EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-0000003665-69-04/F Helsinki, 31 March 2014

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

For 2,5-bis-isocyanatomethyl-bicyclo[2.2.1]heptane, CAS No 74091-64-8 (EC No 411-280-2), 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 proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for 2,5-bis-isocyanatomethyl-bicyclo[2.2.1]heptane, CAS No 74091-64-8 (EC No 411-280-2), by [redacted] (Registrant).

- Mammalian Erythrocyte Micronucleus Test (OECD 474); and
- *In vivo* Comet assay

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of [redacted]. 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.

The examination of the testing proposals was initiated upon the date when receipt of the complete registration dossier was confirmed on 4 June 2012. On 23 October 2012 the Registrant updated his registration dossier.

ECHA held a third party consultation for the testing proposals 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.

On 18 December 2012 ECHA received comments from the Registrant.

On 24 January 2013 the Registrant updated the dossier, withdrawing the initially submitted testing proposal for Unscheduled DNA Synthesis (UDS) test with Mammalian Liver Cells *in*

vivo (test method: EU B. 39/OECD 486/ mutagenicity), and proposing instead an *in vivo* Comet assay.

ECHA considered the Registrant's comments received as well as the registration updates received before 20 June 2013. On this basis, 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 in Section II, but modified Section III to include a reminder related to germ cell mutagenicity.

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

On 26 August 2013 the Registrant provided comments on the proposals for 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 26 September 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

The Registrant shall carry out the following 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:

- Mutagenicity – *in vivo* Mammalian Erythrocyte Micronucleus Test (Annex IX, 8.4., test method: EU B.12/OECD 474);

- *In vivo* Comet assay (Annex IX, 8.4.), in accordance with the protocol provided by the Registrant in his registration dossier, and attached to this decision.

The Registrant should carefully consider the choice of testing strategy, in particular the order in which the two studies will be performed. Indeed, the Guidance on information requirements and chemical safety assessment (May 2008) states that if the substance is classified in accordance with the CLP Regulation (EC) No 1272/2008 as Category 1A or 1B mutagen ((Dangerous Substances Directive 67/548/EEC (DSD) Category 1 or 2 mutagen), or as a CLP Category 1A carcinogen, no further *in vivo* testing on mutagenicity is needed. Accordingly, if the Registrant considers that the criteria for classification for CLP Category

1A or 1B (DSD Category 1 or 2) for mutagenicity are fulfilled, based on the results obtained from the first conducted *in vivo* assay, no further testing will be necessary.

Pursuant to Articles 40(4) and 22 of the REACH Regulation, the Registrant shall submit to ECHA by **31 March 2016** an update of the registration dossier containing the information required by this decision.

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.

1. Mutagenicity – *in vivo* Mammalian Erythrocyte Micronucleus Test

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 Registrant has submitted a positive *in vitro* mammalian cell gene mutation test (OECD test guideline 476), indicating the potential for the substance to cause chromosome aberration *in vitro*. According to Annex IX, section 8.4. of the REACH Regulation, a mammalian erythrocyte micronucleus test is part of the standard information requirements if 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.

The Registrant has justified his testing proposal on the need to investigate further the potential effects on chromosomal aberrations of the subtance by referring to the positive results obtained in the *in vitro* mammalian cell gene mutation study.

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.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study: *in vivo* Mammalian Erythrocyte Micronucleus Test (test method: EU B.12/OECD 474) using the registered substance.

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.

2. *In vivo* Comet 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.

ECHA notes that the *in vitro* gene mutation activity of the substance was confirmed by a positive *in vitro* mammalian cells gene mutation test (OECD test guideline 476). Based on the available data, the Registrant concludes that the substance shows an alert for gene

mutation and proposed an *in vivo* Comet assay to address the potential gene mutation effects of the registered substance.

According to the ECHA guidance (Guidance on information requirements and chemical safety assessment R.7a (November 2012), chapter R7.7., p. 347, 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, or with a Comet assay.

Following ECHA's initial draft decision sent to the Registrant on 20 November 2012, the Registrant updated his registration on 24 January 2013 and proposed a Comet Assay instead of the initially proposed *in vivo* Unscheduled DNA Synthesis (UDS) test with Mammalian Liver Cells (test method: EU B. 39/OECD 486/ mutagenicity) in order to address the potential gene mutation effects of the registered substance. The Registrant comments to the draft decision support ECHA's view that the Unscheduled DNA Synthesis Test is not an appropriate test given the physicochemical properties of the registered substance. In his comments the Registrant indicates that *"the alkaline Comet assay identifies the broadest spectrum of DNA damage by detecting double- and single-strand breaks, alkaline-labile lesions that are expressed as single-strand breaks and single-strand breaks arising as DNA repair intermediates (e.g. after point mutations) ("Minimum Criteria for the acceptance of in vivo alkaline Comet Assay Reports", European Food Safety Authority (EFSA), EFSA Journal 2012; 10 (11):2977). Therefore, the applicability of the Comet assay is of equal value compared to the TGR assay for assessment of in vivo genotoxicity and for classification and labelling purposes. (...)Although no validated OECD guideline for the Comet assay is available yet, this assay will be performed under GLP conditions following the International Validation of the In Vivo Alkaline Comet Assay for the Detection of Genotoxic Carcinogens (Version 14.2; 2009; http://cometassay.com/JaCVAM.pdf) and the recent recommendations published by EFSA ("Minimum Criteria for the acceptance of in vivo alkaline Comet Assay Reports", European Food Safety Authority (EFSA), EFSA Journal 2012; 10 (11):2977) to ensure high quality test data"*

The details on the proposed test protocol to be applied by the Registrant for the *in vivo* Comet assay were included in the updated registration dossier, and are attached to this decision. Thus, although there is no internationally agreed guideline for the Comet assay, ECHA considers that the proposed detailed protocol for the Comet Assay to be conducted in rats in blood, liver and stomach tissue is adequate to further investigate the potential gene mutation effects of the registered substance.

According to section 8.4. of Annexes IX and X, column 2 of the REACH Regulation, if the result of the *in vivo* somatic cell mutation study is positive, the Registrant should consider the potential for germ cell mutagenicity on the basis of all available data, including toxicokinetic evidence. The Registrant should document these considerations as part of the endpoint summary in the technical IUCLID dossier and in the hazard assessment of the chemical safety report as this is relevant information concerning the substance subject to the present decision. If no clear conclusions about germ cell mutagenicity can be made, the Registrant shall consider additional investigations and may need to submit further testing proposals accordingly.

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:in vivo Comet Assay, according to the test method protocol provided in the dossier submission [redacted], using the registered substance subject to the present 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.

ECHA emphasises that the two tests (*In vivo* Mammalian Erythrocyte Micronucleus Test (Annex IX, 8.4., test method: EU B.12/OECD 474 and *in vivo* Comet assay (Annex IX, 8.4.), in accordance with the protocol provided by the Registrant in his registration dossier, and attached to this decision) may be performed in combination to minimise vertebrate animal testing. The Registrant may combine the Comet assay with the *in vivo* Mammalian Erythrocyte Micronucleus Test provided that the combined test is fulfilling the requirements of the aforementioned test method (EU B.12/OECD 474) and the Comet assay protocol. In case the two studies are performed in a combined way, the Registrant has to ensure that the outcome of such a combined test is sufficiently documented to allow the assessment whether the data generated meets all conditions of the two individual studies.

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 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 evaluation 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.

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. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Furthermore, there must be adequate information on substance identity for the sample tested and the grades 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://echa.europa.eu/regulations/appeals. 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: Test protocol to be applied when performing the Comet-assay