~~Confidential~~

Helsinki, 12 August 2022

Addressees
Registrants of JS_Direct_Black_22 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
04/06/2021

Registered substance subject to this decision ("the Substance")
Substance name: Reaction products of diazotised 4,4-diaminodiphenylamine-2-sulfonic acid, subsequently coupled with 6-amino-4-hydroxynaphthalene-2-sulfonic acid, further diazotised and coupled with metaphenylendiamine, sodium salts
EC number: 939-382-7

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 ***18 November 2024***.

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 vivo mammalian alkaline comet assay (triggered by Annex VII, Section 8.4., column 2), same as under "2."

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

2. In vivo mammalian alkaline comet assay (triggered by Annex VIII, Section 8.4., column 2; test method: OECD TG 489) in rats, or if justified, other rodent species, oral route, on the following tissues: 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.

In the list of requests above, the same information requirement is mentioned under different headings. This is because some information requirements may be triggered at different tonnage band(s) under different conditions. In such cases, only the reasons why the information requirement is triggered are provided in the pertinent section of Appendix 1 for the lower tonnage band. The reasons why the standard information requirement is actually not met as well as the specification of the study design are then provided in the pertinent section for the highest tonnage band. 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 VII of REACH

1. In vivo mammalian alkaline comet assay

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, 2006) which raise the concern for gene mutations.

3 In the comments to the draft decision submitted by one of the addressees the reliability of the OECD TG 471 study (2006) available in the dossier is being questioned. The comments indicate that the study has not been performed according to OECD TG 471 as not all five strains have been tested from a GLP laboratory. Moreover, these comments mention that the positive result is only noted in one strain (TA 1537). Therefore the conduct of a new OECD TG 471 study is proposed.

4 These comments also refer to the negative result obtained in the *in vitro* gene mutation study in mammalian cells (OECD TG 476, 1994) provided in your dossier.

5 Indeed, the results of the OECD TG 471 study (2006) are from a publication and the study has been assigned with a reliability score of 2; as reported in the dossier the study is 'well documented' and generally meets the scientific principles of OECD TG 471. We note that the study (2006) has some deviations from the OECD TG 471, such as the missing fifth strain and the limited information provided on the study results. However, based on the information reported in the dossier, the method and procedure used follow the main principles of the Ames study. Therefore, ECHA cannot disregard the positive results obtained for *S. typhimurium* TA 1537 with metabolic activation, TA 1538 and TA 98 with and without metabolic activation, in the study (2006) reported in your dossier.

6 Furthermore, although the *in vitro* gene mutation test in bacteria and the *in vitro* gene mutation test in mammalian cells both investigate gene mutations, they are considered complementary as they cover different gene mutation mechanisms. Therefore, the negative results obtained in the OECD TG 476 study (1994) provided in your dossier, and referred to in the above comment, cannot be used to supersede the positive results obtained in the OECD TG 471 study (2006) and do not remove the concern for gene mutation.

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

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

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

2. In vivo mammalian alkaline comet assay

9 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.

10 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 2006) which raise the concern for gene mutations. For related comments, and ECHA's reflection on them, we refer to section 1 above.

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

2.2. Test selection

14 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 mutations.

2.3. Specification of the study design

15 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 (OECD TG 489, para. 23).

16 You did not specify the route for testing. 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.

17 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.

2.3.1. Germ cells

18 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

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

20 In the comments submitted by the lead registrant agreement with the requested study was indicated.

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 started the testing proposal evaluation in accordance with Article 40(1) on 18 October 2021.

ECHA held a third party consultation for the testing proposal from 25 November 2021 until 10 January 2022. ECHA did not receive information from third parties.

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 took into account the two sets of comments and did not amend the request.

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.

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.

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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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 identify all the constituents as far as possible as well as their concentration (OECD GLP (ENV/MC/CHEM(98)16) and EU Tests Methods Regulation (EU) 440/2008 (Note, Annex). Also any constituents that have harmonised classification and labelling according to the CLP Regulation must be identified and quantified using the appropriate analytical methods.

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

This information is needed to assess 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].

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