Decision number: TPE-D-0000002149-74-05/F Helsinki, 11 July 2012

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

For Tert-butyl 2-ethylperoxyhexanoate, CAS No 3006-82-4 (EC No 221-110-7), 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 testing proposals set out in the registration dossier for Tert-butyl 2-ethylperoxyhexanoate, CAS No 3006-82-4 (EC No 221-110-7) submitted by [redacted] (Registrant), latest submission number [redacted], for 1000 tonnes or more per year.

In accordance with Articles 10(a)(ix) and 12(1)(e) of the REACH Regulation, the Registrant submitted the following testing proposals as part of the registration dossier to fulfil the information requirements set out in Annexes IX and X:

- Mutagenicity, Unscheduled DNA synthesis assay with mammalian liver cells *in vivo*, EU Method B.39, Annex X, 8.4.;
- Sub-chronic toxicity study (90 day) EU Method B.26, in rats by oral route, Annex IX, 8.6.2.), including additional tests with special focus on sexual organs;
- Pre-natal developmental toxicity study EU Method B.31., Annex IX, 8.7.2.

The examination of the testing proposals was initiated on 27 October 2010.

ECHA opened a third party consultation for the testing proposals including testing on vertebrate animals that was held from 15 April 2011 until 30 May 2011. ECHA did not receive any comments from third parties.

On 30 November 2011 ECHA sent a draft decision to the Registrant for comments. The Registrant did not provide any comments on the draft decision.

On 20 January 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 23 February 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 received and amended the draft decision accordingly.

On 5 March ECHA referred the draft decision to the Member State Committee.

On 7 March 2012 the Registrant provided comments on the proposed amendments. The Member State Committee took the comments of the Registrant into account.

On 19 March 2012 following an informal discussion the Member State Committee modified the draft decision.

After discussion in the Member State Committee meeting on 24-27 April 2012, a unanimous agreement of the Member State Committee on the draft decision as amended by ECHA was reached on 26 April 2012 and ECHA took the decision pursuant to Article 51(6) 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 present dossier at a later stage.

II. Testing required

Pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant shall carry out the following additional test using the indicated test method and the registered substance concerned by the present decision:

1. Mutagenicity, *in vivo* (Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, Annex X, 8.4., test method: OECD 488). The test shall be conducted in mice treated for 42 days, and tissues (forestomach, intestine, liver, kidney and developing germ cells from the seminiferous tubules) shall be harvested three days after the cessation of treatment. Mutation frequency shall be assessed in forestomach, intestine, liver, kidney, and developing germ cells from the seminiferous tubules

while the Registrant's proposal to carry out an Unscheduled DNA Synthesis (UDS) test with mammalian liver cells in vivo (Annex X, 8.4.; test method: EU B.39/OECD 486) is rejected in accordance with Article 40(3)(d) of the REACH Regulation.

Pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant shall carry out the following proposed tests using the indicated test method and the registered substance concerned by the present decision

2. Sub-chronic toxicity study (90-day) in rats, oral route (Annex IX, 8.6.2.; test method: EU B.26/OECD 408). It is at the Registrant's discretion to perform the proposed additional examinations during the testing program; and

3. Pre-natal developmental toxicity study in rats, oral route (Annex IX, 8.7.2.; test method: EU B.31/OECD 414).

The Registrant shall determine the appropriate order of the studies taking into account the possible outcomes and considering the possibilities for adaptations of the standard

information requirements according to column 1 or 2 provisions of the relevant Annexes of the REACH Regulation.

Pursuant to Articles 40(4) and 22 of the REACH Regulation, the Registrant shall submit to ECHA by **11 July 2014** an update of the registration dossier containing the information required by this decision.

Data from a second pre-natal developmental toxicity study on another species is a standard information requirement according to Annex X, 8.7.2. of the REACH Regulation. The Registrant should firstly take into account the outcome of the pre-natal developmental toxicity on a first species and all other relevant available data to determine if the conditions are met for adaptations according to Annex X, 8.7. column 2, or according to Annex XI. If the Registrant considers that testing is necessary to fulfil this information requirement, he should include in the update of his dossier a testing proposal for a pre-natal developmental toxicity study on a second species.

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.

1. Mutagenicity, *in vivo*

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

According to Annex X, section 8.4. of the REACH Regulation, in case any *in vitro* test required at Annexes VII or VIII revealed positive results, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data.

ECHA notes that the genetic toxicity of the substance was tested in three *in vitro* tests and one *in vivo* test: two *in vitro* Ames tests with the substance revealed negative and positive results and one *in vitro* gene mutation study (test method: EU B.17) revealed positive results. In addition to the *in vitro* assays the registered substance was tested in an *in vivo* mouse micronucleus assay. The test item did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow. Based on the available data the Registrant concluded that the substance has an alert for gene mutation and proposed an Unscheduled DNA Synthesis (UDS) test with mammalian liver cells *in vivo* (test method: EU B.39/OECD 486) to confirm the genotoxicity test results.

In its draft decision communicated to the Registrant on 30 November 2012 ECHA proposed to accept the Registrant's testing proposal. However, a Member State Competent Authority proposes in accordance with Article 51(1) of the REACH regulation that ECHA reject the testing proposal and instead require the Registrant to perform a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD 488) to fulfil the information requirement of Annex X, 8.4.

The Registrant provided comments to the proposal for amendment providing arguments supporting its initial testing proposal and considerations why the OECD 488 test is not appropriate.

For the reasons set out below ECHA and its Member State Committee, taking into account the Registrant's comments as well as the proposal for amendment of the Member State Competent Authority, concluded that for the registered substance the proposed test is not appropriate to fulfil the standard information requirement of Annex X, 8.4, and that the Registrant should accordingly perform a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD 488).

ECHA agrees that the information requirement for gene mutation at Annex X, 8.4., must be addressed by an *in vivo* test. Based on the structure (reactive peroxide), rapid hydrolytic instability and properties of the registered substance, specifically irritancy and sensitising properties, ECHA considers that there is a concern that the substance is a short-lived, reactive, *in vitro* mutagen which may be mutagenic at the site of contact with the body. As stated in the ECHA Guidance (Guidance on information requirements and chemical safety assessment R.7a (paper version, May 2008), chapter R.7.7, p. 392) "For substances that are short-lived, reactive, *in vitro* mutagens, or for which no indications of systemic availability have been presented, an alternative strategy involving studies to focus on tissues at initial sites of contact with the body should be considered. Expert judgement should be used on a case-by-case basis to decide which tests are the most appropriate." Moreover, ECHA considers that the Unscheduled DNA synthesis assay with mammalian liver cells *in vivo* (UDS), test method EU B.39/OECD 486, is not an appropriate test to detect mutagens that are active at the site of contact with the body (i.e. which do or may not reach the liver), and hence ECHA concludes that the proposed test is not suitable for the registered substance subject to the present decision. The UDS test is an indicator assay measuring DNA repair, and should be considered as a surrogate test for an *in vivo* gene mutation test. Accordingly, in this case the UDS test proposed by the Registrant would not fulfill the information requirement for Annex X, 8.4., mutagenicity, and is therefore non-compliant and is rejected in accordance with Article 40(3)(d).

According to the ECHA guidance (Guidance on information requirements and chemical safety assessment R.7a (paper version May 2008), chapter R.7.7, p. 392), substances that are short-lived, reactive, *in vitro* mutagens, may be investigated with a transgenic rodent gene mutation assay, according to OECD test guideline 488. This test guideline is able to detect stable mutations (in contrast to TG 486, the UDS test measuring DNA repair) and has the advantage that it can detect mutagenesis not only in the site of contact of the substance with the body, but also gives the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance. Therefore testing according to the OECD guideline 488 is most appropriate in this case where the organs to be investigated are the site of contact (the forestomach and intestine) and those organs already identified in the 28-day study (liver and kidney). In addition, the developing germ cells from the seminiferous tubules are to be investigated to characterise germ cell mutagenicity.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant is requested to carry out the additional study: Mutagenicity, *in vivo* (Annex X, 8.4., test method: OECD 488). The test shall be conducted in mice treated for 42 days, and tissues (forestomach, intestine, liver, kidney, and developing germ cells from the seminiferous tubules) shall be harvested 3 days after the cessation of treatment. Mutation frequency shall be assessed in forestomach, intestine, liver, kidney, and developing germ cells from the seminiferous tubules.

2. Sub-chronic repeated dose toxicity study

A sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, section 8.6.2. of the REACH Regulation. The information on this endpoint is not

available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to generate the data for this endpoint.

The Registrant proposed testing by the oral route in rats. In the light of the physico-chemical properties of the substance (e.g. low vapour pressure and the information provided on the uses and human exposure, ECHA considers that testing by the oral route in rats is appropriate.

The Registrant has based his testing proposal on the findings observed in the 28-day repeated dose toxicity study, where the registered substance showed test-substance related effects in both sexes. Additionally, the study showed clear differences in the response of both sexes to the test substance. Therefore, the Registrant proposes to include additional parameters. The Registrant proposed to extend the sub-chronic toxicity study (90 day) by including additional parameters on sexual organs to support the test proposal with regards to reproduction toxicity. ECHA notes, that it is at the Registrant's discretion to perform the intended additional examinations during the testing program and use the results to ensure the safe use of the substance. However, the Registrant is reminded that the proposed extension of this study does not fulfil the standard information requirements in the registration dossier for reproductive toxicity set out in Annex X, 8.7.3. unless Annex X, 8.7. column 2 adaptation is applied and justified.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study: Sub-chronic toxicity study (90-day) in rats, by the oral route (test method: EU B.26/OECD 408) using the registered substance.

3. Pre-natal developmental toxicity study

Pre-natal developmental toxicity studies are part of the standard information requirements as laid down in Annexes IX and X, section 8.7.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to generate the data for this endpoint.

The Registrant did not specify the species and route to be used for testing. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat as a first species to be used.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study: Pre-natal developmental toxicity study in rats, by the oral route (test method: EU B.31/OECD 414) using the registered substance.

When considering the need for a testing proposal for a prenatal developmental toxicity study in a second species, the Registrant should take into account the outcome of the pre-natal developmental toxicity study on the first species and all available data to determine if the conditions are met for adaptations according to Annex X, 8.7. column 2, or according to Annex XI; for example if the substance meets the criteria for classification as toxic for reproduction Category 1B: May damage the unborn child (H360D), and the available data are adequate to support a robust risk assessment, or alternatively, if Weight of Evidence assessment of all relevant available data provides scientific justification that the study in a second species is not needed.

IV. Adequate identification of the composition of the tested material

The process of evaluation of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the generation of information is tailored to real information needs in order to prevent unnecessary testing. The information submitted in the registration dossier was sufficient to confirm the identity of the substance for the purpose of assessing the testing proposal. It is noted, however, that this information, or the information submitted by other registrants of the same substance, has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In relation to the proposed tests, the sample of substance used for the new studies must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is within the specifications of the substance composition that are given by the joint registrants. It is the responsibility of all the joint registrants of the same substance to agree with the tests proposed in the testing proposal (as applicable to their tonnage level) and to document the necessary information on its composition. The substance identity information of the registered substance and of the sample tested must enable ECHA to confirm the relevance of the testing for the substance actually registered by each joint registrant. Finally, the studies must be shared by the joint registrants concerned.

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 a. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

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Geert Dancet
Executive Director