EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114301941-59-01/F Helsinki, 30 June 2015

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

For tert-pentyl hydroperoxide, CAS No 3425-61-4 (EC No 222-321-7), 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 the following testing proposal submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(c) thereof for tert-pentyl hydroperoxide, CAS No 3425-61-4 (EC No 222-321-7, submitted by [redacted] (Registrant).

- *In vivo* mammalian erythrocyte micronucleus test (OECD 474), in rats, inhalation route.

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 10 to 100 tonnes per year. This decision does not take into account any updates after 15 January 2015, 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 REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

On 5 April 2013, pursuant to Article 40(1) of the REACH Regulation, ECHA initiated the examination of the testing proposal set out by the Registrant in the registration dossier for the substance mentioned above.

ECHA held a third party consultation for the testing proposal from 21 March 2014 until 5 May 2014. ECHA did not receive information from third parties.

On 25 June 2014 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision. That draft decision was based on submission number [redacted].

On 1 August 2014 ECHA received comments from the Registrant on the draft decision.

On 8 October 2014 the Registrant updated his registration dossier with the submission number [redacted].

The ECHA Secretariat considered the Registrant's comments and update. On basis of this information, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

On 15 January 2015 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 for amendment of the draft decision within 30 days of the receipt of the notification.

Subsequently, proposals for amendment to the draft decision were submitted.

On 20 February 2015 ECHA notified the Registrant of the proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposals for amendment received and did not amend the draft decision.

On 2 March 2015 ECHA referred the draft decision to the Member State Committee.

By 23 March 2015, in accordance to Article 51(5), the Registrant provided comments on the proposals for amendment. The Member State Committee took the comments of the Registrant on the proposals for amendment into account.

After discussion in the Member State Committee meeting on 20-23 April 2015, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 22 April 2015.

ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

A. Tests required pursuant to Article 40(3)

The Registrant shall carry out the following test pursuant to Article 40(3)(c) of the REACH Regulation using the indicated test method and the registered substance subject to the present decision:

> *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD 489, in rats, oral route, with examination of liver, forestomach and glandular stomach.

while the originally proposed test for an *in vivo* mammalian erythrocyte micronucleus test (OECD 474), in rats, inhalation route, proposed to be carried out using the registered substance is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

Note for consideration by the Registrant:

The Registrant may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring to and conforming with the appropriate rules in the respective Annex, and an adequate and reliable documentation.

Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, shall result in a notification to the Enforcement Authorities of the Member States.

B. Deadline for submitting the required information

Pursuant to Articles 40(4) and 22(2) of the REACH Regulation, the Registrant shall submit to ECHA by **7 July 2016** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposals submitted by the Registrant for the registered substance.

Tests required pursuant to Article 40(3)

In vivo mammalian alkaline comet assay

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 other tests in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "Appropriate in vivo mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII."

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and chromosomal aberrations is not available for the registered substance but shall be proposed by the Registrant. Consequently, there is an information gap and the Registrant proposed to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for an *in vivo* mammalian erythrocyte micronucleus test to be performed with the registered substance with the following justification: *"In order to clarify the positive results obtained in the in vitro micronucleus assay, it is proposed to perform an in vivo micronucleus assay in the bone marrow of rats exposed by inhalation"*.

ECHA notes that an *in vivo* mammalian erythrocyte micronucleus test is as such an appropriate test to investigate further effects on chromosomal aberrations as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.1. and figure R.7.7-1 (August 2014). However, the high reactivity of the substance in question raises a concern regarding the potential for the registered substance and/or its metabolites to reach the bone marrow. This is the tissue analysed in the *in vivo* mammalian erythrocyte micronucleus test and thus, the bone marrow needs to be reached by the substance for the results to be conclusive. Likewise, the high reactivity of the substance also brings a concern on potential mutagenic effects at the first site of contact that needs to be addressed and the micronucleus assay cannot be used for that purpose because site of contact tissue is not used in the test. Therefore, ECHA considers that it is unlikely that the test proposed by the Registrant will provide meaningful results since it cannot address the concern at the site of contact and the substance might not reach the bone marrow.

According to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (August 2014), the *in vivo* mammalian alkaline comet assay (OECD 489) is suitable to follow up positive result *in vitro* for gene mutation and for

chromosomal aberrations. ECHA considers that the comet assay is more appropriate since it can be performed on several tissues including sites of contact with the registered substance.

The comet assay shall be performed in rats because rats are routinely used for this test and this species was also used in other toxicity studies for the registered substance.

In light of the physicochemical properties of the substance (liquid with a vapour pressure of 4320 Pa at 25 °C) and relevant human inhalation exposure (e.g. spraying processes), ECHA considers that testing by the inhalation route is appropriate. However, considering the corrosive properties of the substance as well as its high solubility, the upper respiratory tract (i.e. the first site of contact) will most likely be the main target of the substance and thus the tissue to be analyzed in a comet assay as site of contact. However, there is currently a technical difficulty associated with the study of this tissue (i.e. nose epithelium) in the comet assay which might prevent the validation of the data generated by such a study. An alternative tissue exposed via inhalation could be considered, i.e. the lung. However, it is expected that this second site of contact tissue will be exposed to a lower dose considering that a fraction of the substance will have reacted at the first site of contact (i.e. upper respiratory tract) given the high solubility and high reactivity of the registered substance. Therefore, ECHA considers that testing by inhalation route will not provide relevant data to address the concern identified *in vitro*. The alternative route of administration is the oral route. Based on the above, ECHA considers that performing the comet assay by the oral route is more appropriate for this substance.

The test shall be performed by using the following tissues: liver as primary site of xenobiotic metabolism, and both forestomach and glandular stomach as sites of direct contact. The request of testing in both forestomach and glandular stomach is justified by the need to address the uncertainty, associated with the administration by oral gavage, on the actual first site of contact. It is particularly important to address the concern on potential genotoxic effects at the first site of contact for this highly reactive substance.

b) Outcome

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant is requested to carry out the following study with the registered substance subject to the present decision: *In vivo* mammalian alkaline comet assay (OECD TG 489), in rats via oral route, with examination of liver, forestomach and glandular stomach. Pursuant to Article 40(3)(d) the proposed i*n vivo* mammalian erythrocyte micronucleus test is rejected.

Note for the consideration of the Registrant

The Registrant is reminded that according to the column 2 of section 8.4 of Annex IX of the REACH Regulation, if positive results from an in vivo somatic cell study are available, "*the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered*". ECHA notes that the examination of gonadal cells would optimize the use of animals. Positive results in whole gonad that contains a mixture of somatic and germ cells are not necessarily reflective of germ cell damage, but they indicate that tested substance(s) and/or its metabolites have reached the gonad and caused genotoxic effects. This type of evidence may still be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

IV. Adequate identification of the composition of the tested material

The process of examination of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the new studies meet real information needs. Within this

context, the Registrant's dossier was sufficient to confirm the identity of the substance to the extent necessary for examination of the testing proposal.

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 joint registrants of the same substance to agree to the tests proposed (as applicable to their tonnage level) and to document the necessary information on their substance composition.

In addition, 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 by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new studies must be suitable to assess these grades.

Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the studies to be assessed.

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

ECHA 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).

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://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

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