EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-0000003795-64-06/F Helsinki, 3 February 2014

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

For Reaction mass of (E)-1-chlorobut-2-ene and 3-chlorobut-1-ene, EC No 908-820-9, 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)/(e) thereof for Reaction mass of (E)-1-chlorobut-2-ene and 3-chlorobut-1-ene, List No 908-820-9, by [redacted] (Registrant).

Mammalian Erythrocyte Micronucleus Test (OECD 474) with an integrated Comet Assay in vivo

This decision is based on the registration dossier as submitted with submission number [redacted], for general registration purposes in the tonnage band of 1 to 10 tonnes per year as well as for a transported isolated intermediate for 1000 tonnes or more per year. This decision does not take into account any updates after 20 June 2013, 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 present dossier at a later stage.

On 23 March 2012, 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 16 July 2012 until 30 August 2012. ECHA did not receive information from third parties.

On 20 November 2012 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 20 December 2012 ECHA received comments from the Registrant. On 23 January 2013 and subsequently on 7 May 2013 the Registrant updated his registration dossier proposing an alternative testing strategy to that originally proposed, by integrating Comet Assay in vivo into the originally proposed Mammalian Erythrocyte Micronucleus Test in vivo.

ECHA considered the Registrant's comments and dossier updates received.
On basis of the comments and dossier updates, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

On 20 June 2013 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 26 July 2013 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 decided not to amend the draft decision.

On 5 August 2013 ECHA referred the draft decision to the Member State Committee.

On 23 August 2013 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 25-27 September 2013, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 27 September 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

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

- Mammalian Erythrocyte Micronucleus Test (test method:OECD 474) with an integrated Comet Assay in vivo, in accordance with the protocol described in the study outline provided by the Registrant in their registration dossier, and attached to this decision.

Pursuant to Articles 40(4) and 22 of the REACH Regulation, the Registrant shall submit to ECHA by **3 February 2015** an update of the registration dossier containing the information required by this decision.

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 submitted by the Registrant for the registered substance.

In vivo gene mutation assay

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.

The current dossier is registered at the tonnage level of 1-10 tonnes per year for general registration purposes as well as a transported isolated intermediate for 1000 tonnes or more per year. In order to satisfy the information requirement of Annex VII, 8.4., the Registrant provided in his dossier the results for a bacterial reverse mutation assay (Ames test, OECD 471), in which the registered substance yielded positive results. According to Annex VII, 8.4., column 2 of the REACH Regulation, further mutagenicity studies shall be considered in case of a positive result.

Subsequently, the Registrant provided the results for an in vitro mammalian cell gene mutation test (OECD 476) and an in vitro mammalian chromosome aberration test (OECD 473), and considered the results of these two tests as negative. ECHA notes that in the in vitro chromosome aberration test positive results were obtained at high concentrations causing cytotoxicity, but that this was considered as not biologically relevant by the Registrant.

According to Annex VIII, 8.4., column 2 of the REACH Regulation, 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. During dossier evaluation, in view of the available results and in order to clarify the genotoxic potential of the registered substace, ECHA invited the Registrant to submit a testing proposal addressing genetic toxicity in vivo.

Initially, in their dossier submission ([redacted]) the Registrant had proposed to perform a Mammalian Erythrocyte Micronucleus Test (test method: OECD 474) in order to clarify the mutagenic potential of the substance.

However, ECHA considered that the proposed mammalian erythrocyte micronucleus test, does not address gene mutations (which needs further investigation based on the positive result of Ames test) but detects structural and numerical chromosome aberrations, having the potential to detect clastogenic and aneugenic substances. ECHA considered, in accordance with its Guidance on information requirements and chemical safety assessment, a transgenic rodent gene mutations assay (TGR) (OECD test guideline 488) as an appropriate test to detect gene mutations in vivo, and therefore an appropriate in vivo study, according to Annex VIII, 8.4., column 2 of the REACH Regulation to be performed in this case. A draft decision requesting a transgenic rodent gene mutations assay (TGR) was sent to the Registrant on 20 November 2012.

Following ECHA's draft decision, the Registrant submitted comments indicating their intention to propose an alternative testing strategy to that originally submitted. The Registrant commented that *'it has been clarified in the meanwhile that uses of the substance other than intermediate under SCC conditions are not being performed'* and that in their view *'Taking into account that the substance is a sole intermediate the performance of the high priced TGR test is not appropriate with regard to the economic proportionality of the measure.'*.

In their comments and in their subsequent dossier updates (23 January and 7 May 2013) the Registrant still proposed to perform a Mammalian Erythrocyte Micronucleus Test (OECD 474), but to combine it with an integrated Comet assay (single cell gel electrophoresis assay) in vivo. The Registrant stated that *'The in vivo alkaline Comet Assay is a widely accepted method to assess the genotoxic potential of a great variety of chemicals by detecting primary*

DNA damage in various organs and tissues of exposed animals. Although for the Comet Assay in vivo no OECD guideline is available yet there are internationally agreed protocols for performing this test. Regulatory relevance of the Comet assay in vivo has recently been approved by the European Food Safety Authority (Scientific Report of EFSA, Minimum Criteria for the acceptance of in vivo alkaline Comet Assay Reports, EFSA Journal 2012; 10(11):2977).'

The details on the proposed test protocol were included in the updated registration dossier ([redacted]) and are attached to this decision. Although there is no internationally agreed guideline for the Comet assay, ECHA considers that the proposed protocol for the Mammalian Erythrocyte Micronucleus Test (test method:OECD 474) with an integrated Comet Assay in vivo to be conducted in liver and stomach tissue is adequate to further investigate the potential gene mutation effects of the registered substance and is therefore an appropriate in vivo study according to Annex VIII, 8.4., column 2 of the REACH Regulation to be performed in this case.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the following study using the registered substance:

Mammalian Erythrocyte Micronucleus Test (test method:OECD 474) with an integrated Comet Assay in vivo according to the study outline submitted together with the registration dossier (submission number [redacted]) and as described above.

IV. 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.

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://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.

[redacted]
Leena Ylä-Mononen
Director of Evaluation

Attachment: Testing protocol to be applied including the Comet-assay