EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114300801-66-01/F Helsinki, 12 June 2015

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

For 2-methoxyethyl acrylate, CAS No 3121-61-7 (EC No 221-499-3), 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-methoxyethyl acrylate, CAS No 3121-61-7 (EC No 221-499-3, submitted by [redacted] (Registrant).

- OECD Test Guideline 114 (Viscosity of Liquids);
- OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test), rat, oral route (gavage).

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 registration dossier containing the above-mentioned testing proposal for further examination pursuant to Article 40(1) on 27 March 2013.

ECHA held a third party consultation for the testing proposal from 21 March 2014 until 5 May 2014. ECHA did not receive information from third parties.

On 28 October 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.

On 17 November 2014 ECHA received comments from the Registrant agreeing to ECHA's draft decision.

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, a proposal for amendment to the draft decision was submitted.

On 20 Februay 2015 ECHA notified the Registrant of the proposal for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposal for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposal for amendment received and amended 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 proposal for amendment. The Member State Committee took the comments of the Registrant on the proposal for amendment into account.

After discussion in the Member State Committee meeting on 20-23 April 2015, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 22 April 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 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. Viscosity (Annex IX, Section 7.17.; test method OECD 114);
2. *In vivo* mammalian bone marrow chromosomal aberration test (IX, Section 8.4., column 2;OECD 475);
   or
   In vivo mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2; OECD 474).
   or
   In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD 489), in rats, oral route, with examination of liver, forestomach and glandular stomach.

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 **20 June 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 proposals submitted by the Registrant for the registered substance.

Tests required pursuant to Article 40(3)

1. Viscosity (Annex IX, Section 7.17.)

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.

"Viscosity" is a standard information requirement as laid down in Annex IX, Section 7.17. of the REACH Regulation. The information on this endpoint is not available for the registered substance subject to the present decision 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 provide information for this endpoint.

The Registrant has submitted a testing proposal for a OECD Test Guideline 114 (Viscosity of Liquids).

ECHA considers the proposed test appropriate and testing should be performed with the registered substance.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed test using the registered substance: Viscosity of liquids (test method: OECD 114)

2. *In vivo* somatic cell genotoxicity study

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."

The technical dossier contains two *in vitro* studies performed in mammalian cells with the registered substance, an *in vitro* mammalian chromosome aberration test performed

according to the OECD 473 test guideline and an *in vitro* mammalian cells gene mutation test using the thymidine kinase gene performed according to the OECD 476 test guideline. Both of these tests show positive results. The increases in mutant frequency observed in the OECD 476 test were mainly due to small colony formation, which is indicative of a clastogenic effect. The positive results in these two tests indicate that the substance is inducing chromosomal aberrations under the conditions of the tests.

An appropriate *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations is not available for the registered substance but shall be proposed by the Registrant. Consequently, there is an information gap.

Hence, the Registrant has submitted a testing proposal for an *in vivo* mammalian bone marrow chromosome aberration test (OECD 475) with the following justification: "*There are no data available on genetic toxicity, in-vivo. In order to meet the standard information requirements according to Regulation (EC) 1907/2006, Annex IX, Column II, 8.4, as there are positive results in the in-vitro chromosome aberration test and in the mouse lymphoma assay, a GLP-compliant genetic toxicity study in the rat via the oral route following OECD 475 is proposed*".

ECHA notes that this test is an appropriate test to investigate effects on chromosomal aberration *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment, chapter R.7.7.1. and figure R.7.7-1 (August 2013).

ECHA further observes that there is currently no information on the potential aneugenicity of the substance subject to this decision in the technical dossier. According to the ECHA Guidance document on information requirements and chemical safety assessment table R.7.7-3 (August 2014), the *in vivo* mammalian erythrocyte micronucleus test (test method B.12./OECD 474) is adequate to measure structural and numerical chromosome aberrations and has the potential to detect clastogenic and aneugenic effects. This test is also suitable to adequately further investigate the findings obtained in the *in vitro* mammalian chromosome aberration test performed according to the OECD 473 test guideline and the *in vitro* mammalian cells gene mutation test performed according to the OECD 476 test guideline included in the technical dossier. Moreover, the *in vivo* mammalian erythrocyte micronucleus test may also provide additional information on an aspect of genotoxicity not yet explored for this substance. Specifically, the revised OECD 474 test guideline, published on 26 September 2014, paragraph 42 mentions protocol adaptations (i.e. protocol including the use of a chromosome centromere labeling method, e.g. FISH, CREST) that may enable the determination of the mechanism of micronucleus induction and allow the distinction between clastogenic and aneugenic effects.

ECHA would also like to note that considering the available data set for the registered substance, e.g. high reactivity, there is a concern regarding potential mutagenic effects at the first site of contact. These mutagenic effects at the first site of contact can be addressed neither by the micronucleus test nor by the chromosomal aberration test because no site of contact tissue is studied in those tests. According to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (August 2014), the *in vivo* mammalian alkaline comet assay (OECD 489) is suitable to follow up positive result *in vitro* showing gene mutation or chromosomal aberrations. Therefore, this test is also suitable to adequately further investigate the findings obtained in the *in vitro* mammalian chromosome aberration test performed according to the OECD 473 test guideline and the *in vitro* mammalian cells gene mutation test using the *tk* gene performed according to the OECD 476 test guideline included in the technical dossier. Moreover, the *in vivo* mammalian alkaline comet assay enables the generation of information regarding potential genotoxic effects at the first site of contact.

In light of the physicochemical properties of the substance (liquid with a vapour pressure of 281 Pa at 25°C) and the absence of reported human inhalation exposure, ECHA considers that testing by the oral route would be most appropriate. Therefore, ECHA considers that performing the comet assay by the oral route is the most appropriate for this substance.

If the Registrant decides to perform the in vivo mammalian alkaline comet assay, the test shall be performed by using the following tissues: liver as primary site of xenobiotic metabolism, and both forestomach and glandular stomach as sites of direct contact. The request of testing in both forestomach and glandular stomach is justified by the need to address the uncertainty, associated with the administration by oral gavage, on the actual first site of contact. It is particularly important to address the concern on potential genotoxic effects at the first site of contact for this highly reactive substance.

In his comment on the MSCAs proposal for amendment, the Registrant proposed to perform the in vivo mammalian bone marrow chromosomal aberration test or the in vivo mammalian erythrocyte micronucleus test 'in peripheral blood and bone marrow cells'. ECHA notes that the test guideline for the mammalian erythrocyte micronucleus test (OECD 474) does allow the study of both peripheral blood and bone marrow, whereas the one for mammalian bone marrow chromosomal aberration test (OECD 475) only foresees the study of bone marrow, not of peripheral blood. ECHA also notes that micronuclei observed in peripheral blood or in bone marrow cells (in the OECD 474) are both the consequence of the same original event, i.e. a genotoxic effect of a substance on the bone marrow.

Based on the above considerations, ECHA provides the Registrant with a choice between conducting the proposed *in vivo* mammalian bone marrow chromosomal aberration test (test method: OECD 475) or an *in vivo* mammalian erythrocyte micronucleus test (test method: OECD 474) or an *in vivo* mammalian alkaline comet assay (OECD 489, in rats, via oral route, with examination of liver, forestomach and glandular stomach).

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out either of the following studies with the registered substance subject to the present decision: *in vivo* mammalian bone marrow chromosomal aberration test (test method: OECD 475) or *in vivo* mammalian erythrocyte micronucleus test (test method: OECD 474) or an *in vivo* mammalian alkaline comet assay (OECD 489, in rats, via oral route, with examination of liver, forestomach and glandular stomach).

Notes for the consideration of the Registrant

If the Registrant decides to perform either OECD 474 or OECD 475:

Due to the nature of the substance, the Registrant is reminded that, according to paragraph 10 of the OECD 474 (Mammalian Erythrocyte Micronucleus Test, updated on 26 Sept 2014) and to paragraph 6 of the OECD 475 (Mammalian bone marrow chromosomal aberration test, updated on 26 Sept 2014), "If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test". Additionally, according to paragraph 48 (d) of the OECD 474 and to paragraph 44 (d) of the OECD 475, a test chemical is considered clearly negative if "Bone marrow exposure to the test substance(s) occurred". Accordingly, if a substance is negative in this test, and if it is not possible to demonstrate that bone marrow exposure to the substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the substance and whether to request any further information.

Regarding follow up testing, 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.

If the Registrant decides to perform OECD 489:

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 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, or the information submitted by other registrants of the same substance, has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In relation to the proposed tests, the sample of substance used for the new studies must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is within the specifications of the substance composition that are given by the joint registrants. It is the responsibility of all joint registrants of the same substance to agree to the tests proposed (as applicable to their tonnage level) and to document the necessary information on their substance composition.

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

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.

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