~~Confidential~~

Helsinki, 27 May 2024

Addressee(s)
Registrant(s) of Disperse Red 367 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
31 May 2023

Registered substance subject to this decision ("the Substance")
Substance name: 2-ethoxyethyl-2-(4-(2,6-dihydro-2,6-dioxo-7-phenyl-1,5-dioxaindacen-3-yl)phenoxy)acetate
EC/List number: 403-960-2

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)

Under Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***3 June 2026***.

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 (Annex VII, 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 addressee(s) of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

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.

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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 related to the information under Annex VII of REACH

1. In vivo mammalian alkaline comet assay

1 Under Annex VII, Section 8.4., Column 2, an appropriate in vivo mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4.4, must be performed in case of a positive result in any of the in vitro studies referred to in Annex VII, Section 8.4. The in vivo study must address the concern(s) raised by the in vitro study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

1.1. Triggering of the information requirement

2 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 1989) which raise the concerns for gene mutations.

3 Therefore, the information requirement is triggered.

1.2. Information provided

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

5 Your dossier also contains the following in vivo studies:

(i) in vivo MN test with the registered substance (EC 403-960-2) (OECD TG 474, 1989).
(ii) in vivo unscheduled DNA synthesis (UDS) assay with the registered substance (EC 403-960-2) (OECD TG 486, 1991).

6 Regarding the UDS assay (ii), this indicator test only detects some DNA repair mechanisms and does not investigate the gene mutation concern identified from the in vitro data. In particular, a negative result in the UDS assay is not a proof that a substance does not induce gene mutations.

7 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 for which testing is proposed. ECHA has taken these considerations into account.

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

1.3. Test selection

9 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.

10 The negative results obtained in the in vivo MN test (i) are considered adequate to conclude on the chromosomal aberration properties of the Substance and no additional testing is required to investigate the chromosomal aberration effects.

1.4. Specification of the study design

11 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.

12 You proposed testing on one sex only, in male animals. If no toxicologically relevant differences were observed between males and females, it is appropriate to test only males.

13 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.

14 In line with the test method OECD TG 489, the test must be performed by analysing tissues from the 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.

1.4.1.1. Germ cells

15 In your testing proposal, you indicated that "No germ cells will be collected, as the [comet] assay is not validated for germ cells, the TG states "this guideline is not considered appropriate to measure DNA strand breaks in mature germ cells. Since high and variable background levels in DNA damage were reported in a literature review on the use of the comet assay for germ cell genotoxicity"".

16 You may still 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.

17 In your comments to the draft decision, you indicated that you *"still [do] not consider it meaningful to collect male gonadal cells because – as the guideline states – the standard alkaline comet assay as described in OECD TG 489 is not considered appropriate to measure DNA strand breaks in mature germ cells for various reasons. According to the guideline, it should also be considered that "gonads contain a mixture of somatic and germ cells. For this reason, positive results in whole gonad (testis) are not necessarily reflective of germ cell damage"".* You also quoted Gajski et al. (2021), who concluded that *"in sperm the DNA is differently packed than in somatic cells"*, and you consider that *"the standard protocol of the comet assay needs to be adapted when it is applied to sperm"*. Moreover, you remind that *"Dirven et al. (2023) developed an approach to distinguish specific germ cells from other cells of the testicle to provide germ cell-specific DNA damage level assessments. This approach would have to be validated and included into TG 489 to produce reliable data on germ cell DNA damage"*.

18 ECHA wants to clarify that the collection of male gonadal cells in the comet assay i) is only a recommendation, and ii) is not aimed at investigating genotoxic effects specifically in mature germ cells but on the mixture of somatic and germ cells.

19 As specified in paragraph 10 of OECD TG 489, the inclusion of such examination may bring relevant information for the overall assessment of germ cell mutagenicity, for instance with respect to gonad exposure to the Substance and/or its metabolites. Furthermore, the

feasibility of the analysis of cells from the gonads has been demonstrated in the literature (Speit et al, 2009[2]; Zheng and Olive, 1997[3]; Cordelli et al, 2003[4]; Dirven et al., 2023[5]).

1.5. 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.

[2] Speit, G, M. Vasquez, A. Hartmann (2009), The comet assay as an indicator test for germ cell genotoxicity, Mutation Research, Vol. 681/1, pp. 3-12

[3] Zheng, H., P.L. Olive (1997), Influence of oxygen on radiation-induced DNA damage in testicular cells of C3H mice, International Journal of Radiation Biology, Vol. 71/3, pp. 275-282

[4] Cordelli, E. et al. (2003), Evaluation of DNA damage in different stages of mouse spermatogenesis after testicular X irradiation, Journal of Radiation Research, Vol. 160/4, pp. 443-451

[5] Dirven, Y., Eide, D.M., Henriksson, E.W., Hjorth, R., Sharma, A.K., Graupner, A. et al. (2023) Assessing testicular germ cell DNA damage in the comet assay; introduction of a proof-of-concept. Environmental and Molecular Mutagenesis, 64(2), 88–104. https://doi.org/10.1002/em.22527

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).
Guidance for monomers and polymers; ECHA (2023).
Guidance on intermediates; ECHA (2010).

All guidance documents are 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 2 June 2023 and started the testing proposal evaluation in accordance with Article 40(1).

ECHA held a third-party consultation for the testing proposal(s) from 3 October 2023 until 17 November 2023. ECHA received information from third parties (see corresponding Appendix/Appendices).

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 amended the request and the deadline.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. For performing and reporting the in vivo mammalian alkaline comet assay, ECHA considers 12 months adequate. 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 adopted the decision under Article 51(3) of REACH.

Appendix 3: Addressee(s) 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]

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[6].

(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[7].

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