

Decision number: TPE-D-2114301475-58-01/F

Helsinki, 30 July 2015

DECISION ON TESTING PROPOSAL SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006**For tert-butyl peroxyisobutyrate, CAS No 109-13-7 (EC No 203-650-5),
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 tert-butyl peroxyisobutyrate, CAS No 109-13-7 (EC No 203-650-5), submitted by [REDACTED] (Registrant).

- *In vivo* rodent alkaline comet assay

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 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.

ECHA received the updated registration dossier containing the above-mentioned testing proposal for further examination pursuant to Article 40(1) on 22 March 2013.

ECHA held a third party consultation for the testing proposal from 15 July 2014 until 29 August 2014. ECHA did not receive information from third parties.

On 5 November 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.

By 12 December 2014 the Registrant did not provide any comments on the draft decision to ECHA.

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 amended section III of 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.

A unanimous agreement of the Member State Committee on the draft decision was reached on 7 April 2015 in a written procedure launched on 26 March 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 proposed test pursuant to Article 40(3)(a) and 13(4) of the REACH Regulation using the indicated test method and the registered substance subject to the present decision:

- *In vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (Comet assay) (Annex IX, Section 8.4., column 2, OECD Guideline 489) in rat via the oral route, with examination of liver and either (glandular) stomach or duodenum/jejunum.

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 in this decision, or to fulfil otherwise the information requirement with a valid and documented adaptation, will 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 **8 August 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 proposal submitted by the Registrant for the registered substance.

A. Tests required pursuant to Article 40(3)

- *In vivo* mammalian alkaline Comet assay (Annex IX, Section 8.4., column 2, OECD 489)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "[I]f there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant."

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations 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 a Rodent alkaline single cell gel electrophoresis (Comet) assay (at the time of the testing proposal under draft by the OECD) with the following justification: "*Tert-butyl peroxyisobutyrate revealed positive results in a bacterial reverse mutation assay (Ames) and in a HPRT assay in vitro. Based on the available in vitro data, an alkaline in vivo Comet assay is proposed to assess the mutagenic properties of the test substance in vivo. The in vivo Comet assay is considered to be the appropriate test system to investigate short-lived substances at the first site of contact. Generally, organic peroxides undergo hydrolysis when getting in contact with water and thus disappear quickly if applied orally. The Comet assay is an already established in vivo mutation assay performed in a wide range of CRO's in EU. As a result, historical control data are available for various tissues while the availability of labs with sufficient experience with the Transgenic Rodent Mutation Assay (TRM) is limited.*"

ECHA notes that the test guideline of *In vivo* mammalian alkaline Comet assay is now an adopted OECD test guideline (OECD 489).

As regards the route of administration, paragraph 39 of the OECD 489 test guideline states that "[t]he anticipated route of human exposure should be considered when designing an assay" and "[i]n any case the route should be chosen to ensure adequate exposure of the target tissue(s)". In light of the physicochemical properties of the substance (liquid), ECHA considers that testing by the *oral* route is appropriate.

As regards the species to be used, paragraph 23 of the OECD 489 test guideline states that "[t]he choice of rodent species should be based on (i) species used in other toxicity studies (to be able to correlate data and to allow integrated studies), (ii) species that developed tumours in a carcinogenicity study (when investigating the mechanism of carcinogenesis), or (iii) species with the most relevant metabolism for humans, if known. Rats are routinely used in this test." ECHA notes that an OECD 422 study in rat has been performed using the registered substance. ECHA considers that testing in the rat is appropriate.

As regards the tissues to be studied, paragraph 42 of the OECD 489 test guideline states that “[t]he liver has been the tissue most frequently studied and for which there are the most data. Therefore, in the absence of any background information, and if no specific tissues of interest are identified, sampling the liver would be justified as this is a primary site of xenobiotic metabolism and is often highly exposed to both parent substance(s) and metabolite(s). In some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant.” Therefore ECHA considers that the Comet assay should be performed in liver and either glandular stomach or duodenum/jejunum.

ECHA notes that, in his comments on the proposals for amendment from Competent Authorities of the Member States, the Registrant agrees to analyse three tissues (i.e. ‘liver, forestomach and duodenum/jejunum’) in the *in vivo* mammalian alkaline Comet assay. ECHA points out that the (glandular) stomach should be analysed instead of the forestomach..

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: *In vivo* mammalian alkaline Comet assay (OECD 489 Guideline) in rat via the oral route, with examination of liver and either stomach or duodenum/jejunum.

Note for consideration by 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 study 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. The Registrant must note, however, that this information has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In addition, is important to ensure that the particular sample of substance tested in the new study 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 study must be suitable to assess these.

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

V. 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.

Authorised^[1] by Leena Ylä-Mononen, Director of Evaluation

^[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.