

Helsinki, 8 December 2017

Addressee: [REDACTED]

Decision number: TPE-D-2114382059-43-01/F
Substance name: 3-(Trimethoxysilyl)propyl (2E,4E)-hexa-2,4-dienoate
EC number: 642-902-2
CAS number: 163802-53-7
Registration number: [REDACTED]
Submission number: [REDACTED]
Submission date: 20/12/2016
Registered tonnage band: 1-10

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

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

- 1. In vivo mammalian alkaline comet assay (Annex VIII, 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 VIII, Section 8.4., column 2; test method: EU B.58./OECD TG 488) in transgenic mice or rats, 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 glandular stomach and of the liver are negative.

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 to the REACH Regulation.

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 have to submit the requested information in an updated registration dossier by **17 June 2019**. You also have to update the chemical safety report, where relevant. The reasons for this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and 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, has to 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>.

Authorised¹ by Kevin Pollard, Head of Unit, Evaluation E1

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 proposals submitted by you and scientific information submitted by third parties.

1. **In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays (Annex VIII, 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 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."

The technical dossier contains an *in vitro* study In Vitro Mammalian Cell Gene Mutation Test performed according to *OECD 476* with the registered substance that show positive results. *OECD Guideline 476* (In Vitro Mammalian Cell Gene Mutation Test) was performed with mouse lymphoma L5178Y cells with and without metabolic activation. Positive genotoxicity result with metabolic activation and a dose-response relationship was observed. The positive results indicate that the substance is inducing gene mutations under the conditions of the test.

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

Hence, you have submitted a testing proposal for a **In vivo mammalian alkaline comet assay** to be performed with the registered substance 3-(Trimethoxysilyl)propyl (2E,4E)-hexa-2,4-dienoate (EC No 642-902-2).

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity *in vivo*. ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA notes that the proposed test is an appropriate test to further 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.7.7.1. 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*) and the *in vivo* mammalian alkaline comet assay ("comet assay", *OECD TG 489*) are suitable to follow up positive *in vitro* result for gene mutation. Hence, ECHA considers that those tests are appropriate to address the concern. Therefore, ECHA provides you the choice to perform one of those tests.

You did not specify the species to be used for testing. Furthermore, you did not specify the route for testing .

According to the test method OECD TG 489, 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.

In case you decide to perform the comet assay according to 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

According to the test method EU B.58./OECD TG 488, the test shall be performed in transgenic mice or rats and the substance is usually administered orally.

In case you decide to perform a 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 sample 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 analysed if the results of the glandular stomach and of the liver are negative.

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°C). This duration is sufficient to allow the Registrant 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 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

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; germ cells and duodenum 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.

Notes for your consideration

You are 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".

In case you decided to perform the comet assay, you 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.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 21 December 2016.

ECHA notes that the tonnage band for one member of the joint submission is 10 to 100 tonnes per year.

ECHA held a third party consultation for the testing proposals from 10 March 2017 until 25 April 2017. ECHA did not receive information from third parties.

This decision does not take into account any updates after **23 August 2017**, 30 calendar days after the end of the commenting period.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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.

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

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-57 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 requests in this decision, or to otherwise fulfil the information requirements 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 tests 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 tests 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 tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.