~~Confidential~~

Helsinki, 28 April 2023

Addressees
Registrants of RECONSILE EC# 946-755-8 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
23/02/2022

Registered substance subject to this decision ("the Substance")
Substance name: Trimethoxy(methyl)silane and its reaction products with 3-aminopropyltriethoxysilane and [3-2,3-epoxypropoxy)propyl]trimethoxysilane
EC/List number: 701-410-9

Decision number: Please refer to the REACH-IT message which delivered this communication (in format TPE-D-XXXXXXXXXX-XX-XX/F)

DECISION ON TESTING PROPOSAL(S)

Under Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***2 February 2026***.

Requested information must be generated using the Substance unless otherwise specified.

Information required from all the Registrants subject to Annex VIII of REACH

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test also requested below (triggered by Annex VIII, Section 8.4., column 2)

Information required from all the Registrants subject to Annex IX of REACH

2. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., Column 2; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, or if justified, in mice, oral route.

- For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.
- For the micronucleus test: centromere staining must be performed if the Substance induces an increase in the frequency of micronuclei in the OECD TG 474; target tissue exposure must be demonstrated if the result of the OECD TG 474 is negative.

3. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats.

4. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit).

The reasons for the decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[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 for the decision

Contents

Reasons for the decision(s) related to the information under Annex VIII of REACH

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

1 Appropriate in vivo mutagenicity studies must be considered under Annex VIII to REACH (Section 8.4., Column 2) in case of a positive result in any of the in vitro genotoxicity studies under Annex VII or VIII to REACH.

2 Your dossier contains positive results for the *in vitro* chromosomal aberration test (OECD TG 473, 2013) which raise the concern for chromosomal aberrations.

3 ECHA considers that an *in vivo* follow-up study is necessary to address the identified concern.

4 For the assessment of the testing proposal, see Section 2.

Reasons for the decision(s) related to the information under Annex IX of REACH

2. In vivo mammalian alkaline comet assay combined with In vivo mammalian erythrocyte micronucleus test

5 An appropriate in vivo somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if (1) there is a positive result in any of the in vitro genotoxicity study under Annex VII or VIII to REACH and (2) there are no results available from an in vivo study.

6 Your dossier contains positive results for the *in vitro* chromosomal aberration test (OECD TG 473, 2013) which raise the concern for chromosomal aberrations.

2.1. Information provided to fulfil the information requirement

7 You have submitted a testing proposal for an in vivo mammalian erythrocyte micronucleus test to be performed with the Substance.

8 ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. 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.

9 ECHA agrees that an appropriate in vivo follow up genotoxicity study is necessary to address the concerns identified in vitro.

2.2. Test selection

10 You have proposed to perform an in vivo mammalian erythrocyte micronucleus test ("MN test", OECD TG 474), as an in vivo follow-up test, to address the concern for chromosomal aberration.

11 Under OECD TG 474, the MN test can be combined with an in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) in a single study (OECD TG 474, para. 37c; Guidance on IRs & CSA, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations.

12 The combined study, together with the results of the in vitro mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing in vivo mutagenicity and lack thereof. Furthermore, the combined study can detect effects in both distant organs, such as the bone marrow or the liver, and at site(s) of contact, such as the glandular stomach, the duodenum or the lung. Investigating several genotoxic endpoints and different tissues in a combined study is necessary to reduce the uncertainties of not testing all organs and to generate complementary information that provides a comprehensive overview of the genotoxic potential of the Substance. Moreover, the combined study can help reduce the number of tests performed and the number of animals used.

13 Therefore, the comet assay combined with the MN test is the most appropriate study for the Substance.

2.3. Specification of the study design

14 You did not specify the species to be used for testing. According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.

15 You proposed testing by the oral route. 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.

16 In line with the test method OECD TG 489, the test must 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.

17 According to the test method OECD TG 474, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen (OECD TG 474, paragraph 25, Table 1).

18 The combination of OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen et al. 2011 [1]).

[1] Bowen D.E. et al. 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. Mutation Research 722 7–19.

2.3.1. Assessment of aneugenicity potential

19 If the result of the in vivo MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance. In line with the OECD TG 474 (paragraph 42), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragments) and/or aneugenic events (i.e. micronuclei contain whole chromosomes).

2.3.2. Investigation of target tissue exposure

20 The applicable test method OECD TG 474 states that "If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test". Additionally, a negative test result can be considered reliable only if "Bone marrow exposure to the test substance(s) occurred".

21 Therefore, to ensure that the data generated are adequate for hazard identification, you must take blood samples at appropriate times and measure plasma levels of the Substance and/or its metabolites (OECD TG 474, paragraph 40), unless exposure of the bone marrow can be demonstrated through other means, e.g., by showing a depression of immature to mature erythrocyte ratio (OECD TG 474, paragraph 48).

22 If the Substance is negative in this test, but it is not possible to demonstrate that bone marrow exposure to the Substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the Substance and whether to request any further information.

2.3.3. Germ cells

23 A subsequent germ cell genotoxicity study (CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an in vivo genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

24 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal 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.

2.4. Outcome

25 Under Article 40(3)(b) your testing proposal is accepted under modified conditions, and you are requested to conduct the test with the Substance, as specified above.

3. Sub-chronic toxicity study (90-days)

26 A sub-chronic toxicity study (90 day) is an information requirement under Annex IX to REACH (Section 8.6.2.).

3.1. Information provided to fulfil the information requirement

27 You have submitted a testing proposal for a Sub-chronic toxicity study (90 day) according to OECD TG 408 with the Substance.

28 ECHA requested your considerations for alternative methods to fulfil the information requirement for Repeated dose toxicity. 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.

29 ECHA agrees that a 90-day study is necessary.

3.2. Specification of the study design

30 You proposed testing in the rat. ECHA agrees with your proposal because the rat is the preferred species according to the OECD TG 408. Therefore, the study must be conducted in the rat.

31 You proposed testing by the oral route. ECHA agrees with your proposal because this route of administration is appropriate to investigate systemic toxicity; Guidance on IRs and CSA, Section R.7.5.4.3.2.

3.3. Outcome

32 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test, as specified above.

4. Pre-natal developmental toxicity study

33 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX to REACH (Section 8.7.2.).

4.1. Information provided to fulfil the information requirement

34 You have submitted a testing proposal for a PNDT study according to the OECD TG 414 by the oral route with the Substance.

35 ECHA requested your considerations for alternative methods to fulfil the information requirement for Developmental toxicity. 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.

36 ECHA agrees that a PNDT study in a first species is necessary.

4.2. Specification of the study design

37 You proposed testing in the rat as a first species. You may select between the rat or the rabbit because both are preferred species under the OECD TG 414 (ECHA Guidance R.7a, Section R.7.6.2.3.2.).

38 You proposed testing by the oral route. ECHA agrees with your proposal because this route of administration is the most appropriate to investigate reproductive toxicity (ECHA Guidance R.7a, Section R.7.6.2.3.2.).

4.3. Outcome

39 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test, as specified above.

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).

All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF); ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs); ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 21 April 2022.

ECHA held a third party consultation for the testing proposal(s) from 16 June 2022 until 1 August 2022. ECHA did not receive information from third parties.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA did not receive any comments within the commenting period.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third-party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. Test methods, GLP requirements and reporting

(1) Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

(2) Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[2].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

(1) Selection of the Test material(s)
The Test Material used to generate the new data must be selected taking into account the following:
- the variation in compositions reported by all members of the joint submission,
- the boundary composition(s) of the Substance,
- the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

(2) Information on the Test Material needed in the updated dossier
- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include the careful identification and description of the characteristics of the Tests Materials in accordance with OECD GLP (ENV/MC/CHEM(98)16) and EU Test Methods Regulation (EU) 440/2008 (Note, Annex), namely all the constituents must be identified as far as possible as well as their concentration. Also, any constituents that have harmonised classification and labelling according to the CLP Regulation

[2] https://echa.europa.eu/practical-guides

must be identified and quantified using the appropriate analytical methods.

With that detailed information, ECHA can confirm whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[3].

2. General recommendations for conducting and reporting new tests

References to Guidance on REACH and other supporting documents can be found in Appendix 1.

[3] https://echa.europa.eu/manuals