EUROPEAN CHEMICALS AGENCY

Helsinki, 19 July 2018

Addressee: [redacted]

Decision number: TPE-D-2114432413-60-01/F
Substance name: triethoxy(3-thiocyanatopropyl)silane
EC number: 252-161-3
CAS number: 34708-08-2
Registration number: [redacted]
Submission number: [redacted]
Submission date: 04.04.2013
Registered tonnage band: 100-1000T

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. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats using the registered substance.**
2. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rats or rabbits), oral route using the registered substance.**

While your originally proposed test for In vivo mammalian erythrocyte micronucleus test (EU B.12./OECD TG 474) using the registered substance is rejected, you are requested to perform:

3. **In vivo mammalian alkaline comet assay (Annex IX, 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.**

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 July 2020**. You shall also update the chemical safety report, where relevant.

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

1. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.)

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 sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, Section 8.6.2. 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.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats by the oral route according to EU B.26/OECD TG 408.

You proposed testing by the oral route. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA agrees that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is a liquid of very low vapour pressure and no uses with spray application are reported that could potentially lead to aerosols of inhalable size.

Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.

You proposed testing in rats. According to the test method EU B.26/OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation.

The third party has indicated the following (summary): *For animal welfare and economic reasons genotoxicity testing should preferably be incorporated into a required repeated dose toxicity study. Hence, at the tonnage level of the registered substance a sub-acute toxicity study according to OECD TG 407 (and a screening test for reproduction toxicity effects) is needed but not the proposed 90-day sub-chronic toxicity study.*

ECHA notes that the total tonnage band published in the related disseminated dossier does not reflect the registered tonnage band(s) and associated information requirement obligations. For the total tonnage band of the disseminated dossier, compiled data is calculated from the non-confidential quantities of a substance manufactured and/or imported by all registrants, excluding any quantity directly used as an intermediate to produce a different chemical.

ECHA agrees with the third party observation that you shall consider the possibility to incorporate the genetic toxicity test into the repeated dose toxicity study, as outlined in more detail in section 3, and in particular in the "Notes for your consideration" on "Combining a comet assay with a repeated dose toxicity test".

In your comments to the draft decision you agree to perform the requested study.

c) 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: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD TG 408).

Notes for your consideration

ECHA notes that a revised version of OECD TG 408 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

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.

You have submitted a testing proposal for a pre-natal developmental toxicity study in rats according to EU B.31/OECD TG 414.

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

According to the test method EU B.31/OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default consideration, ECHA considers testing should be performed with rats or rabbits as a first species.

You did not specify the route for testing.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you agree to perform the requested study.

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

Notes for your consideration

For the selection of the appropriate species you are advised to consult ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6.2.3.2 (July 2017).

ECHA notes that a revised version of OECD TG 414 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

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

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other tests in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

"Mutagenicity" is an information requirement as laid down in 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 *In vitro* Mammalian Chromosome *Aberration Test* performed according to *OECD TG 473* and an *In vitro* Mammalian Cell Gene Mutation Test performed according to OECD TG 476 with the registered substance that show positive results. The studies were performed in compliance with GLP.

The chromosome aberration test found that the test substance is clastogenic (causes structural aberrations in chromosomes) under the conditions of the test both with and without metabolic activation. No evidence of polyploidy was observed.

Based on the results of the gene mutation test it was concluded that the test substance is mutagenic in the *in vitro* mammalian cell gene mutation assay (thymidine kinase locus) in mouse lymphoma L5178Y cells. No indication of clastogenic effects (increase in number of small colonies) was observed in this study. The result was positive in a dose related manner and with metabolic activation.

The positive results thus indicate that the substance is inducing gene mutations and chromosomal aberrations under the conditions of the tests.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations or chromosomal aberrations is not available for the registered substance.

Consequently, there is an information gap, and you proposed to generate information for this endpoint.

For this purpose you submitted a testing proposal for a Mammalian Erythrocyte Micronucleus Test (OECD TG 474).

You did not specify the species to be used for testing. You did not specify the route for testing. Following proposals for amendment (PfAs) from two Member State Competent Authorities (MSCAs) it was noted that the proposed study is not an appropriate test to further investigate effects seen with the registered substance. Specifically, due to the high reactivity of the substance there is a concern for chromosomal aberrations in the initial site of contact tissues, which cannot be evaluated by performing a Mammalian Erythrocyte Micronucleus Test (OECD TG 474), since the latter only measures effects in the bone marrow (distant tissue). You have proposed that the hydrolysis half-life of the substance at 37.5^{0}C and pH 2 is approximately 5 seconds, and ECHA considers that this is evidence that the registered substance will not reach the target tissue of the micronucleus test. In accordance with paragraph 10 of OECD TG 474, it may not be appropriate to use this test. Also, as described in the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017), a Mammalian Erythrocyte Micronucleus Test (OECD TG 474) investigates effects on chromosome aberrations and does not address gene mutations *in vivo*. For all the above reasons, the Mammalian Erythrocyte Micronucleus Test (OECD TG 474) is not an appropriate test, and so your testing proposal is rejected.

In view of the above concerns ECHA considers that, according to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (version 6.0, July 2017), the *in vivo* mammalian alkaline comet assay (OECD TG 489) is the suitable study to follow up the positive result *in vitro* showing gene mutation and chromosomal aberrations for substances of high reactivity. The *in vivo* mammalian alkaline comet assay is appropriate to address the concerns noted in the *in vitro* studies (OECD TG 473 and 476). Moreover, it enables the generation of information regarding potential genotoxic effects at the site of contact.

According to the test method OECD TG 489, the comet assay shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the comet assay by the oral route is appropriate.

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.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation.

The third party has indicated the following (summary): "*The registrant proposed a mammalian erythrocyte micronucleus test according to OECD Test Guideline 474 on the basis of positive results in an in vitro mammalian chromosome aberration test in Chinese*

hamster lung fibroblasts and a mammalian cell gene mutation assay in mouse lymphoma L5178Y cells. As evidence both for gene mutations and chromosome aberrations is reported the in vivo mammalian alkaline Comet assay according to the recently adopted OECD Test Guideline 489 could be an appropriate alternative to the mammalian erythrocyte micronucleus test. For animal welfare and economic reasons genotoxicity testing should preferably be incorporated into a required repeated dose toxicity study. Hence, at the tonnage level of the registered substance a sub-acute toxicity study according to OECD TG 407 (and a screening test for reproduction toxicity effects) is needed but not the proposed 90-day sub-chronic toxicity study."

ECHA notes that the total tonnage band published in the related disseminated dossier does not reflect the registered tonnage band(s) and associated information requirement obligations. For the total tonnage band of the disseminated dossier, compiled data is calculated from the non-confidential quantities of a substance manufactured and/or imported by all registrants, excluding any quantity directly used as an intermediate to produce a different chemical.

In the third party comments it was proposed that the comet assay should be performed instead of a micronucleus assay. As explained above, following a PfA from MSCAs, ECHA has agreed to request only the *in vivo* mammalian alkaline comet assay (OECD 489). As regards combining this assay to a repeated dose toxicity study you may consider this option (see "*Notes for your consideration*").

c) Outcome

You are requested to carry out, pursuant to Article 40(3)(c) of the REACH Regulation, the additional study with the registered substance subject to the present decision:

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

While your originally proposed test for a Mammalian Erythrocyte Micronucleus Test (OECD TG 474) is rejected according to Article 40(3)(d) of the REACH Regulation.
Notes for your consideration

Germ cell testing

You are reminded that according to Annex IX, 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".

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.

Combining a comet assay with a repeated dose toxicity test

You may consider to combine a comet assay with a repeated dose toxicity study as long as this will not impair the validity of and the results from each individual study.

Hence, if you decide to combine both assays you should consider a number of practical aspects, which may prove challenging, such as (i) the selection of dosing, which should use the maximum tolerated dose (as defined in OECD TG 489, para. 36) or maximum (limit) dose, and which should avoid administration via feed or drinking water (OECD TG 489, para. 12 and Annex 3(2)); (ii) historical control values should take into account the different age of test animals; (iii) careful consideration should be given to the tissue sampling for comet analysis alongside the requirements of tissue sampling for other types of toxicological assessments; harvesting 24 hours after the last dose, which is typical of a general toxicity study, is not appropriate for the comet assay where samples are usually collected 2-6 h after the last treatment (see OECD TG 489, para. 33); and (iv) address OECD TG 489 para. 34.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal(s) for examination pursuant to Article 40(1) on 4 April 2013.

ECHA held a third party consultation for the testing proposal(s) from 16 October 2014 until 1 December 2014. ECHA received information from third parties (see Appendix 1).

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. In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request(s).

You were notified that the draft decision does not take into account any updates after **6 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 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 during its MSC-60 meeting 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.