

Helsinki, 15 June 2022

**Addressees**

Registrant as listed in Appendix 3 of this decision

**Date of submission of the dossier subject to this decision**

05/05/2021

**Registered substance subject to this decision ("the Substance")**

Substance name: Bis[2-[2-(1-methylethyl)-3-oxazolidinyl]ethyl] hexan-1,2-diylbiscarbamate  
EC number: 261-879-6

**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 **22 June 2023**.

Requested information must be generated using the Substance unless otherwise specified.

**Information required from all the Registrants subject to Annex IX of REACH**

1. In vivo mammalian alkaline comet assay (Annex IX, 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.

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<sup>1</sup> 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

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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 1: Reasons for the decision**

### **Contents**

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**Reasons for the decision(s) related to the information under Annex IX of REACH****1. In vivo mammalian alkaline comet assay**

1 An appropriate in vivo somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if (1) there is a positive result in any of the *in vitro* genotoxicity study under Annex VII or VIII to REACH and (2) there are no results available from an *in vivo* study.

2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 2003). Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

*1.1. Information provided to fulfil the information requirement*

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

4 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.

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

*1.2. Test selection*

6 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.

*1.3. Specification of the study design*

7 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 (OECD TG 489, para. 23).

8 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.

9 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.

*1.3.1. Germ cells*

- 10 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.
- 11 Therefore, 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, in accordance with Annex IX, Section 8.4., column 2, 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.

#### 1.3.2. Cross-linking properties

- 12 You are reminded that you may decide to consider the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay to detect cross links. Therefore, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23 [1] in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS); or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39 [2-5] in the OECD TG 489 or Pant et al. 2015 [6]). To ensure the robustness of the test result a specific positive control group of animals would be needed.

#### References:

- [1] Nesslany *et al.* (2007) *In vivo* comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds *Muta Res*;630(1-2):28-41.
- [2] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.
- [3] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.
- [4] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.
- [5] Spanswick *et al.* (2010) Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay. *Methods Mol Biol*;613:267-282.
- [6] Pant K *et al.* (2015) Modified *in vivo* comet assay detects the genotoxic potential of 14-hydroxycodone, an  $\alpha,\beta$ -unsaturated ketone in oxycodone. *Environ Mol Mutagen*;56(9):777-87.

#### 1.4. Outcome

- 13 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test, as specified above.
- 14 In your comments to the draft decision you agreed with conducting the requested study, as specified above.

## 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 20 May 2021.

ECHA held a third party consultation for the testing proposal from 1 July 2021 until 16 August 2021. 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.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request or the deadline.

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 Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

<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.

## 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<sup>2</sup>.
- (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<sup>3</sup>.

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<sup>2</sup> <https://echa.europa.eu/practical-guides>

<sup>3</sup> <https://echa.europa.eu/manuals>