~~Confidential~~

Helsinki, 12 October 2022

Addressee
Registrant of JS_CAS_2259360-19-3 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
31/03/2022

Registered substance subject to this decision ("the Substance")
Substance name: pentasodium 4-amino-3-[{4-[(2,4-disulfonatophenyl)diazenyl]-3-methylphenyl}diazenyl]-5-hydroxy-6-[(4-nitro-2-sulfonatophenyl)diazenyl]naphthalene-1,7-disulfonate
EC/List number: [redacted]

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)

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***20 October 2025***.

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

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

1. In vitro cytogenicity study in mammalian cells (test method: OECD TG 473) or In vitro micronucleus study (test method: OECD TG 487) (triggered by Annex VII, Section 8.4., column 2).

2. *In vivo* genetic toxicity study (triggered by Annex VII, Section 8.4., column 2) to be selected according to the following specifications:

    a. If the results of the *in vitro* test requested under section 1 are **negative**:

    In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified, other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

    b. If the results of the *in vitro* test requested under section 1 are **positive**:

    In vivo mammalian alkaline comet assay (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.

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.

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

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2. In vivo genetic toxicity study 4

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Reasons for the decisions related to the information under Annex VII of REACH

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

1 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result (Section 8.4., Column 2).

2 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 2020).

3 The Guidance on IRs & CSA, Section R.7.7.6.3, further specifies that "REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII." This is for the reason that the in vitro cytogenicity test under Section 8.4.2 will allow to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy. The obtained in vitro data will inform on the genotoxic concern(s) associated with the substance and help identify the most adequate follow-up in vivo study (as explained in section 2 of this Appendix).

1.1. Information provided to fulfil the information requirement

4 You have submitted a testing proposal for an *In vivo* mammalian alkaline comet assay to be performed with the Substance to further investigate the mutagenicity of the substance.

5 However, no information from an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study on the Substance is available in the dossier.

6 ECHA therefore considers that an appropriate in vitro cytogenicity in mammalian cells or an in vitro micronucleus study is necessary to further investigate the mutagenicity of the Substance and to help identify the most adequate follow-up in vivo study.

1.2. Specification of the study design

7 Either the in vitro cytogenicity study in mammalian cells (test method OECD TG 473) or the in vitro micronucleus study (test method OECD TG 487) are considered suitable.

1.3. Outcome

8 Under Article 40(3)(c) you are requested to carry out the additional test with the Substance, as specified above.

2. In vivo genetic toxicity study

9 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result (Section 8.4., Column 2).

10 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 2020) which raise the concern for gene mutations.

2.1. Information provided to fulfil the information requirement

11 You have submitted a testing proposal for an In vivo mammalian alkaline comet assay to be performed with the Substance.

12 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.

13 ECHA received third party information concerning the testing proposal during the third-party consultation. The third party information notes that further in vitro data is needed with the Substance before performing the in vivo test. The third party comments are further addressed under section 2.2. below.

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

2.2. Test selection

15 According to the Guidance on IRs & CSA, Section R.7.7.6.3, the in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) is suitable to follow up a positive in vitro result on gene mutation.

16 The third party information however mentions that "the potential of the registered substance to cause structural or numerical chromosomal aberrations has not been investigated experimentally, despite the positive (Q)SAR predictions noted for this endpoint." Therefore, the third party indicates that further in vitro testing is required to "fully characterise the genotoxic activity" of the Substance before the in vivo testing is performed, "in order to minimise the extent of in vivo testing required.".

17 As explained above, under section 1 of this Appendix, in the dossier there is indeed no information from an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study. Therefore, by this decision, ECHA also requests an in vitro cytogenicity study or an in vitro micronucleus study, which may raise a concern for chromosomal aberration in case of positive results.

18 In case there is also a concern for chromosomal aberration, the comet assay can be combined with an in vivo mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) in a single study (see OECD TG 489 para. 33; OECD TG 474 para. 37c; Guidance on IRs & CSA, Section R.7.7.6.3). While the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations, the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy). A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

19 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 help reduce the number of tests performed and the number of animals used while addressing (structural and numerical) chromosomal aberrations as well as gene mutations.

20 Therefore, you must wait for the results of the in vitro test requested under section 1 and, depending on these results, to conduct either a) Comet assay if the test results of request 1 are negative; or b) Comet assay combined with MN test if the test results of request 1 are positive. The deadline set in this decision allows for sequential testing.

2.3. Specification of the study design

*2.3.1. Comet assay (if the test results of request 1 are **negative**)*

21 You proposed testing in the rat. According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified (OECD TG 489, para. 23).

22 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.

23 You proposed to analyse the following tissues: liver, glandular stomach, duodenum/jejunum and bone marrow. 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. ECHA notes that the collection and analysis of the proposed additional tissue (bone marrow) is at your discretion.

2.3.1.1. Germ cells

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.

*2.3.2. Comet assay combined with MN test (if the test results of request 1 are **positive**)*

25 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.

26 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.

27 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.

28 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]).

2.3.2.1. Germ cells

29 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.

[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.

2.4. Outcome

30 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.

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).

All Guidance on REACH is 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 received your testing proposal(s) on 14 December 2021 and started the testing proposal evaluation in accordance with Article 40(1).

ECHA held a third party consultation for the testing proposal(s) from 1 April 2022 until 16 May 2022. ECHA received information from third parties (see Appendix 1, Section 2).

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

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

ECHA did not receive any comments within the commenting period.

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) of the REACH Regulation.

Appendix 3: Addressee 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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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

(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 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 all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance.

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

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