ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114303496-52-01/F Helsinki, 27 July 2015

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

For 1,6-hexanediyl-bis(2-(2-(1-ethylpentyl)-3-oxazolidinyl)ethyl)carbamate, CAS No 140921-24-0 (EC No 925-259-5), 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 in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for 1,6-hexanediyl-bis(2-(2-(1-ethylpentyl)-3-oxazolidinyl)ethyl)carbamate, CAS No 140921-24-0 (EC No 925-259-5), submitted by [redacted] (Registrant).

- Comet assay (Draft OECD Guideline for the Testing of Chemicals (2012) Rodent alkaline single cell gel electrophoresis (Comet) assay).

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 10 to 100 tonnes per year. This decision does not take into account any updates after 5 March 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 dossiers containing the above-mentioned testing proposal for further examination pursuant to Article 40(1) on 23 May 2013 and 27 June 2013.

ECHA held a third party consultation for the testing proposals for comet assay, repeated dose toxicity and prenatal developmental toxicity based on the earlier submission of the dossier with submission number [redacted] from 16 May 2014 until 1 July 2014. ECHA did not receive information from third parties.

On 13 November 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 19 November 2014 ECHA received comments from the Registrant agreeing to ECHA's draft decision concerning test "*in vivo* mammalian alkaline comet assay" (section II(1) of this decision). However, the Registrant indicated that the production volume had decreased from 100-1000 tonnes per annum to 10-100 tonnes per annum. The Registrant commented

further that for this reason the testing for sub-chronic and pre-natal developmental toxicity was no longer necessary.

On 17 December 2014 the Registrant updated his registration dossier (submission number [redacted]).

The ECHA Secretariat considered the Registrant's comments and update.

On basis of this information, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

On 5 March 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 10 April 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 did not amend the draft decision.

On 20 April 2015 ECHA referred the draft decision to the Member State Committee.

By 11 May 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.

A unanimous agreement of the Member State Committee on the draft decision was reached on 26 May 2015 in a written procedure launched on 13 May 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 test pursuant to Article 40(3)(a) and 13(4) of the REACH Regulation using the indicated test method and the registered substance subject to the present decision:

> *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 and either glandular stomach or duodenum/jejunum.

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 in this decision, or to fulfil otherwise the information requirement 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 **3 August 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.

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed tests.

Tests required pursuant to Article 40(3)

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

a) Examination of the testing proposal

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "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."

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and chromosomal aberrations is not available for the registered substance and the Registrant considered it necessary to generate further information on mutagnicity.

Hence, the Registrant has submitted a testing proposal for an *in vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (Comet assay) with the following justification: "[redacted] [ECHA: the registered substance subject to the present decision] *revealed positive results in an HPRT assay* [ECHA: Mammalian cell HPRT gene mutation assay analogue to test method EU B.17.] in vitro *in the presence of a metabolic activation system. Based on the available* in vitro *data, an alkaline* in vivo *Comet assay is proposed to assess the mutagenic properties of the test substance* in vivo. *The* in vivo *Comet assay is considered to be the appropriate test system to investigate short-lived substances at the first site of contact. Generally,* [redacted] *immediately hydrolyses when getting in contact with water and thus disappears quickly if applied orally."*

ECHA notes that the proposed comet assay is an appropriate test to investigate further effects on gene mutations and chromosomal aberrations *in vivo* following the positive results in *in vitro* studies. ECHA shares the considerations of the Registrant that further investigation of mutagenicity is necessary even at the tonnage band of 10 to 100 tonnes per annum because no clear conclusion on this intrinsic property can be made based on the available data and there is a concern for mutagenic effects indicated by the positive *in vitro* data. This is in line and further described in the ECHA Guidance document on information

requirements and chemical safety assessment, chapter R.7.7.1. and table R.7.7-3 (August 2014).

The Registrant proposed oral administration by gavage. It is noted that the substance hydrolysis rapidly in water. Therefore, the test will mainly detect the effects of the hydrolysis products (liver) and maybe first site of contact effects of the parent compound (stomach and duodenum/jejunum).

As regards the route of administration, paragraph 39 of the OECD 489 test guideline states "*The anticipated route of human exposure should be considered when designing an assay*" and "*In any case the route should be chosen to ensure adequate exposure of the target tissue(s)*". In light of the physicochemical properties of the substance, ECHA considers that testing by the oral route is appropriate.

The Registrant specified the forestomach and the liver as tissues to be sampled. ECHA notes that paragraph 42 of the OECD 489 test guideline states: "*The liver has been the tissue most frequently studied and for which there are the most data. Therefore, in the absence of any background information, and if no specific tissues of interest are identified, sampling the liver would be justified as this is a primary site of xenobiotic metabolism and is often highly exposed to both parent substance(s) and metabolite(s). In some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant.*" Therefore ECHA considered in the initial draft decision that the comet assay should be performed in liver and either glandular stomach or duodenum/jejunum.

However, ECHA received from a Member State a proposal for amendment requesting the examination of 3 organs, i.e. liver, stomach and duodenum/jejunum, with the justification that '*the [fore]stomach is the site of first contact and should therefore be examined. As the substance may potentially also reach the intestines [duodenum/jejunum], these should be analysed as well*'. ECHA notified the Registrant of the proposal for amendment, who provided a comment '*agreeing with the MSCA to additionally include the organ duodenum/jejunum in the proposed comet assay in vivo*'. Taking account of the above, ECHA considers that the comet assay shall be performed in 2 organs, i.e. liver and either glandular stomach or duodenum/jejunum. It is at the Registrant's discretion to perform an analysis of all three organs as proposed by the Member State.

The Registrant specified the rat as the species to be tested. ECHA notes that this is in line with the OECD test method to perform the comet assay (OECD 489): "*Common laboratory strains of healthy young adult rodents (6-10 weeks old at start of treatment though slightly older animals are also acceptable) are normally used. The choice of rodent species should be based on (i) species used in other toxicity studies (to be able to correlate data and to allow integrated studies), (ii) species that developed tumours in a carcinogenicity study (when investigating the mechanism of carcinogenesis), or (iii) species with the most relevant metabolism for humans, if known. Rats are routinely used in this test. However, other species can be used if ethically and scientifically justified.*" ECHA considers this species as being appropriate and testing should be performed with the rat.

b) Outcome

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 in accordance with the OECD test guideline 489, in rats via oral route, with examination of liver and either glandular stomach or duodenum/jejunum.

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 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 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 Ofelia Bercaru, Head of Unit, 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.