis products of the target substance. Metilox carboxylic acid, CAS 20170-32-5, one of those predicted metabolites, is of low oral acute toxicity, not irritant to skin and eyes with no mutagenic potential in bacterial and mammalian in vitro tests and no acute or delayed neurotoxic potential. Upon repeated dosing, the liver and thyroid were identified as the major target organs. In both organs, weight increases and hypertrophy/hyperplasia were observed. *"The stimulatory effects seen in liver and the thyroid gland are known to be rodent-specific without any implications for man."* In addition, kidney (weight increase, tubular droplets) and lung (focal accumulation of foam cells) were observed. The NOAEL was established at 30 mg/kg/day.

  Thiodiglycol, CAS 111-48-8, another predicted metabolite of the target substance, is of low oral acute toxicity (LD over 9900 mg/kg bw/d) with depression of the central nervous system at doses near to or exceeding the LD50 value. It is not irritating and not sensitizing. In a study performed according to OECD guideline 407 (1981), repeated exposure of rats by gavage to 1000 mg/kg body weight for 28 days resulted in no effects of toxicological relevance. In a 90-day gavage study (comparable to the current OECD guideline 408; 0, 50, 500, 5000 mg/kg body weight), effects on body and kidney weight (without a histopathological effect) as well as altered parameters of the urine analysis were observed in males and females at 5000 mg/kg. Thiodiglycol is not mutagenic in bacteria and in the mouse lymphoma assay (OECD guideline 476) and no clastogenic activity was detected in a mouse bone marrow micronucleus assay at oral doses up to and including 2000 mg/kg body weight (OECD guideline 474). In two gavage studies on the prenatal developmental toxicity in Wistar rats (OECD 414), the NOAEL for maternal and developmental toxicity was 400 mg/kg body weight and there were borderline effects concerning a certain type of skeletal variations (dumbbell ossification of thoracic vertebral bodies) at oral doses of 1000 mg/kg which resulted also in marginal toxicity.

  Triethylene Glycol, CAS 112-27-6 is one of the metabolites of the source substance. It has low oral acute toxicity, it is not irritant to skin and eyes, and has no mutagenic potential in bacterial and mammalian in vitro tests. In a 90-d feeding study a NOAEL

of 1522 mg/kg bw/d and 1699 mg/kg bw/d was obtained for males and females respectively based on small increase in kidney weight (high dose group females) and kidney weight relative to body weight (all groups of males and mid and high dose females), decrease in urine pH at all doses in males and mid and high dose females and an increase in urine volume inmales of the high dose group. In a two generation study in mice no reproductive effects were observed. However, developmental toxicity was noted in the first generation as reduced pup body weight (NOAEL for F1 was 6780 mg/kg bw/d). In a rat study fetotoxicity was observed at 10 mL/kg bw and in a developmental toxicity study in mice, there was maternal toxicity at 10 mL/kg and fetotoxicity at 10 and 5 mL/kg. NOEL for developmental toxicity was 0.5 ML/kg bw (565 mg/kg bw).

ECHA also notes that no data are available for the carboxylic acid metabolite of the source substance. You assume that its toxicological properties are very similar to Metilox carboxylic acid due to structural similarity.

For the target substance monoester metabolite and source substance monoester metabolite no data are available. "*However, since the toxicological effects are most likely related to the hindered phenol moieties, it is not expected that these compounds differe significantly from the respective parent compounds.*"

- Finally you provide the impurity profiles for the target and source substance explaining that both compounds share a closely related impurity composition and that it is expected that the impurities are similar with regard to their overall contribution to toxicity.

You conclude that "*The toxicological potential of both the target and the source compound as discussed above are very likely related to the respective carboxylic residues. This is supported by the repeated dose toxicity data available for Metilox carboxylic acid, which show similar adaptive liver toxicity and rodent sepcific effects if the liver-thyroid axis. It is notable that Metilox carboxylic acid is more potent regarding the effects seen in the liver than the source and the target substance. This might be related to the hydrolysis rate of the parent compound; the faster the hydrolysys takes place, the more metabolite is present systemically, being able to induce the adaptive liver response.*"

As an integral part of this prediction, you propose that the source and registered substances have similar properties for the above-mentioned information requirements. ECHA considers that this information is your read-across hypothesis.

ECHA's evaluation and conclusion

Your proposed adaptation argument is that the similarity in chemical structure and in some of the physico-chemical and toxicological properties between the source and registered substance is a sufficient basis for predicting the properties of the registered substance for other endpoints. Structural similarity is a prerequisite for applying the grouping and read-across approach. However similarity in chemical structure and similarity of some of the physico-chemical toxicological properties does not necessarily lead to predictable or similar human health properties in other endpoints.

- Your justification based on structural similarity, similar physico-chemical, and toxicological properties has not established why the prediction is reliable for the reproductive endpoints for which the read across is claimed. Several important aspects were not sufficiently addressed, more specifically:You did not suficiently demonstrate that the hazard for reproductive toxicity can be predicted from the data

provided with the source. The repeated dose toxicity data provided in the data matrix suggest that the target substance is more potent than the source. From the 2-generation study with the source substance there is concern for developmental effects (F1: **Pup losses** of mid (60-80 mg/kg bw) and high dose groups (120-160 mg/kg bw), **retardation of pup development** in the high dose group, **mean body weights reduced** in mid and high dose groups; F2a: **lower mean numbers of pups found alive on day 1 post-partum** in mid and high dose groups ; F2b: **moderately reduced mean number of pups found alive on day 1 post-partum** in high dose group, **retardation of pup development** in the high dose group; for all F2: mean pup weight was slightly reduced in low dose group (21-26 mg/kg bw), **slightly to moderately reduced in mid dose group, and markedly reduced weight** in high dose group). Furthermore, the concern is not clarified even for the source substance by the prenatal developmental toxicity study provided, as the reduction in the number of live foetuses was not examined. Therefore, for the developmental toxicity endpoint it cannot be excluded that the target substance would induce more severe effects than the source substance and there is a concern for developmental toxicity.

- The metabolites that you identify for the source substance are triethylene glycol, a monoester metabolite and a carboxylic acid metabolite. Triethylene glycol is not a developmental toxicant and no data are available for the monoester and carboxylic acid. The predicted metabolites from the target substance are thiodiglycol, a monoester metabolite and metilox carboxylic acid. Thiodiglycol has some developmental toxicity with borderline effects concerning a certain type of skeletal variations (dumbbell ossification of thoracic vertebral bodies) at oral doses of 1000 mg/kg which resulted also in marginal toxicity. No data on reproductive toxicity data are available for the monoester and metilox carboxylic acid. Data are available for Metilox (CAS 6386-38-5, OECD 421 study), an analogue of the metilox carboxylic acid metabolite, which indicates that it causes similar developmental effects to those seen in the 2-generation study (decreased litter size, viability and weight) with the source substance[2]. ECHA concludes that the available information on the developmental toxicity of the metabolites does not support that similar toxicological properties would be expected for the source and target substances in the context of developmental toxicity.
- No hydrolysis data and hydrolysis rates are provided for the target substance and so the contribution of the predicted metabolites to the target substance toxicity cannot be evaluated. Furthermore, there seems to be some difference between the target and source substances with regard to toxicokinetics: *"the low water solubility and the high log Pow value suggests a poor absorption through the GI tract"* for the target substance while *"the source compound is rapidly absorbed in considerable amounts (50%) after ingestion"*. This seem contradictory to the claim of similar toxicokinetics on the basis of similar chemical structure and similar physico-chemical properties. You also stated that the proposed toxicokinetics mechanism for the target chemical involving hydrolysis in the liver was predicted by the liver simulator of OECD toolbox 4.0 followed by further modifications. However, these data were not provided in the dossier. Consequently, ECHA considers that the provided hydrolysis and toxicokinetics data are not sufficient to verify the assumption of similar toxicokinetics for the source and target substances.

[2] SIAM 13, 6-9 November 2001 for metilox: "*No effects on fertility were observed. Effects on pups (**decreased litter size, viability and weight**) were reported at 250 mg/kg bw per day. The NOAEL for developmental effects is 100 mg/kg bw per day.*"

As explained above there is a concern for developmental toxicity for the target substance which cannot be clarified on the basis of the available data with the source. Therefore, the read-across is rejected for the reproductive endpoints.

Additionally, ECHA has taken into account all of your arguments together. ECHA firstly notes that you have not provided a reasoning as to why these arguments add to one another to provide sufficient basis for read-across. Secondly, the defects of each individual argument are not mitigated by the other arguments you have provided, and so ECHA considers that the arguments when taken all together do not provide a reliable basis for predicting the properties of the registered substance.

Therefore, ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the reproductive toxicity of the registered substance may be predicted from data for reference substances within the group. Hence, this approach does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation.

As described above, further elements are needed to establish a reliable prediction for a toxicological or ecotoxicological property, based on recognition of the structural similarities and differences between the source and registered substances. This could be achieved (if it is possible) by a well-founded hypothesis of (bio)transformation to a common compound(s), or that the registered and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties, i.e. reproductive toxicity.

For the genotoxicity endpoint although you did not make an endpoint specific hypothesis, ECHA considers that the presented data is supportive of the read-across due to the following main reasons:

- No genotoxicity was reported in bacterial cells for target and source substances as well as for the predicted or actual metabolites.
- No genotoxicity was reported in mammalian cells in vitro for methilox carboxylic acid.
- No genotoxicity was reported in a mouse lymphoma assay (OECD 473) and an in vivo micronucleus study (OECD 474) with thiodiglycol.
- No genotoxicity was reported in mammalian cells in vitro for triethylene glycol.

Therefore, ECHA considers that for the genotoxicity endpoints this grouping and read-across approach does provide a reliable basis whereby the properties of the registered substance may be predicted from data for reference substances within the group.

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

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.

You have sought to adapt this information requirement according to Annex VIII, Section 8.4.2., column 2. You provided the following justification for the adaptation "*In accordance with Annex VIII (8.4.2) of the REACH legislation, the test does not need to be conducted if adequate data from a reliable in vivo cytogenicity assay is available. In this case, a valid*

micronucleus study in vivo is 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 the provided in vivo cytogenicity study was not deemed reliable. More specifically, in the technical dossier you have provided a study record for an in vivo OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) with the registered substance showing several deficiences which make the study unreliable:

- Only one time point was observed.
- 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 testing 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 testing guideline. In the provided study only 1000 cells per animal were assessed.
- The provided table does not show the PCE/NCE (polychromatic/normchromatic cells) ratio which would provide information on whether the bone marrow has been reached.

Due to the issues highlighted above, the validity of the provided test cannot be confirmed.

In your comments on the draft decision, you agree that certain aspects of the OECD TG 474 are not addressed and therefore there is a data gap for *in vitro* cytogenicity. To supplement the available information you provided a QSAR analysis to address *in vitro* cytogenicity using the "Chromosomal Aberrations S9 activated" module in which the prediction was "negative" for both the main compound all potential metabolites.

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 has evaluated the provided QSAR information and 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. Furthermore, information on internal validation and external validation is not documented. On the availability of the training set, the QMRF says that almost half of the data set is proprietary. The applicability domain is specified in broad ranges for calculated parameters like molecular weight and log Kow but the distribution of the 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 considers that the QSAR model and the resulting prediction, is not sufficiently described. Based on available and missing information and observed inconsistencies in documentation, ECHA considers that the prediction for *in vitro* mammalian chromosomal aberration cannot be considered valid.

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

2. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

"Screening for reproductive/developmental toxicity" (test method OECD TG 421 or 422) is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of a screening for reproductive/developmental toxicity in the dossier that would meet the information requirement of Annex VIII, Section 8.7.1.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a two-generation reproductive toxicity study (test method OECD TG 416) with the analogue substance Benzenepropanoic acid, 3-(1,1-dimethylethyl)-4-hydroxy-5-methyl-,1,2-ethanediylbis(oxy-2,1-ethanediyl) ester) (EC no 253-039-2). However, as explained above in Appendix 1, under the *Grouping of substances and read-across approach* section, your adaptation of the information requirement is rejected.

Therefore, your adaptation of the information requirement is rejected.

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 the test methods OECD TG 421/422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

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:

- Reproductive/developmental toxicity screening test (test method: OECD TG 421) *or* Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

In your comments on the proposal for amendment (PfA) submitted by one of the Member States Competent Authorities you indicated your disagreement to include this request as you intend to use the OECD TG 414 study (requested under section 3 of the present decision) to waive the OECD TG 421 study.

ECHA notes that indeed according to Annex VIII Section 8.7.1., the screening for reproductive/developmental toxicity study (OECD TG 421 or 422) does not need to be conducted if "*a pre-natal developmental toxicity study (Annex IX, 8.7.2) [...] is available.*" However, currently in the technical dossier there is no available PNDT study that can be used to adapt this information requirement according to Annex VIII Section 8.7.1, column 2.

Furthermore, ECHA notes that according to the ECHA Guidance Chapter R.7a "*where information from a reproductive toxicity study addressing a fertility endpoint is not available, it is strongly recommended that a screening study is considered to fulfil this endpoint.*"

Notes for your considerations

For the selection of the appropriate test, please consult ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.5 and 7.6 (version 6.0, July 2017).

You should also carefully consider the order of testing of the requested screening (OECD TG 421/422) and the developmental toxicity studies (OECD TG 414) to ensure that unnecessary animal testing is avoided, paying particular attention to the endpoint specific guidance (https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf) Section R.7.6.2.3.2., pages 484 to 485 of version 6.0 – July 2017."

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

A "pre-natal developmental toxicity study" (test method OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. 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.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a pre-natal developmental toxicity study" (test method OECD TG 414) with the analogue substance Benzenepropanoic acid, 3-(1,1-dimethylethyl)-4-hydroxy-5-methyl-,1,2-ethanediylbis(oxy-2,1-ethanediyl) ester) (EC no 253-039-2).

However, as explained above in Appendix 1, under the *Grouping of substances and read-across approach* section, your adaptation of the information requirement is rejected. Therefore, your adaptation of the information requirement is rejected.

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 the test method OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits as a first species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

In your comments on the draft decision you agreed to perform the requested test.

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: Pre-natal developmental toxicity study (test method: OECD TG 414) in a first species (rat or rabbit) by the oral route.

4. 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 < 1 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 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 environmental 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 and 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).

5. 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 the model, the transformation reliability was low for most of these metabolites. The low reliabilities (between 0 to 0.47) 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 to 0.47). 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 producst 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.

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 7 % 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 30 months from the date of adoption of the decision. A competent authority of a Member State submitted proposals for amendment (PfAs) to reduce the time and to add requests for information on Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1), Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1) and Identification of degradation products (Annex IX, Section 9.2.3). Considering all the requests included in the decision and the time needed to carry out the studies, the timeline of 18 months is considered to be sufficient.

In your comments on the PfAs, you indicated that the "*30-month timeline is essential to ensure an on-time dossier update with the requested studies*" due to limited laboratory

capacity, time-consuming negotiations with co-registrants and also due to a dose range finding (DRF) study required for the PNDT study (OECD TG 414).

As regards the laboratory capacity ECHA requested you to submit documentary evidence from the selected test laboratory indicating the scheduling timelines for the studies in question of the laboratory facility. You have submitted a statement from your laboratory indicating the following: "*Based on our current workload and considering that a range finder study plus characterisation of the test material is required before the main study can be started, we estimate that the study could be finalised within 30 months*". However, you have not provided any scheduling timelines to support this statement.

ECHA notes that the 18 months timeline already allows for the performance of a DRF study before the OECD TG 414 is initiated. ECHA also understands that the laboratory has limited capacities. Therefore, ECHA has only partially granted the request and set the deadline to 24 months.

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 04 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.