Decision number: TPE-D-2114331312-66-01/F Helsinki, 09 June 2016

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

For Bis(2-chloroethoxy)methane 1,15-dichloro-3,5,8,11,13-penta-oxa pentadecane 1-(2-chloroethoxy)-2-(2-chloroethoxymethoxy)ethane, List No 940-783-4, 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)(e) thereof for Bis(2-chloroethoxy)methane 1,15-dichloro-3,5,8,11,13-penta-oxa pentadecane 1-(2-chloroethoxy)-2-(2-chloroethoxymethoxy)ethane, List No 940-783-4, submitted by [redacted] (Registrant).

- In vivo Mammalian Erythrocyte Micronucleus Test (OECD 474)
- In vivo Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells (OECD Guideline 486)
- Two-generation reproduction toxicity study (OECD 416)
- Daphnia reproduction toxicity test (OECD 211)
- Fish Early-Life Stage toxicity test (OECD 210)

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 1000 tonnes or more per year. This decision does not take into account any updates after 21 January 2016, 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.

The examination of the testing proposals was initiated upon the date when receipt of the complete registration dossier was confirmed on 21 February 2014.

ECHA held a third party consultation for the testing proposals from 2 June 2014 until 17 July 2014. ECHA received information from third parties (see section III below).

On 27 August 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 2 October 2014 ECHA received comments from the Registrant on the draft decision. On 09 October 2014, the Registrant updated his registration dossier (submission number [redacted]). Subsequently, following a request from ECHA to provide further documentation, the Registrant updated again his registration dossier on 27 January 2015 ([redacted]). Following a request from ECHA to correct an inconsistency in the dossier, the Registrant updated again his registration dossier on 7 May 2015 ([redacted]) and on 9 June 2015 ([redacted]). In his update the Registrant removed the testing proposals for Two-generation reproduction toxicity study (OECD 416), Daphnia reproduction toxicity test (OECD 211) and Fish Early-Life Stage toxicity test (OECD 210) from his dossier. ECHA has accordingly terminated the evaluation process concerning these testing proposals.

The Registrant has also updated the proposals for In vivo Mammalian Erythrocyte Micronucleus Test (OECD 474) and In vivo Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells (OECD Guideline 486) by replacing the original proposals with a single proposal for a combined test, *In vivo* mammalian erythrocyte micronucleus test (test method: OECD 474) combined with a comet (single cell gel electrophoresis) assay (OECD TG 489).

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

On 21 January 2016 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 26 February 2016 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 7 March 2016 ECHA referred the draft decision to the Member State Committee.

By 29 March 2016, 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 12 April 2016 in a written procedure launched on 1 April 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 proposed tests 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 erythrocyte micronucleus test (Annex IX, Section 8.4, column 2; test method: OECD TG 474)[1] combined with an In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489)[2]; in rats, with oral administration. For the comet assay, the following tissues shall be analysed: liver, glandular stomach and duodenum. For the micronucleus test, the bone marrow shall be analysed. The test design should consider combination aspects such as dosing and sampling as described in the literature (see OECD test guideline 489 and e.g. Bowen et al. 2011[3]).

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 **16 June 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 proposals submitted by the Registrant for the registered substance and scientific information submitted by third parties.

[1] Only the OECD TG is mentioned since it has recently been updated while the corresponding EU test method has not yet been updated.
[2] Only the OECD TG is mentioned since it has recently been adopted and the corresponding EU test method has not yet been published.
[3] 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.

A. Tests required pursuant to Article 40(3)

1. In vivo mammalian erythrocyte micronucleus test (Annex IX, Section 8.4, column 2; test method:OECD TG 474) combined with an In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489); in rats, with oral administration.

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 "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, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant." Furthermore, Column 2 of Annex X, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annexes VII or VIII, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data."
There are positive results in an in vitro Mammalian Chromosome Aberration test, and in an in vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay). The Registrant has provided in vivo micronucleus robust study summaries for read-across from supporting substances. An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations or chromosomal aberrations is not available for the registered substance. The Registrant has provided documentation in the technical dossier to demonstrate that the registered substance is used under Strictly Controlled Conditions (SCC). ECHA has evaluated the information and considers it as fulfilling the requirements of Annex XI, Section 3.2(b). Thus the Registrant has provided a valid adaptation for the information requirements of Annex IX and X. However, the Registrant has a valid concern for gene mutagenicity arising from positive results of in vitro tests, and has proposed to generate information for this endpoint in accordance with Annex VIII, Section 8.4 and column 2 of Section 8.4 of Annexes IX and X and in agreement with ECHA Guidance (R.7.7.6.3; Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance Version 4.1 October 2015).

Article 13(1) of the REACH Regulation states that testing in accordance with Annex IX and X may be omitted where justified by information on exposure and implemented risk management measures as specified in Annex XI, section 3. As suggested by the Registrant, ECHA considers that in this case and taking into account the type of concern, it is appropriate to address this identified concern with a combined in vivo test even in the presence of valid adaptation based on Annex XI, Section 3.2.

In their initial dossier submission ([redacted]) the Registrant had proposed to perform a separate Mammalian Erythrocyte Micronucleus Test (OECD 474) in order to clarify the mutagenic potential of the substance in respect of the positive result in an in vitro mammalian chromosome aberration test OECD 473. In addition, the Registrant had originally proposed to perform a separate in vivo Unscheduled DNA synthesis (UDS) Test with Mammalian Liver Cells (OECD TG 486) in order to clarify the mutagenic potential of the substance in respect of the positive gene mutation result obtained in vitro (i.e. in bacterial reverse mutation assay OECD 471).

In ECHA's draft decision sent to the Registrant, ECHA noted that the OECD 474 micronucleus test is an appropriate test to investigate further effects on chromosomal aberrations *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 (February 2014). However, ECHA considered that according to this ECHA Guidance, the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (TGR), the Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells and the Comet assay were suitable to follow-up the positive gene mutation result obtained *in vitro* (i.e. in bacterial reverse mutation assay OECD 471). Regarding the Comet assay, no adopted OECD guideline was available at the time when the draft decision was sent to the Registrant. Concerning the UDS, this assay has only the liver as target organ, while the TGR is able to detect gene mutations, and the comet assay, DNA damage, also in other tissues. Furthermore, the TGR measures permanent mutations whereas the UDS and the Comet assays only detect putative DNA lesions. Based on the above, ECHA considered that the most appropriate test to follow-up in vivo gene mutation concern was the TGR assay.

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 *'The testing proposal to perform an mammalian erythrocyte micronucleus test OECD 474 was removed from the dossier in the update and replaced by a combination of the mammalian erythrocyte micronucleus test (OECD 474) and Comet Assay (OECD 489). Per ECHA endpoint specific guidance update from august 2014: The comet assay and the in vivo micronucleus test can be combined into a single acute study. This combination saves resources and numbers of animals used. This will provide information on mutagenicity and clastogenicity.'*

In their comments and in their subsequent dossier updates (9 October 2014, 27 January 2015 and 9 June 2015) the Registrant still proposed to perform an In vivo Mammalian Erythrocyte Micronucleus Test (OECD 474), but to combine it with an In Vivo Mammalian Alkaline Comet assay (OECD 489). ECHA accepts that the In Vivo Mammalian Alkaline Comet assay (OECD 489) is an appropriate test to address the concern for gene mutation. ECHA notes that the amended testing proposal made by the registrant in the latest dossier update (submission [redacted] of 9 June 2015), i.e. to combine the micronucleus test in vivo (OECD 474) and the comet assay in vivo (OECD 489) is in agreement with the current ECHA guidance (Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance, version 4.1, October 2015). Consequently, ECHA accepts this testing proposal and the draft decision has been amended accordingly.

ECHA notes that the Registrant did not indicate the route of administration. As regards the route of administration, in light of the physicochemical properties of the substance (liquid with low vapor pressure of 0.1 hPa and a boiling point of 225 °C) and of the toxicokinetic profile (absorption study shows that after oral administration nearly complete absorption from the gastrointestinal tract was observed), ECHA considers that testing by the oral route is appropriate.

ECHA notes that the Registrant did not indicate the test species. As regards the species to be used, pargaraph 19 of OECD 474 states that "Mice, rats, or another appropriate mammalian species may be used", and paragraph 23 of OECD 489 mentions that "Rats are routinely used in this test." ECHA considers that testing in the rat is appropriate.

As regards the tissues to be studied, according to the test method (OECD 489), the Comet assay can be performed by analysing tissues from liver, glandular stomach and duodenum. 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 are recommended as tissues to examine site of contact effects after oral exposure.

In view of several expected or possible variables (different tissue structure and function of the glandular stomach and duodenum; different pH conditions; variable physico-chemical properties and fate of the substance; and probable different absorption rates of the substance and its possible breakdown product(s) between these two tissues) ECHA considers that it is necessary to increase the reliability of the analysis of genotoxicity at the site of contact by sampling both tissues.

For the micronucleus test (OECD 474), the bone marrow shall be analysed.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the initial testing proposal during the third party consultation proposing to conduct a comet assay study, in place of the proposed mammalian erythrocyte micronucleus test (OECD 474), in combination with the unscheduled DNA synthesis test (OECD 486). After receipt of the draft decision, the Registrant indeed proposed to perform a combined test, but proposed a mammalian erythrocyte micronucleus test (OECD 474) in combination with a comet assay (OECD 489). Although ECHA considers the strategy proposed by the third party as scientifically valid, ECHA is of the opinion that the OECD 474 test as now proposed by the Registrant in combination with the OECD 489 test is the most appropriate to fulfil the information requirements for mutagenicity, specifically because the ECHA Guidance (Chapter R.7a, version 4.1, October 2015) advises against the use of the UDS assay except in particular circumstances (R.7.7.6.3).

c) 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 erythrocyte micronucleus test (Annex IX, Section 8.4, column 2; test method: OECD TG 474) combined with an In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489); in rats, with oral administration. For the comet assay, the following tissues shall be analysed: liver, glandular stomach and duodenum. For the micronucleus test, the bone marrow shall be analysed. The test design should consider combination aspects such as dosing and sampling as described in the literature (see OECD test guideline 489 and e.g. Bowen et al. 2011).

d) Notes for consideration by the Registrant

The Registrant is reminded that according to Annex X, Section 8.4., column 2 of the REACH Regulation, "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".

Concerning the comet assay, the Registrant may consider examining gonadal cells, as it would optimise the use of animals. 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 (EC) No 1272/2008.

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. 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 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.

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. 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[4] by Ofelia Bercaru, Head of Unit, Evaluation E3

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