Decision number: TPE-D-2114322496-49-01/F Helsinki, 29 March 2016

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

For (3E)-2-chloro-3-(hydroxymethylene)cyclohexene-1-carbaldehyde, EC No 801-656-8 (CAS No 1416808-92-8), 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 (3E)-2-chloro-3-(hydroxymethylene)cyclohexene-1-carbaldehyde, EC No 801-656-8 (CAS No 1416808-92-8), submitted by [redacted] (Registrant).

- *In vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay) (Annex VII, Section 8.4., column 2; test method: OECD 489) combined with i*n vivo* mammalian erythrocyte micronucleus test (Annex VII, Section 8.4., column 2; test method EU B.12./OECD 474)

This decision is based on the registration as submitted with submission number [redacted], for the tonnage band of 1 to 10 tonnes per year.

This decision does not take into account any updates after 2 October 2015, i.e. 30 calendar days after the end of the commenting period.

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.

The examination of the testing proposal was initiated on 27 February 2015.

ECHA held a third party consultation for the testing proposal from 17 April 2015 until 4 June 2015. ECHA did not receive information from third parties.

On 27 July 2015 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 2 September 2015 the Registrant did not provide any comments on the draft decision to ECHA.

On 29 October 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 4 December 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 the draft decision.

On 14 December 2015 ECHA referred the draft decision to the Member State Committee.

By 4 January 2016 the Registrant did not provide any comments on the proposals for amendment.

After discussion in the Member State Committee meeting on 2–4 February 2016, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 3 February 2016.

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)(a) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

1. *In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD 489); which may be combined with an *in vivo* mammalian erythrocyte micronucleus test (Annex VII, Section 8.4., column 2; test method: EU B.12./OECD 474); in rats, oral route. For the comet assay, the following tissues shall be analysed: liver, glandular stomach, and duodenum/jejunum. For the micronucleus test, if performed, the bone marrow shall be analysed.

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, 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 **5 April 2017** 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 VII, Section 8.4., column 2); which may be combined with an *in vivo* mammalian erythrocyte micronucleus test (Annex VII, Section 8.4., column 2)

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 VII, Section 8.4. of the REACH Regulation. Column 2 of Annex VII, Section 8.4. provides that "Further mutagenicity studies shall be considered in case of a positive result.

The technical dossier contains an *in vitro* study, Bacterial reverse mutation assay, performed according to OECD Guideline 471 with the registered substance that show positive results. The positive results indicate that the substance is inducing gene mutations under the conditions of the test.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance. The Registrant considered it necessary to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for an *in vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay) (test method: OECD 489) combined with i*n vivo* mammalian erythrocyte micronucleus test (test method EU B.12./OECD 474), to be performed with the registered substance subject to the present decision with the following justification:
"Because of the positive result in the genetic toxicity *in vitro* (7.6.1) further mutagenicity testing is required in order to be REACH compliant.
Concerning further mutagenicity testing we propose a combined in vivo micronucleus/comet assay in order to detect all other potential modes of mutagenicity action besides the already detected base pair substitutions in 7.6.1. The in vivo comet assay alone cannot conclude on aneuploidy but the in vivo micronucleus can do so.
No info on systemic uptake in blood is available, but our company medicin believes that, due to the potential dermal exposure, systemic uptake can not be avoided."

ECHA considers that the Registrant has adequately demonstrated the need to perform the comet assay. ECHA considers that this assay is appropriate to investigate effects on gene mutation *in vivo* 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).

The Registrant did not specify the species to be used for testing, neither did he specify the route for testing. According to the test method (OECD 489), the test shall be performed in rats by the oral route considering the anticipated routes of human exposure and adequate exposure of the target tissue(s).

According to the test method (OECD TG 489), the test shall be performed by analysing tissues from liver, glandular stomach and duodenum/jejunum. As set out in the OECD TG 489, the liver is recommended as the primary site of xenobiotic metabolism, and an often highly exposed tissue. The glandular stomach and duodenum/jejunum are recommended as tissues to examine site of contact effects after oral exposure.

In view of the different pH, different tissue structure and function of the stomach and duodenum/jejunum, and known examples of differential absorption of substances between these two tissues, ECHA considers that it is necessary to sample both tissues to have a sufficient analysis of genotoxicity at the site of contact.

Although in this case there were no data available in the registration dossier for either the *in vitro* mammalian chromosomal aberration or for the *in vitro* micronucleus test, the Registrant proposed to combine the *in vivo* comet assay with an *in vivo* micronucleus test. The micronucleus test is suitable to investigate other potential modes of genotoxicity such as clastogenicity and aneuploidy, as expressed in the Registrant's testing proposal. 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. The Registrant should nevertheless take into account the combination aspects such as dosing and sampling following the principles described in the literature (see e.g. Bowen et al. 2011[1]).

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the registrant is requested to submit the following information derived with the registered substance subject to the present decision:

In vivo mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD 489); which may be combined with an in vivo mammalian erythrocyte micronucleus test (Annex VII, Section 8.4., column 2; test method: EU B.12./OECD 474); in rats, oral route. For the comet assay, the following tissues shall be analysed: liver, glandular stomach, and duodenum/jejunum. For the micronucleus test, if performed, the bone marrow shall be analysed.

Note for consideration by the Registrant

When performing the comet assay, the Registrant may consider examining gonads for analysis of germ cell mutagenicity. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

[1] Bowen D.E. et al. 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the Comet assay and the flow-cytometric peripheral blood micronucleus test. Mutation Research, 722, 7–19.

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, it 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[2] by Guilhem de Seze, Head of Unit, Evaluation, E1.

[2] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.