

Helsinki, 08 June 2021

**Addressees**

Registrants of JS\_DisperseRed302-1 listed in the last Appendix of this decision

**Date of submission of the dossier subject of a decision**

19/12/2018

**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 phenyl carbonate

EC number: 248-882-8

CAS number: 28173-59-3

**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**.

The requested information must be generated using the Substance unless otherwise specified.

**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. 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.

**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<sup>1</sup> under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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<sup>1</sup> 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 combined with In vivo mammalian erythrocyte micronucleus test**

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 for the *in vitro* gene mutation study in bacteria and *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 a testing proposal for an *In vivo* mammalian erythrocyte micronucleus test (OECD TG 474) to be performed with the Substance. In IUCLID section 7.6.2., there is also a testing proposal for "*OECD 486: Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo*", however ECHA understands that you propose to conduct an OECD TG 474 study.

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.

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. *Test selection*

You have proposed to perform an *In vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474). However, the positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

We note that in the testing proposal you also refer to the Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (OECD TG 486). This test provides an indication of induced damage to DNA followed by DNA repair (measured as unscheduled DNA synthesis in liver cells) however, as reminded in the ECHA Guidance, a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutations in the conditions of the test.

The third party noted that in your dossier there is a testing proposal for UDS, which is not an appropriate test for following up the concern for gene mutation. Also, the third party notes that there are both concerns for both gene mutation and chromosomal aberration, and that either a comet assay or a combination of comet/micronucleus study should be performed.

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 suitable test to follow up the positive *in vitro* result for chromosomal aberration and gene mutation. However, only the MN test detects both structural and numerical chromosomal aberrations, and therefore provides direct evidence of *in vivo* chromosomal mutagenicity.

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.

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

### 1.3. 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 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.

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.

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<sup>2</sup> [1]).

Finally, as notified to you in a separate testing proposal decision (TPE-D-2114557582-43-01/F), for the (target) substance, 2-[(1-amino-9,10-dihydro-4-hydroxy-9,10-dioxo-2-anthryl)oxy]ethyl ethyl carbonate (EC number 254-959-7) the same study is requested with your Substance. As indicated in the decision of the target substance the top dose of the combination study must be set high enough for a correction for differences in molecular weight between your Substance and the target 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.

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<sup>2</sup> 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.

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.

*1.4. Outcome*

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.

In your comments to the draft decision you agreed to perform the comet assay combined with the MN test, in rats, oral route.

## **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<sup>3</sup>.

### **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<sup>4</sup>.

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<sup>3</sup> <https://echa.europa.eu/practical-guides>

<sup>4</sup> <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.

In your comments you agreed to the draft decision. ECHA took your comments into account 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<sup>5</sup> 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)<sup>6</sup>

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)<sup>7</sup>

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.

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<sup>5</sup> <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

<sup>6</sup> <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

<sup>7</sup> [https://echa.europa.eu/documents/10162/13630/raaf\\_uvcb\\_report\\_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316](https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316)



OECD Guidance documents<sup>8</sup>

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.

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<sup>8</sup> <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.

<b>Registrant Name</b>	<b>Registration number</b>	<b>Highest REACH Annex applicable to you</b>
[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.