ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114310297-55-01/F Helsinki, 15 October 2015

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

For Reaction mass of 2,2'-[(4-methylphenyl)imino]bisethanol and Ethanol 2-[[2-(2-hydroxyethoxy)ethyl](4-methylphenyl)amino]-, EC No 911-490-9, 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 proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for Reaction mass of 2,2'-[(4-methylphenyl)imino]bisethanol and Ethanol 2-[[2-(2-hydroxyethoxy)ethyl](4-methylphenyl)amino]-, EC No 911-490-9, submitted by [redacted] (Registrant).

- 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 (EFSA Journal 2012) via oral route with analysis of blood, liver and stomach
- Prenatal Developmental Toxicity Study (OECD Guideline 414)

This decision is based on the registration 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 10 August 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.

ECHA received the registration dossier containing testing proposals for further examination pursuant to Article 40(1) on 24 May 2013. The registration was subsequently updated on 21 January 2014 containing the above-mentioned testing proposals.

ECHA held a third party consultation for the testing proposals from 18 November 2014 until 2 January 2015. ECHA received information from third parties (see section III below).

On 3 June 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 10 July 2015 the Registrant did not provide any comments on the draft decision to ECHA.

On 3 September 2015, ECHA notified the competent authorities of the Member States of its draft decision and invited them to propose amendments to the draft decision under Article 51 of the REACH Regulation. As no amendments were proposed, ECHA took the decision pursuant to Article 51(3) 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 alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD 489) in rats, oral route, on the following two tissues: liver, and either glandular stomach or duodenum/jejunum. It is at the Registrant's discretion to perform the intended additional examinations on blood during the testing program,
2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414) in rats or rabbits, oral route.

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 requests in this decision, or to fulfil otherwise the information requirements 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 **23 October 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report. The timeline has been set to allow for sequential testing as appropriate.

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.

A. Tests required pursuant to Article 40(3)

1. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2)

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, an Ames test performed according to OECD guideline 471 and an *in vitro* mammalian cell gene mutation study performed according to OECD guideline 476 with the registered substance, that show positive results. A further *in vitro* mammalian chromosome aberration test carried out according to OECD guideline 473 shows negative results. The positive results indicate that the substance is inducing gene mutations under the conditions of the tests.

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

Hence, the Registrant has submitted a testing proposal for a 'In Vivo Alkaline Comet Assay for the Detection of Genotoxic Carcinogens' to be performed with the registered substance subject to the present decision. He provided the following justification: '*The available in vitro Chromosome aberration test shows no effects of Accelerator (PT 25E or PT 25E/2) on the number of polyploid cells and cells with endoreduplicated chromosomes. Therefore clastogenicity or polyploidy do not seem to be induced by the test substance. The test substance was found to be mutagenic in the AMES test and showed significant increases in the MLA in the mutation frequency of both the small and large colonies in the absence of S9-mix, as compared with the solvent controls. This indicates increases in both chromosome aberrations and gene mutations. 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; EFSA Journal 2012). Furthermore, when comparing the performance of the Comet assay with other in vivo gentox tests, the Comet assay was shown to be superior in all aspects, including sensitivity, specificity, concordance, as well as positive and negative predictivity. 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 (EFSA Journal 2012) to ensure high quality test data. Furthermore, following ECHA Guidance R7a (Section R7.7, draft version August 2013) the COMET assay is found to be adequate for addressing the information requirement of Annexes IX and X, 8.4. Based on these consideration, to address the potential to induce point mutations and/or chromosome aberrations of the test substance an in vivo Comet assay is proposed'.* ECHA notes that since the submission of this testing proposal the comet assay test guideline (OECD 489) has been adopted.

ECHA notes that the proposed test is an appropriate test to investigate effects on gene mutations and 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 (August 2014).

According to the test method (OECD 489), the test shall be performed in rats by a route considering the anticipated routes of human exposure and adequate exposure of the target tissue(s). ECHA notes that there is no spray application indicating high inhalation exposure and therefore ECHA considers that testing by the oral route is appropriate.

According to the test method OECD 489, the test, by oral route, shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, and either glandular stomach or duodenum/jejunum as site of direct contact. Paragraph 42 of the OECD 489 test guideline indicates that "*Additional or alternative tissues should be selected based on the specific reasons for the test is being conducted*". ECHA notes that in the subacute repeated dose toxicity study included in the registered dossier it is concluded that "*No toxicologically relevant changes occurred in haematological parameters of treated rats*". Therefore ECHA considers it is at the Registrant's discretion to perform the intended blood examinations during the comet assay.

b) 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 alkaline comet assay (test method: OECD 489) in rats, oral route, on the following two tissues: liver, and either glandular stomach or duodenum/jejunum. It is at the Registrant's discretion to perform the intended additional examinations on blood during the testing program.

Note for consideration by the Registrant

The Registrant is reminded that according to Annex IX/X, Section 8.4., column 2 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".

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(s) 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.

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2)

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.

A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. 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 provide information for this endpoint.

The Registrant has submitted a testing proposal for a pre-natal developmental toxicity study according to EU B.31/OECD 414 with the following justification: *'In accordance with column 1 of REACH Annex IX, a pre-natal developmental toxicity study (OECD 414) is a standard requirement for >100T/year substances'.*

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

The Registrant did not specify the species to be used for testing. He did not specify the route for testing. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat or the rabbit as a first species to be used.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

The third party has indicated that a sequential testing process is recommended which gives priority to the additionally proposed test on genetic toxicity (in vivo mammalian alkaline comet assay). If a positive result will be obtained, the substance self-classified as a germ cell mutagen, and appropriate risk management measures be implemented a prenatal developmental toxicity study will not be required (REACH Guidance R.7.6.6.3).

ECHA notes that it is the Registrant's responsibility to consider and justify in the registration dossier any adaptation of the information requirements in accordance with Annex IX, Section 8.7., column 2, second indent. This adaptation specifies that in case the substance is known to be a germ cell mutagen (which correspond to a classification as germ cell mutagen category 1A or 1B) and appropriate risk management measures are implemented, the pre-natal developmental toxicity study does not need to be conducted.

However, ECHA notes that results of a positive in vivo comet assay may contribute to a classification as germ cell mutagen, but this test is usually not sufficient on its own for classification as germ cell mutagen category 1B.

Therefore, the information provided by the third party is currently not adequate to adapt the standard information requirement of Annex IX section 8.7.2.

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: Pre-natal developmental toxicity study in rats or rabbits, oral route (test method: EU B.31/OECD 414).

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.

Authorised[1] by Leena Ylä-Mononen, Director of Evaluation

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