EUROPEAN CHEMICALS AGENCY

Helsinki, 16 March 2020

Addressee
Registrant of Joint_Submission_EC_402-170-5 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
16/12/2014

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Trisodium 7-(4-(4-fluoro-6-(2-(2-vinylsulfonylethoxy)ethylamino)-1,3,5-triazine- 2-ylamino)-2-ureidophenylazo)naphthalene-1,3,6-trisulfonate
EC number: 402-170-5
CAS number: 106359-91-5

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

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***23 March 2021***.

A. Requirements applicable to all the Registrants subject to Annex VII of REACH

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method EU B.13/14. / OECD TG 471) with the Substance;

B. Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487) with the Substance;

Conditions to comply with the requests

You are bound by the requests for information corresponding to the REACH Annexes applicable to your own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.

To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa.

Registrants are only required to share the costs of information that they must submit to fulfil the information requirements for their registration.

The Appendices A and B state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

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

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.

Approved[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[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 A: Reasons for the requests to comply with Annex VII of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 1 to 10 tonnes or more per year must contain, as a minimum, the information specified in Annex VII to REACH.

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

An *In vitro* gene mutation study in bacteria is a standard information requirement in Annex VII to REACH.

You have provided the following key studies in your dossier:

i. An *in vitro* gene mutation study with the following strains, TA 98, TA 100, TA 1535, and TA 1537, which all gave negative results, [redacted] (1987).
ii. An *in vitro* gene mutation study with the following strains TA 98, TA 100, TA 1535, and TA 1537, which all gave negative results, [redacted] (1986).

We have assessed this information and identified the following issue:

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471 (1997)[2]. The key parameters of this test guideline include:

a) If the Substance is an azo-dye or a diazo-compound, the test in presence of metabolic activation must be performed following the Prival modification.
b) The test must be performed with 5 strains: four strains of S. typhimurium (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101)

However, the reported data for the studies you have provided did not include:

a) the Prival modification, in spite of the fact that the tested substance is an azo-dye.
b) the appropriate 5 strains, as the information provided does not include results in the required fifth strain, S. typhimurium TA 102 or E. coli WP2 uvrA or E. coli or E. coli WP2 uvrA (pKM101).

Hence, the information provided does not cover key parameters required by the relevant OECD TG 471.

Therefore, the information requirement is not fulfilled.

In your comments on the draft decision you indicated your agreement to perform the requested test.

[2] ECHA Guidance R.7a, Table R.7.7-2, p.557

Appendix B: Reasons for the requests to comply with Annex VIII of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 10 to 100 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII and VIII to REACH.

1. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

An *In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is a standard information requirement in Annex VIII to REACH.

You have provided the following key studies in your dossier:

- An *in vitro* chromosomal aberration test in Chinese hamster lung fibroblasts with a positive result with metabolic activation, [redacted] (1987).
- An *in vivo* micronucleus test in mouse with a negative result, [redacted] (1987).

In your comments you also refer to a "*higher tier mammalian cell gene mutation confirmation assay*", "*conducted according to OECD guideline 476 with negative results*".

We have assessed this information and identified the following issues:

In vitro studies

To fulfil the information requirement, the study has to be an *in vitro* chromosomal aberration test or an in vitro micronucleus test, conducted in mammalian cells in accordance with OECD TG 473 or OECD TG 487, respectively[3]. The key parameters of these test guidelines include that:

1) At least 300 well-spread metaphases must be scored per concentration.

   The reported data for the *in vitro* study you have provided did not include information on the number of cells scored per concentration.

2) Data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures must be reported.

   The reported data for the *in vitro* study you have provided did not include data on the cytotoxicity and/or the frequency of cells with structural chromosomal aberrations for the treated and control cultures.

In your comments to the draft decision you submitted further information and the original study report to clarify the above deficiencies. ECHA has assessed the information in your comments and from the original study report. From the provided study report ECHA notes that "*There were relevant enhancements of cells with structural aberrations after treatment with the test article at fixation intervals 7 h and 28 h with metabolic activation by S9 mix.*" However, at these fixation intervals the doses exceeded the acceptable treshold for cytotoxicity of 50 % (Table 3 in the report shows mitotic indeces of 12.3% for 2.50 mg at 7h and 31.5% for 2.50 mg at 28h). Therefore, the information provided still does not cover the second key parameter highlighted in the present decision.

Hence, the information provided does not cover key parameters required by the relevant

[3] ECHA Guidance R.7a, Table R.7.7-2, p.557

OECD TG.

With reference to the *in vitro* gene mutation in mammalian cells study (OECD TG 476) ECHA notes that this information is not an *in vitro* cytogenicity study in mammalian cells nor an *in vitro* micronucleus study. Therefore, this information does not cover the key parameters required by the OECD TG 473/487.

Therefore, the information requirement is not fulfilled.

In vivo study

To be considered adequate, the *in vivo* study you submitted has to meet the requirements of OECD TG 474[4] , and the key parameters of this test guideline include:

a) Samples of bone marrow taken at least twice with sampling starting not earlier than 24 hours after treatment but not extending beyond 48 hours after treatment in case animals are treated once with the test substance.

   The reported data for the *in vivo* study you submitted had only one sampling between 24 and 48 hours.

b) At least 4000 immature erythrocytes per animal must be scored for the incidence of micronucleated immature erythrocytes.

   The reported data for the *in vivo* study you submitted did not include the data on number of scored immature erythrocytes per animal.

c) The proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes must be reported for each group of animals.

   You did not provide this information in your dossier.

d) It is not appropriate to perform the test if there is evidence that the test substance, or a relevant metabolite, will not reach the target tissue.

   You did not demonstrate that the testing material reached the bone marrow and the information available is insufficient to conclude that it did.

In your comments to the draft decision you argue that, as per the former version of the guideline the parameters listed in a) and b) comply.

However, with regard to the key parameter a), the fact that only one sample was taken is not according to either the original or the updated testing guideline. The OECD 474 (May 1983) stated: *"Therefore, using the highest dose, samples of bone marrow are taken at least three times, starting not earlier than 12 hours after treatment, with appropriate intervals following the first sample, but not exceeding beyond 72 hours."* This is an important deficiency as it is essential to have the bone marrow harvesting at times at which the treatment-related induction of micronucleated immature erythrocytes can be detected.

[4] ECHA Guidance R.7a, Table R.7.7-3, p.558

By selecting only one dose, there is an increased probability that a time-window detection was missed leading to a false negative result. Therefore, the provided *in vivo* micronucleus test is still not considered reliable to clarify the hazard for this endpoint.

Consequently, the provided *in vivo* test is not adequate.

Further, in your comments you also refer to a negative *in vivo* micronucleus study (OECD TG 474) with an analogue substance Reactive Yellow 181 (EC no. 406-860-7). However, ECHA notes that in the current dossier of the Substance and in your comments, you did not provide any documentation following the read-across approach as described in Annex XI section 1.5. Therefore, ECHA cannot use the study with the analogue substance to "*confirm the results*" of the *in vivo* micronucleus study performed with the Substance.

Appendix C: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The compliance check was initiated on 7 March 2019.

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 within 30 days of the notification.

ECHA took into account your comments and did not amend the requests.

Included in your comments, you outlined your tonnage volumes. As this matter does not affect the decision making process of this decision, ECHA dealt with this matter in a separate communication.

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 D: Observations and technical guidance

1. This compliance check decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

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. Test guidelines, GLP requirements and reporting

   Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.
   Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses shall be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.
   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: 'How to report robust study summaries'[5].

4. Test material

   Selection of the test material(s)

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account 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.

   Technical reporting of the test material

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

   Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[6].

5. List of references of the ECHA Guidance and other guidance/ reference documents[7]

[5] https://echa.europa.eu/practical-guides
[6] https://echa.europa.eu/manuals
[7] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

Evaluation of available information

Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 in this decision.

QSARs, read-across and grouping

Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)[8]

Physical-chemical properties

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment

Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

OECD Guidance documents[9]

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.

Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

[8] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

[9] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix E: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.