EUROPEAN CHEMICALS AGENCY

For final decision: TPE-D-0000002563-76-04/F Helsinki, 29 January 2013

DECISION ON A TESTING PROPOSAL SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For 2,4-di-tert-butylphenol, CAS No 96-76-4 (EC No 202-532-0), registration number: [redacted]

Addressee: [redacted]

The European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 51 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

I. Procedure

Pursuant to Article 40(1) of the REACH Regulation, ECHA has examined the following testing proposal submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for 2,4-Di-tert-butylphenol, CAS No 96-76-4 (EC No 202-532-0), by [redacted] (Registrant).

- Annex IX, 8.6.2: Subchronic toxicity study (90-day) by oral route, according to OECD Guideline 408, on the read-across substance (2,6-di-tert-butylphenol (EC No 204-884-0));
- Annex IX 9.1.5. Long-term toxicity testing on aquatic invertebrates, according to OECD Guideline 211 (Daphnia magna Reproduction Test) on the read-across substance (2,6-di-tert-butylphenol (EC No 204-884-0)).

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 100 to 1000 tonnes per year. This decision does not take into account any updates after 6 September 2012, the date upon which ECHA notified its draft decision to the Competent Authorities of the Member States pursuant to Article 51(1) of the REACH Regulation.

This decision does not imply that the information provided by the Registrant in his registration dossier is in compliance with the requirements of the REACH Regulation. The decision does not prevent ECHA to initiate a compliance check on the registration dossier at a later stage.

The examination of the testing proposals was initiated on 28 October 2010.

ECHA opened a third party consultation for the testing proposals including testing on vertebrate animals that was held from 16 May 2011 until 30 June 2011 and received comments (see Section III below).

On 17 October 2011 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision. This draft decision was based on the registration dossier as submitted with the submission number [redacted].

On 15 November 2011 ECHA received comments from the Registrant stating his disagreement with the rejection of the read-across argumentation.

On 9 March 2012 ECHA received a dossier update (submission number [redacted]) including a justification document for the read-across.

ECHA considered the Registrant's comments received. On basis of the comments, Section II was amended (request for a Sub-chronic toxicity study (90-day) on 2,4-di-tert-butylphenol and for Long-term toxicity testing on aquatic invertebrates on the analogue substance 2,6-di-tert-butylphenol). The Statement of Reasons (Section III) was changed accordingly.

On 06 September 2012 ECHA notified the Competent Authorities of the Member States of its draft decision and invited them pursuant to Article 51(1) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, Competent Authorities of the Member States submitted proposals for amendment to the draft decision.

On 10 October 2012 ECHA notified the Registrant of proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

ECHA reviewed the proposals for amendment and the Registrants comments received and decided to amend the draft decision (Section II: request for both a Sub-chronic toxicity study (90-day) and for Long-term toxicity testing on aquatic invertebrates on 2,4-di-tert-butylphenol; Section III amended accordingly).

On 22 October 2012 ECHA referred the draft decision to the Member State Committee.

On 23 October 2012 the Registrant provided comments on the proposed amendments. The Member State Committee took the comments of the Registrant into account.

After discussion in the Member State Committee meeting on 10-14 December 2012, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 12 December 2012. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

Pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant shall carry out the following tests using the indicated test method and the registered substance (2,4-di-tert-butylphenol):

1. Sub-chronic toxicity study (90-day) in rats: oral route, (Annex IX, 8.6.2., EU method B.26 or OECD 408);
2. Long-term toxicity testing on aquatic invertebrates (Annex IX 9.1.5., EU method C.20 or OECD 211 – *Daphnia magna* Reproduction Test);

while the originally proposed tests using 2,6-di-tert-butylphenol (EC No 204-884-0) are rejected in accordance with Article 40(3)(d) of the REACH Regulation.

Pursuant to Articles 40(4) and 22 of the REACH Regulation, the Registrant shall submit the information in the form of an updated IUCLID dossier to ECHA by **29 July 2014**.

The registered substance subject to the present decision is currently listed on the draft Community rolling action plan for Substance Evaluation. Without prejudice to this possible Substance Evaluation, once the results of the proposed test on long-term toxicity to aquatic invertebrates are available, the Registrant shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation. In case the Substance Evaluation indicates the need for further testing in order to clarify a concern with regard to the registered substance subject to the present decision, any further testing requirements will be proposed by the evaluating member state Competent Authority. The Registrant shall therefore discuss his further envisaged testing needs with the evaluating Competent Authority before submitting further testing proposals in that regard.

At any time, the Registrant shall take into account that there may be an obligation to make every effort to agree on sharing of information and costs with other registrants.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposal of the Registrant for the registered substance and scientific information submitted by third parties.

1. Sub-chronic toxicity study (90-day)

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out additional tests in cases of non-compliance of the testing proposal with Annexes IX, X and XI.

Sub-chronic toxicity study (90-day) is a part of the information requirements as laid down in section 8.6.2. of Annex IX of the REACH Regulation. As the information for this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements, it follows that there is an information gap and that it is necessary to provide information for this endpoint.

(1) Proposal to test on a read-across substance

The Registrant proposed to perform the sub-chronic toxicity study on the substance 2,6-di-tert-butylphenol (2,6-DTBP), instead of the registered substance 2,4-di-tert-butylphenol (2,4-DTBP) thus applying a read-across approach. The substances are structurally similar. However, ECHA considers that from the toxicological point of view these substances have some distinct properties, and accordingly, there is no adequate basis for considering that the human health effects may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach in accordance with Annex XI, 1.5. of the REACH Regulation). The substances differ significantly in their eye irritation potential: 2,6-DTBP is not irritating, while 2,4-DTBP is moderately irritating and confers a risk of serious eye damage. Furthermore, even though for both compounds one of the target organs was liver, 2,4-DTBP and 2,6-DTBP have differences in toxicological properties. In addition, for the 2,4-DTBP the adverse effects seen in the oral 28-day study in the kidney (at 300 mg/kg there were cellular infiltration (neutrophils), dilatation of the distal tubules and/or hypertrophy of the proximal tubules) were not only

different from those of 2,6-DTBP (at 600 mg/kg slight accumulations of tubular eosinophilic inclusion bodies), but in the study with 2,4-DTBP effects were seen in both males and females, whereas for 2,6-DTBP there were male-specific effects. Kidney effects observed both in males and in females (as in 2,4-DTBP) cannot be mediated by the rat-specific alpha-2u-globulin nephropathy mechanism, whereas for effects seen in males only (as in 2,6-DTBP), it is possible that they are mediated by this mechanism which is not relevant to humans.

The Registrant, in his comments submitted according to Article 51(1) of the REACH Regulation and later in a dossier update that included a read-across justification document, has provided further justification for the read-across from 2,6-DTBP to 2,4-DTBP that he proposed for both endpoints (i.e. the present one and the long-term aquatic toxicity to aquatic invertebrates). The Registrant addressed the following issues that are relevant for assessing the proposed read-across for the sub-chronic toxicity study: 1) steric hindrance 2) bulk density and melting point 3) eye irritation 4) NOAEL differences in 28-day study.

ECHA agrees with the Registrant that the two substances, being region-isomers, are structurally similar. However, concerning steric hindrance, ECHA considers that the presence of two t-butyl groups next to the phenolic OH in 2,6-DTBP makes the hydroxyl group hindered compared to the 2,4-DTBP isomer, where the two t-butyl groups are in ortho- and para- position of the phenyl ring. Concerning the argument of the Registrant on chemical reactivity, ECHA notes that the Registrant has considered only electronic effects, not steric effects. Chemical reactivity is a result of both electronic and steric effects. This steric hindrance could play a significant role in terms of potential binding to biological receptors and enzymes resulting therefore in potentially different (eco)toxicological profile. This structural difference is supported by some physical-chemical properties, like the water solubility that is 8 times higher for the 2,4-DTBP due to the phenolic OH group being less hindered and more accessible for H bond interactions with other molecules (in this case the water solvent). Accordingly, ECHA's believes that the (eco)toxicological properties of 2,4-DTBP cannot be predicted with sufficient certainty from the (eco)toxicological properties of 2,6-DTBP by interpolation (read-across approach).

Furthermore, ECHA considers that the Registrant's argumentation for the read-across for the endpoint of section 8.6.2. of Annex IX of the REACH Regulation is not adequate for sub-chronic toxicity, and so does not fulfil the Annex XI, 1.5. criterion that adequate and reliable documentation of the applied method shall be provided. ECHA agrees with the Registrant that quantitative differences in NOAEL in the 28-day study between substances are small. However, this information does not outweigh the fact that – as explained above – the substances have significant differences in eye irritation and in histopathological effects in kidney, and can therefore – with regard to their toxicological properties – not be considered to be sufficiently similar so as to be able to predict the properties of one substance from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach). Therefore, ECHA concludes that based on these reasons, the proposed read-across approach from 2,4-DTBP to 2,6-DTBP has failed to satisfy the requirements of Annex XI, 1.5 for the present endpoint. Consequently, the read-across approach is not justified for the sub-chronic toxicity study, and Section II of the draft decision is not amended in this regard.

(2) Species and route of testing

The Registrant did not specify the species to be tested. According to the test method EU B.26/OECD 408 the rat is the preferred rodent species. ECHA considers this species as being appropriate. The Registrant proposed testing by the oral route. In light of the physico-

chemical properties of the substance and the information provided on the uses and human exposure, ECHA considers that testing by the oral route is appropriate.

b) Consideration of the information received during third party consultation

During the third party consultation, ECHA received following comments/information on the testing proposal for the sub-chronic toxicity study:

(1) A third party proposed that corrosivity should be taken into account when the decision for the 90-day study is made.

ECHA examined the proposal and concluded the following:
Corrosiveness is not a Column 2 waiving argument for a 90-day study. In addition, neither 2,4-DTBP nor 2,6-DTBP are classified as corrosive. Therefore, the argument provided by the third party cannot constitute an acceptable adaptation to standard information requirements.

(2) A third party has provided QSAR model results.

ECHA examined the proposal and concluded the following:

According to Annex XI, 1.3 of the REACH Regulation, the results of the QSARs may be used instead of testing when the following conditions are met: a) the results are derived from a QSAR model whose scientific validity has been established; b) the substance falls within the applicability domain of the QSAR model; c) results are adequate for the purposes of classification and labelling and/or risk assessment; and d) adequate and reliable documentation of the applied method is provided.

The evaluation of the submitted information according to the conditions described above showed that:

- Contrary to point a) above, the dependent variable of the model is in the form "toxic/non-toxic". In the absence of additional information on the meaning of these terms, the predicted result could not be directly used or extrapolated to fill a data gap according to the information requirements of the REACH Regulation.
- Contrary to point b) above, based on the information in the QPRF (QSAR Prediction Reporting Format), the possibility that the substance does not fall in the applicability domain of the model could not be ruled out. In fact, there is only evidence that the parameters of the chemical, used for prediction, fall within the ranges of the individual descriptors, used in the model. The provided QPRF contains analogues, which do not look similar to the two constituents of the registered substance.
- Contrary to point c) above, the results are not adequate for the purposes of classification and labelling and/or risk assessment, because the estimated endpoint does not have adequate and reliable coverage of the key parameters in the corresponding test method as described in the 414 OECD guideline.
- Contrary to point d) above, the level of detail in the documentation of the algorithm in the QMRF (QSAR Model. Reporting Format) was not considered sufficient to transparently describe the model. The algorithm does not appear in the QMRF in formalised mathematical form that can be reproduced from the documentation. In addition, the training, selection and test sets were not found available.

In conclusion, the conditions for using QSAR instead of testing, as required under Annex XI, section 1.3. are not met. Therefore, the third party has not provided scientifically valid information or studies (Annex XI 1.3. and Article 40(2)).

c) Outcome

Therefore, pursuant to Articles 40(3)(c) of the REACH Regulation, the Registrant is required to carry out the study: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD 408) using the registered substance subject to this decision. Pursuant to Article 40(3)(d) of the REACH Regulation, the test using 2,6-di-tert-butylphenol (EC No 204-884-0) is rejected.

2. Long-term toxicity to aquatic invertebrates

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out additional tests in cases of non-compliance of the testing proposal with Annexes IX, X and XI.

According to Section 9.1.5 of Annex IX of the REACH Regulation, long-term toxicity testing on aquatic invertebrates is required to fulfil the standard information requirements. As the proposed test for long-term toxicity to aquatic invertebrates is not available for the registered substance but needs to be present in the technical dossier to meet the information requirement of Section 9.1.5 of Annex IX of the REACH Regulation, it is necessary to provide information for this endpoint.

(1) Proposal to test on a read-across substance

The Registrant proposed to perform the long-term toxicity testing on aquatic invertebrates on the substance 2,6-DTBP, instead of the registered substance 2,4-DTBP thus applying a read-across approach. The Registrant has justified the read-across approach by structural similarity of the substances, physical-chemical and ecotoxicological similarities of the substances. A similar argumentation was also provided by the Registrant during the commenting period on the draft decision and in response to the proposals for amendment (PfAs) received by two Member State Competent Authorities, further arguing for the general uncertainty of estimation models and for the scientific (ir)relevance of steric hindrance for the interpretation of aquatic toxicity.

Furthermore, as explained in the proposal for amendment of a Competent Authority, an alkyl chain in para-position exerted the highest effect on estrogenicity when estrogenicity of alkylphenols was compared in an *in vitro* study (Routledge & Sumpter. Journal of Biological Chemistry. 1997;272:3280-3288). Therefore, since in the 2,4-di-tert-butylphenol one alkylgroup is in the para-position and one in ortho-position and in the 2,6-DTBP both alkylgroups are in ortho-positions, it is likely that 2,4-di-tert-butylphenol has higher estrogenicity than 2,6-di-tert-butylphenol.

In addition, aquatic toxicity profiles of 2,4-DTBP and 2,6-DTBP differ significantly. The comparison of the information provided for both substances indicates some significant differences not addressed by the Registrant which is highlighted below. Fish short-term testing is covered with two studies on the registered substance and four read-across studies on 2,6-DTBP. A read-across OECD TG 204 is considered the key study; all other studies are presented as reliable (level 2) supporting information. These short-term fish toxicity results show, comparing the 48h data which are the only available for 2,4-DTBP, a very steep concentration-response curve for 2,4-DTBP, with LC100/LC0 = 1.25, which is much less pronounced for 2,6-DTBP (48h LC100/LC0 = 3.2). The information on time-effect

relationships is only available for 2,6-DTBP, and would suggest an inflection point in the fish lethality slope at about 48h, creating concerns regarding the extrapolation of the 96h data for 2,4-DTBP. Short-term tests on invertebrates are covered by independent studies, a very steep concentration-response curve, with an EC100/EC10 ratio of 1.55 is observed for 2,6-DTBP, but not for 2,4-DTBP. Regarding algae, the comparison indicates a difference of above one order of magnitude between the measured EC50 values for 2,4-DTBP (biomass) and 2,6-DTBP (cell number, no data for growth rate reported). Therefore, it is not possible to predict the aquatic toxicity of 2,4-DTBP based on aquatic toxicity of 2,6-DTBP.

In conclusion, due to the overall uncertainties in the ecotoxicological profiles of the two substances, it is considered that the two substances are not sufficiently similar so as to be able to predict the properties of 2,4-DTBP from data from 2,6-DTBP.Therefore, ECHA considers that the suggested adaptation by use of a read-across does not meet the criteria of Annex XI, 1.5. of the REACH Regulation and that the proposed test shall be carried out on the registered substance.

According to ECHA Guidance on information requirements and chemical safety assessment (version 1.1., August 2008), Chapter R7b, Figure R.7.8-4 page 53, if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. This is the case for the current dossier. According to the integrated testing strategy, the Daphnia study is to be conducted first. If based on the results of the long-term Daphnia study and an applied assessment factor of 50 no risks are indicated, no long-term fish testing may be needed.

(b) Outcome

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant is, requested to perform the long-term toxicity to aquatic invertebrates study with the registered substance (EC No 202-532-0) as the test substance (Annex IX 9.1.5., test method: EU C.20 or OECD 211 - Daphnia magna Reproduction Test). Pursuant to Article 40(3)(d) of the REACH Regulation, the test using 2,6-di-tert-butylphenol (EC No 204-884-0) is rejected.

IV. Adequate identification of the composition of the tested material

It is important to ensure that the particular sample of substance tested in the new studies 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. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Furthermore, there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the studies to be assessed.

V. General requirements for the generation of information and Good Laboratory Practice

ECHA always reminds registrants of the requirements of Article 13(4) of the REACH Regulation that ecotoxicological and toxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP). National authorities monitoring GLP maintain lists of test facilities indicating the relevant areas of expertise of each facility.

According to Article 13(3) of the REACH Regulation, tests that are required to generate

information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the European Chemicals Agency as being appropriate. Thus, the Registrant shall refer to Commission Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 as adapted to technical progress or to other international test methods recognised as being appropriate and use the applicable test methods to generate the information on the endpoints indicated above.

VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Article 51(8) of the REACH Regulation. Such appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://echa.europa.eu/appeals/app_procedure_en.asp. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

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Jukka MALM
Director of Regulatory Affairs