~~Confidential~~

Helsinki, 08 June 2021

Addressees
Registrants of JS_DisperseRed302 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
06/10/2020

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 2-[(1-amino-9,10-dihydro-4-hydroxy-9,10-dioxo-2-anthryl)oxy]ethyl ethyl carbonate
EC number: 254-959-7
CAS number: 40530-60-7

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 ***15 December 2022***.

A. Information required from the Registrants subject to Annex VIII of REACH

1. *In vivo* mammalian alkaline comet assay (Annex VIII, 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, oral route, with the analogue substance 2-[(1-amino-9,10-dihydro-4-hydroxy-9,10-dioxo-2-anthryl)oxy]ethyl phenyl carbonate (Disperse Red 302:1), EC number 248-882-8. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

Reasons for the request(s) are explained in the following appendix entitled "Reasons to request information required under Annexes VIII of REACH".

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

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

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.

For references used in this decision, please consult the Appendix entitled "List of references".

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 to request information required under Annex VIII of REACH

This decision is based on the examination of the testing proposals you submitted and on scientific information submitted by third parties.

1. In vivo mammalian alkaline comet assay

Under Annex VIII Section 8.4., column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

Your dossier contains positive results from the *in vitro* gene mutation study in bacteria and from the *in vitro* mammalian chromosomal aberration test, which raise the concerns for gene mutations and chromosomal aberrations.

1.1. Information provided to fulfil the information requirement

You have submitted testing proposals for an *In vivo* mammalian alkaline comet assay and an *In vivo* mammalian erythrocyte micronucleus test, both tests to be performed with the analogue substance 2-[(1-amino-9,10-dihydro-4-hydroxy-9,10-dioxo-2-anthryl)oxy]ethyl phenyl carbonate (Disperse Red 302:1), EC number 248-882-8.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity *in vivo*. You provided your considerations and you applied read-across to fulfil the respective information requirement, and no other alternative methods were available. ECHA has taken these considerations into account.

ECHA received third party information concerning the testing proposal during the third party consultation which is addressed in the next section.

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

1.2. Evaluation of read-across approach

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and (eco)toxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

You provided the following justification in support of your hypothesis that the source substance and the Substance have the same type and strength of effects:

1. Both source and target substance have structural similarities and comparable physicochemical properties; and
2. Both source and target substance have consistent mutagenic effects in the bacterial gene mutation assay.

ECHA agrees that based on the read-across justification provided and the other information available in the dossier there is a basis for considering the read across plausible. Therefore, you have plausibly demonstrated that relevant properties of the Substance can be predicted from data on the analogue substance.

However, ECHA emphasises that any final determination on the validity of your read-across adaptation will only be possible when the information on the requested studies will be available in the dossier including the choice of top dose (see 1.4 below).

As notified to you in a separate testing proposal decision TPE-D-2114557578-32-01/F, the same study is requested for the source substance, 2-[(1-amino-9,10-dihydro-4-hydroxy-9,10-dioxo-2-anthryl)oxy]ethyl phenyl carbonate, EC number 248-882-8.

1.3. Test selection

According to the ECHA Guidance R.7a, Section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a genotoxicity indicator test that is suitable to follow up the positive *in vitro* results for both chromosomal aberration and gene mutation. Moreover, the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) is a mutagenicity test that provides evidence on *in vivo* chromosomal mutagenicity, as this study detects both structural and numerical chromosomal aberrations.

You propose to perform both the comet assay and the MN test. The third party notes the following: "*This testing strategy is appropriate (i.e. it addresses both genotoxic endpoints for which activity is seen in vitro; however, the two endpoints should be incorporated in a single assay in order to reduce animal usage.*"

We also note that, as indicated in the ECHA Guidance R.7a, it is possible to combine the comet assay and the MN test into a single study. The combination study can help reduce the number of tests performed and the number of animals used while providing useful information on the potential of the Substance to induce chromosomal aberration and gene mutation.

In your comments to the draft decision you agreed to perform the test according to OECD TG 489 with the analogue substance, but you did not address a possible combination with the MN test in one study. You only confirmed that you would not investigate the *in vivo* mammalian erythrocyte micronucleus test according to OECD TG 474 in a parallel study.

As explained above, both concerns for chromosomal aberration and gene mutation exist and no *in vivo* genotoxicity data are available in the dossier. As agreed at the 70th meeting of the Member States Committee (MSC) (June 2020)[2], the combined study of the comet assay and the MN test would be most suitable as an *in vivo* follow-up test.

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

1.4. Specification of the study design

You did not specify the species to be used for testing. According to the test method OECD TG 489, the test must be performed in rats. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined must be conducted in the rat.

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.

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

[2] Minutes of the of the 70th Meeting of the Member State Committee (MSC-70), 10-12 June 2020, web conference: https://echa.europa.eu/documents/10162/28685870/MinutesofMSC-70_adopted-1.pdf/2972d2e5-6a5b-67ce-efc8-1a67a8e025a9

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.

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[3]). Specifically, the top dose of the combination study must be set high enough for a correction for differences in molecular weight between the source test material and your Substance to ensure that the requirements of the OECD TGs 489 and 474 in setting the top dose are met.

Germ cells
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.

1.5. Outcome

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

[3] Bowen DE 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. Muta Res.;722:7–19.

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. 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[4].

B. 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[5].

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

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 14 August 2020.

ECHA held a third party consultation for the testing proposal(s) from 23 November 2020 until 7 January 2021. ECHA received information from third parties (see corresponding Appendix).

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 your 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.

Appendix D: List of references - ECHA Guidance[6] and other supporting documents

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 where relevant.

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 where relevant.

Read-across assessment framework (RAAF, March 2017)[7]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, 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.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

[6] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[7] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[8] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

OECD Guidance documents[9]

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

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

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

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

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.