EUROPEAN CHEMICALS AGENCY

Helsinki, 19 July 2018

Addressee: [redacted]

Decision number: TPE-D-2114428335-52-01/F
Substance name: [3-(2,3-epoxypropoxy)propyl]trimethoxysilane
EC number: 219-784-2
CAS number: 2530-83-8
Registration number: [redacted]
Submission number: [redacted]
Submission date: 30.06.2015
Registered tonnage band: 1000+T

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA has taken the following decision.

Your testing proposal is accepted and you are requested to carry out:

1. **In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum using the registered substance,**

   or

   Transgenic rodent somatic and germ cell gene mutation assays (Annex X, Section 8.4., column 2; test method: EU B.58./OECD TG 488) in transgenic mice or rats treated for 28 days, oral route on the following tissues: liver and glandular stomach with the registered substance; germ cells and duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the liver and glandular stomach are negative or inconclusive. The test material used should be freshly prepared.

You 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 and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **27 January 2020**. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Ofelia Bercaru, Head of Unit, Evaluation E3

[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.

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposal(s) submitted by you

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

or

Transgenic rodent somatic and germ cell gene mutation assays (Annex X, Section 8.4., column 2)

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 Section 8.4. of Annexes VII to X of the REACH Regulation. 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."

The technical dossier contains several *in vitro* studies performed according to Ames test (OECD TG 471) and mouse lymphoma L5178Y mammalian cell gene mutation test (OECD 476, 1997) with the registered substance that show positive results. The positive results indicate that the substance is inducing gene mutations under the conditions of the tests. ECHA notes that there is also a positive result from an *in vivo* somatic cell study [redacted], 1999, OECD TG 474). The study by [redacted] (1999) demonstrated that the substance causes aberrations *in vivo* when given after intraperitoneal administration. On the other hand, there are two other non-test guideline micronucleus assays, however conducted under GLP, which showed the substance did not induce chromosome damage in the bone marrow cells of mice following intraperitoneal or oral administration.

An appropriate second *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but may be necessary to meet the information requirements. Consequently there is an information gap and you proposed to generate information for this endpoint.

Hence, you have submitted a testing proposal for an OECD TG 489 *in vivo* mammalian alkaline comet assay.

ECHA notes that the proposed test is an appropriate test to investigate effects on gene mutations *in vivo* as described in the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017), Chapter R.7a, section R.7.7.6. and figure R.7.7-1. However, ECHA further notes that according to the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017), Chapter R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) is suitable to follow up a positive *in vitro* result on gene mutation.

In the technical dossier you did not specify the species to be used for testing and you did not specify the route for testing.

According to the test methods OECD TG 489 (comet assay) and OECD TG 488 (TGR assay), the test shall be performed in rats. Having considered the anticipated routes of human

exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

Comet assay:

In line with the test method OECD TG 489, the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

TGR assay:

According to the test method EU B.58/OECD TG 488, the test shall be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum as rapidly proliferating tissue and site of direct contact.

There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum shall be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum shall then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

Moreover, ECHA notes that according to the OECD 488 the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years. Hence, in order to limit additional animal testing male germ cells shall be collected at the same time as the other tissues (liver, glandular stomach and duodenum), and stored up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, in accordance to Annex X, Section 8.4., column 2, to decide on the need for assessment of mutation frequency in the collected germ cells. 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.

Outcome:

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are thus 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 TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

or alternatively

Transgenic rodent somatic and germ cell gene mutation assays (test method: EU B.58/OECD TG 488) in transgenic mice or rats, oral route, on the following tissues: liver and glandular stomach; duodenum and germ cells shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative or inconclusive. The test material used should be freshly prepared.

Notes for your consideration

You are reminded that according to Annex 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".

In case you decided to perform the comet assay, you shall consider examining gonadal cells as the technical dossier contains a positive result from an in vivo micronucleus study. Examination of gonadal cells would also 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.

In case you decided to perform the TGR assay, you should consider using the stored male germ cells for potential further analysis of germ cell mutagenicity as the technical dossier contains a positive result from an *in vivo* micronucleus study.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal for examination pursuant to Article 40(1) on 1 October 2014.

ECHA held a third party consultation for the testing proposal from 16 February 2015 until 2 April 2015. ECHA did not receive information from third parties.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation:

ECHA notified you of the draft decision and invited you to provide comments. ECHA did not receive any comments by the end of the commenting period.

You were notified that the draft decision does not take into account any updates after 06 July 2016, 30 calendar days after the end of the commenting period. However, following your request and justification provided (including interlinked read-across testing strategy on several supposedly related registered substances) ECHA has exceptionally granted you additional time until 30 June 2017 for the update of the IUCLID dossier.

You did not update the dossier by the given deadline.

ECHA did not amend the request(s).

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-60 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your 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.

2. 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.

3. In relation to the information required by the present decision, the sample of the substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new test(s) 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 or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new test(s) 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 test(s) to be assessed.