EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-0000003826-67-04/F Helsinki, 31 March 2014

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

For [3-(2,3-epoxypropoxy)propyl]trimethoxysilane, CAS No 2530-83-8 (EC No 219-784-2), 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 as part of registration dossier in accordance with Articles 10(a)(ix) and 12(1)(e) thereof for [3-(2,3-epoxypropoxy)propyl]trimethoxysilane, CAS No 2530-83-8 (EC No 219-784-2), by [redacted] (Registrant).

- *In vivo* Comet Assay on somatic cells

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 1000 tonnes or more per year. This decision does not take into account any updates after 20 June 2013, 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 present dossier at a later stage.

On 21 May 2012, pursuant to Article 40(1) of the REACH Regulation, ECHA initiated the examination of the testing proposal set out by the Registrant in the registration dossier for the substance mentioned above.

ECHA held a third party consultation for the testing proposal from 1 August 2012 until 17 September 2012. ECHA did not receive information from third parties.

On 15 November 2012 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 11 December 2012 ECHA received comments from the Registrant proposing an alternative testing strategy to that originally proposed.

On 30 January 2013 and subsequently on 14 May 2013 the Registrant updated his registration dossier withdrawing the original testing proposal for a mammalian erythrocyte micronucleus test (OECD 474) with additional toxicokinetic investigations and proposing an in vivo Comet Assay, as well as providing a more detailed protocol for the study.

ECHA considered the Registrant's comments and dossier updates received. On the basis of the comments and dossier updates, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

On 20 June 2013 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 to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, Competent Authorities of the Member States submitted proposals for amendment to the draft decision.

On 26 July 2013 ECHA notified the Registrant of proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

On 5 August 2013 ECHA referred the draft decision to the Member State Committee.

On 23 August 2013 the Registrant provided comments on the proposed amendments. The Member State Committee took the comments of the Registrant into account.

After discussion in the Member State Committee meeting on 25-27 September 2013, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 27 September 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

Pursuant to Article 40(3)(d) of the REACH Regulation the proposal for performing an:

- *In vivo* Comet Assay (Annex X, 8.4.), in accordance with the protocol provided by the Registrant in his registration dossier

is rejected. The Registrant shall consider his further obligations as described in Section III.1. and 2. of the present decision.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposal submitted by the Registrant for the registered substance.

1. Proposed *in vivo* Comet assay on somatic cells (Annex X, 8.4., Column 2, subpara. 1)

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) of the REACH Regulation, ECHA may reject a proposed test.

When assessing the original testing proposal, ECHA noted:

- The Registrant has provided in the technical dossier several bacterial reverse mutation tests (Ames test, OECD 471) and three studies on *in vitro* mammalian cell gene mutation (OECD 476). The selected key studies from the aforementioned tests (with a Klimisch score of 2, for both tests) indicated that the substance is inducing gene mutation under the conditions of the tests.

- In order to evaluate the potential for the substance to cause chromosomal aberrations, the Registrant has provided in the technical dossier three *in vivo* micronucleus tests (OECD 474). No *in vitro* cytogenetic assay was provided in the registration dossier. From the three reported *in vivo* micronucleus tests, two yielded negative results. The first reported study (1982) yielded negative effects and did not specify the test guideline followed, it was done by oral route, and 1000 cells were scored. A second study (1994) also with negative effects, was conducted according to an OECD guideline (not specified), and it was indicated that the single dose given to the animals by intraperitoneal route was too high as it provoked toxic effects and approached the maximum tolerated dose. The third reported study in the dossier (1999) followed the test guideline OECD 474, with no deviations. It is reported as a GLP study with a Klimisch score of 1. The results from this third study show that the registered substance induced chromosome damage in the bone marrow cells of mice (2000 cells scored) at all three dose levels tested (500, 1000 and 2000 mg/Kg), following an intraperitoneal administration of the test substance.

Following the REACH Regulation, if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII, a second *in vivo* somatic cell test may be necessary, depending on the quality and the relevance of all the available data (Annex X, 8.4., column 2 REACH Regulation).

The results from the *in vitro* mutagenicity studies (Ames test and *in vitro* mammalian cell gene mutation assay) indicate that the registered substance induce gene mutation under the conditions of the test. ECHA notes that the information requirement for a somatic cell genotoxicity study at Annex IX, 8.4. has been addressed by an *in vivo* test, i.e. by the OECD 474 GLP study reported with Klimisch score 1. ECHA notes that on the basis of all available data, the substance has an alert for gene mutation and that there is no *in vivo* study addressing gene mutation in the dossier.

Initially, in their dossier submission ([redacted]) the Registrant (pursuant to the first subsection of Annex X, 8.4. Column 2) proposed to perform a Mammalian Erythrocyte Micronucleus Test with additional toxicokinetic investigations. However, ECHA noted that the test proposed was not adequate to address the concern for gene mutation identified in the dossier and requested a transgenic rodent gene mutations assay (TGR) according to OECD test guideline 488 as an appropriate test to detect gene mutations *in vivo*.

Following ECHA's draft decision on 15 November 2012, the Registrant submitted comments indicating their intention to conduct an alternative testing strategy to that originally proposed. In the subsequent dossier updates (30 January and 14 May 2013) the Registrant proposed to perform a Comet assay on somatic cells (single cell gel electrophoresis assay) *in vivo*. The details on the proposed test protocol were included in the updated registration ([redacted]). ECHA took these comments and the update into consideration accepting the alternative testing in the draft decision that was communicated to Member State Competent Authorities pursuant to Article 51(1) of the REACH Regulation.

Proposals for amendment included proposals to reject the Comet Assay, due to the reason that the available data was of sufficient quality and relevance to conclude on the fact that the substance induces mutagenicity in somatic cells. Furthermore it was noted that the Comet Assay does not measure gene mutations but primary DNA damage. The Comet assay on somatic cells would not provide any relevant additional information that would be useful to ensure that adequate risk management measures are applied for this substance.

Having taken these proposals for amendment and the Registrant's comments thereon into account, ECHA reconsidered its position on the testing proposal submitted by the Registrant. In fact, a second *in vivo* somatic cell test may only be necessary, depending on the quality and the relevance of the available data. In the present case, one valid OECD 474 micronucleus study is positive and demonstrates that the substance induces mutagenicity (chromosomal aberrations) in somatic cells. The Registrant has not provided arguments demonstrating that this data is not of sufficient quality or relevance. The negative results are not sufficient to put in doubt the relevance of the positive study, which was of higher quality. The negative studies showed deviations from the guideline. It was for example not demonstrated that the target organ was reached by the tested substance. Moreover, the guidance (R7a, table R.7.7-5) mentions, in the case where both gene mutation and chromosomal aberration endpoints have to be investigated *in vivo*, that "if the first *in vivo* test is positive, a second in vivo test to confirm the other genotoxic endpoint need not be conducted".

ECHA concludes that due to the quality and relevance of the available data further testing on somatic cells would not be useful to counter the available evidence and conclusion on mutagenicity. It also notes that the proposed test would not provide any relevant additional information that would be useful in relation to the conclusion that the substance is an *in vivo* mutagen.

b) Outcome

Therefore, pursuant to Article 40(3)(d) of the REACH Regulation, ECHA rejects the proposal to carry out a Comet Assay *in vivo* according to the test method protocol provided in the dossier submission [redacted].

ECHA notes that the Registrant in his registration dossier has not included a classification of the substance with regard to mutagenicity nor has he provided a justification demonstrating that the available positive data is of insufficient quality or relevance. It notes that the Registrant should either classify or provide scientifically justified reasons why no classification is given for mutagenicity. This information should be included in the technical dossier pursuant to Annex VI, Section 4. and in the Chemical Safety Report pursuant to Annex I, Section 3.

2. Considerations for potential germ cell mutagenicity (Annex X, 8.4., Column 2, subpara. 2)

In proposals for amendment Member State Competent Authorities raised concerns regarding the potential for germ cell mutagenicity of the registered substance.

Indeed, according to subparagraph 2 of section 8.4. of Annexes IX and X, column 2 of the REACH Regulation, if the result of the *in vivo* somatic cell mutation study is positive, the Registrant should consider the potential for germ cell mutagenicity on the basis of all available data. ECHA notes that the Registrant has neither submitted a testing proposal for this second information requirement included in the second subparagraph of Annex, 8.4., Column 2 of the REACH Regulation, nor fulfilled this information requirement in other ways (e.g. weight of evidence based on toxicokinetic and other data).

The Registrant should document his considerations regarding germ cell mutagenicity as part of the endpoint summary in the technical IUCLID dossier and in the hazard assessment of the chemical safety report as this is relevant information concerning the substance subject to the present decision. If no clear conclusions about germ cell mutagenicity can be made, the Registrant shall consider additional investigations and may need to submit further testing proposals accordingly.

The Registrant should submit such considerations, any further data (such as toxicokinetic data) and/or testing proposal(s) by **1 October 2014** . ECHA reserves the right to address the information requirement of subsection 2 of Annex X, 8.4., Column 2 in any future evaluation on the registered substance.

IV. 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://echa.europa.eu/appeals/app_procedure_en.asp. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

[redacted]

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