~~Confidential~~

Helsinki, 10 March 2022

Addressees
Registrant(s) of JS_DisperseViolet057 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
06/05/2021

Registered substance subject to this decision ("the Substance")
Substance name: 1-hydroxy-4-[[4-[(methylsulphonyl)oxy]phenyl]amino]anthraquinone
EC number: 216-475-4

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 ***15 June 2023***.

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 point "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, in 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 requests above, the same study has been required under different conditions under different Annexes. Only one study is to be conducted; all the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants 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

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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 in an *in vitro* gene mutation study in bacteria (Section 8.4., Column 2).

2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471; 1994 & 1979) which raise the concern for gene mutations.

3 ECHA considers that further mutagenicity studies must be considered 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 VIII of REACH

2. In vivo mammalian alkaline comet assay

5 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.

6 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471; 1994 & 1979) which raise the concern for gene mutations.

2.1. Information provided to fulfil the information requirement

7 You have submitted a testing proposal for an *In vivo* mammalian alkaline comet assay 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 received third party information concerning the testing proposal during the third-party consultation. The third party indicated that the dossier includes a negative result for an *in vitro* gene mutation study in bacteria (Ames study, OECD TG 471). In addition, the gene mutation and chromosomal aberration *in vitro* studies in mammalian cells gave negative results. According to the third party, the *in vitro* results, together with the structure of the substance, '*indicate the possibility of a bacterial-specific positive response in the Ames test and further assessment of this possibility (e.g. in nitroreductase-deficient strains) could be recommended prior to embarking on testing in vivo*.'

10 ECHA notes that in the key study (1997), there was an increase in the number of revertant colonies, in the strain *S. typhimurium* TA 1537, with metabolic activation, at 5000 µg/ plate. Furthermore, a slight increase in the number of back-mutants was observed in the strains *S. typhimurium* TA 98 and TA 1535, without metabolic activation, at 5000 µg/plate. The confirmatory experiment revealed a slight increase in the number of revertant colonies in the strain *S. typhimurium* TA 1537, with metabolic activation, at 7205 µg/ plate. In the absence of metabolic activation, a slight increase in the number of back-mutants occurred in the strains *S. typhimurium* TA 98, TA 1535 and TA 1537, at 7205 µg/mL.

11 In addition, the supporting study (1979) exhibited doubling revertants at the highest tested doses (2000 µg/plate), in the first experiment, with *S. typhimurium* TA 1537, in the absence of metabolic activation. A second experiment was performed and a doubling of revertants was observed, over a concentration range of 500 to 4000 µg/plate with *S. typhimurium* TA 1537, in the absence of metabolic activation.

12 Therefore, based on the information provided in the dossier, both OECD TG 471 studies gave positive and not negative results. We acknowledge the negative results obtained in the OECD TG 473 (2016) and OECD TG 476 study (2016). However, the studies in mammalian cells are complementary to a study in bacteria, which are not intended to supersede, so we note that these negative results do not remove the concern for gene mutation.

13 The third party also refers to the structure of the Substance and the '*possibility of a bacterial-specific positive response in the Ames test'.* However, this is only a speculation

made by the third party, as in the dossier there is no evidence that the positive response observed in the bacterial test was due to a specific bacterial metabolism of the Substance.

14 On this basis, 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 mutations.

2.3. Specification of the study design

16 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).

17 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.

18 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

19 You may consider to collect the male gonadal cells from the seminiferous tubules in addition to the other aforementioned 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

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

21 In your comments on the draft decision you agreed to perform the comet assay in rats, on the following tissues: liver, glandular stomach and duodenum.

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

The Substance is listed in the Community rolling action plan (CoRAP) where substance evaluation started in 2019.

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 26 May 2021.

ECHA held a third party consultation for the testing proposal(s) from 1 July 2021 until 16 August 2021. ECHA received information from third parties (see Appendix 1).

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.

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

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

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

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