~~Confidential~~

Helsinki, 15 November 2021

**Addressees**
Registrant(s) as listed in the last Appendix of this decision

**Date of submission of the dossier subject to this decision**
15/12/2016

**Registered substance subject to this decision ("the Substance")**
Substance name: 2,2'-[cyclohexane-1,1-diylbis(4,1-phenyleneoxymethylene)]dioxirane
EC number: 810-464-3
CAS number: 13446-84-9

**Decision number:** Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

## DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information by ***22 May 2023***.

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

### A. Information required from all the Registrants subject to Annex VII of REACH

1. Water solubility (Annex VII, Section 9.1.1.; the column elution method EU A.6/OECD TG 105 is the most appropriate method to fulfil the information requirement).

2. Skin sensitisation (Annex VII, Section 8.3.)

   i. *in vitro/in chemico* skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD TG 442D) and activation of dendritic cells (EU B.71/OECD TG 442E)(Annex VII, Section 8.3.1.); and

   ii. Only if the *in vitro/in chemico* test methods specified under point A.2.i.) are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment, *in vivo* skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429).

3. Transgenic rodent somatic and germ cell gene mutation assay (Annex VII, Section 8.4., column 2; test method: OECD TG 488 from 2020) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.
   OR
   *In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

4. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202)

5. If the results of request A.1. showed a water solubility below 1 mg/L : Long-term toxicity testing on aquatic invertebrates (triggered by Annex VII, Section 9.1.1.,

column 2; test method: EU C.20./OECD TG 211)

6. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201)

Reasons for the request(s) are explained in the following appendix entitled "Reasons to request information required under Annexes VII 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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 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.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

**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 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 VII of REACH

### 1. Water solubility

Water solubility is an information requirement under Annex VII to REACH (Section 7.7).

You have provided the following information:

i. An OECD TG 105, key study, study report (2015).

We have assessed this information and identified the following issue:

To fulfil the information requirement, a study must comply with the OECD TG 105 or the EU Method A.6 (Article 13(3) of REACH). Therefore, the following specifications must be met:

- the shake-flask method is applicable to test material with a water solubility ≥ 10 mg/L;
- a reliable analytical method is available.

Your registration dossier provides a study showing the following:

- an OECD TG 105 Flask method, key study, 2015.
- the water solubility was determined to be 4 mg/L, hence below 10 mg/L;
- You indicate that the TOC method is used as an analytical method.

The shake-flask method described in OECD TG 105 is not applicable to the Substance as its solubility is estimated to be well below 10 mg/L. Furthermore, the analytical method (i.e. TOC method) you used has low sensitivity and low precision which further question the reliability of the water solubility value reported in your dossier.

On the basis of the above, the information requirement is not fulfilled.

*Study design*
Considering the properties of the Substance (solubility < 10 mg/L), the column elution described in EU A.6/OECD TG 105 is the most appropriate method to fulfil the information requirement for the Substance.

### 2. Skin sensitisation

Skin sensitisation is an information requirement under Annex VII to REACH (Section 8.3.). Under Section 8.3., Column 1, the registrants must submit information allowing (1) A) a conclusion whether the substance is a skin sensitiser and B) whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and (2) risk assessment, where required.

You have provided the following information in the technical dossier, based on which you conclude that the Substance is not a skin sensitiser:

i. *in vivo* Buehler test (key study, EU Method B.6/OECD TG 406, GLP, [redacted] 2015).

We have assessed this information and identified the following issue(s):

*A. Non-compliant study*

To be considered compliant and enable concluding whether the Substance causes skin sensitisation, a study has to meet the requirements of the EU Method B.6/OECD TG 406 The following key parameter(s) of this test guideline include:

- Dose level selection rationale
- The induction concentration should be the highest causing mild irritation to the skin and the challenge dose should be the highest non-irritation concentration.

In the provided study:
- No dose level selection rationale was provided
- The concentration used for induction did not cause mild irritation.

Therefore the study does not fulfil the key parameter(s) set in the EU method B.6/OECD TG 406 and does not allow to make a conclusion whether the Substance causes skin sensitisation.

*B. No assessment of potency*

To be considered compliant and enable concluding whether the Substance causes skin sensitisation, in case the substance is considered to cause skin sensitisation, the information provided must allow a conclusion whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

As the currently available data does not allow to conclude whether the Substance causes skin sensitisation (see section A above), this condition cannot be assessed.

On this basis, the information requirement is not fulfilled.

*Study design*
To fulfil the information requirement for the Substance for skin sensitisation, *in vitro/in chemico* studies (OECD TG 442C, OECD TG 442D and EU Method B.71/OECD TG 442E) are considered suitable. In case *in vitro/in chemico* methods are not suitable for the Substance or the results cannot be used for classification and risk assessment an *in vivo* skin sensitisation study must be performed and the murine local lymph node assay (LLNA) (EU Method B.42/OEDC TG 429) is considered as the appropriate study.

**3. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays**

Under Annex VII Section 8.4., column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria which raise a concern for gene mutation.

Also, your dossier contains the following *in vivo* study:
i. OECD TG 474 ([redacted] 2016).

We have assessed this information and identified the following issue:

In order to be appropriate, according to ECHA Guidance R.7a, the *in vivo* somatic cell genotoxicity study must address the specific concern raised by the *in vitro* positive result.

However, the *in vivo* study provided is not addressing the gene mutation concern raised by the *in vitro* data. Therefore, the provided *in vivo* test is not appropriate.

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified *in vitro*.

*i. Test selection*

According to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation.

*ii. Test design*

*Comet assay*
In case you decide to perform the comet assay according to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure and the need for 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.

*TGR assay*
In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally.

Based on the recent update[2] of OECD TG 488, you are requested to follow the new 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals. This updated version provides for a transitional period for the new version. However, ECHA is aware that testing according to the updated OECD TG is already available from CROs and the new study design would provide meaningful germ cell data, so this decision requires the application of the new version.

According to the test method OECD TG 488, the test must be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum as rapidly proliferating tissue and site of direct 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 mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

*iii. Germ cells*

*Comet assay*

[2] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66.

You may consider to collect the male gonadal cells collected 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.

*TGR assay*
You may consider to collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, to decide on the need for assessment of mutation frequency in the collected germ 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.

## 4. Short-term toxicity testing on aquatic invertebrates

Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.).

You have provided the following information:

i. OECD TG 202, key study (study report, [redacted]: Acute Toxicity Test in Daphnia magna,2016)

We have assessed this information and identified the following issue:

To fulfil the information requirement, a study must comply with the OECD TG 202 (Article 13(3) of REACH). Therefore, the following specifications must be met:

Adequate information on the analytical method (including performance parameters of the method) and on the results of the analytical determination of exposure concentrations are provided;

Your registration dossier provides a study conducted according to the OECD TG 202, however no analytical monitoring of exposure was conducted.

As the analysis of the test solution was not performed, there is no information available neither on the performance parameters (i.e. LOQ, LOD) nor on the concentration of the substance throughout the test. Consequently there is no possibility to verify wether the daphnia organisms have been exposed to the Substance.

On this basis, the information requirement is not fulfilled.

## 5. Long-term toxicity testing on aquatic invertebrates (triggered by Annex VII, Section 9.1.1., column 2): If the results of request A.3. showed a water solubility below 1 mg/L

Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.). Long-term toxicity testing on aquatic invertebrates must be considered (Section 9.1.1., Column 2) if the substance is poorly water soluble.

You have provided a study conducted according to the OECD TG 202 study but no information on long-term toxicity on aquatic invertebrates for the Substance.
We have assessed this information and identified the following issue:

Poorly water soluble substances require longer time to reach steady-state conditions. As a result, the short-term tests does not give a true measure of toxicity for this type of substances and the long-term test is required. A substance is regarded as poorly water soluble if, for instance, it has a water solubility below 1 mg/L or below the detection limit of the analytical method of the test material (ECHA Guidance R.7.8.5).

In the provided OECD TG 105 study (2015), the saturation concentration of the Substance in water was 4 mg/L. However, as mentioned under Section 1. of this Appendix, the reliability of the value reported in the dossier is uncertain.
Therefore, if the results will show that the water solubility is below 1 mg/L, the Substance will be considered as poorly water soluble, and information on long-term toxicity on aquatic invertebrates will need to be provided.

*Study design*

For Substances which are difficult to test due to the low water solubility OECD TG 211 specifies that you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 211. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solutions.

While you have specified in IUCLID dossier under Section 1.4. the substance is monoconstituent, you have also provided analytical data that showed that the Substance is multiconstituent.
For multi-constituents/UVCBs, the analytical method must be adequate to monitor qualitative and quantitative changes in exposure to the dissolved fraction of the test material during the test (e.g. by comparing mass spectral full-scan GC or HPLC chromatogram peak areas or by using targeted measures of key constituents or groups of constituents).

If you decide to use the Water Accommodated Fraction (WAF) approach, in addition to the above, you must:

- use loading rates that are sufficiently low to be in the solubility range of most constituents (or that are consistent with the PEC value). This condition is mandatory to provide relevant information for the hazard and risk assessment (ECHA Guidance, Appendix R.7.8.1-1, Table R.7.8-3);
- provide a full description of the method used to prepare the WAF (including, among others, loading rates, details on the mixing procedure, method to separate any remaining non-dissolved test material including a justification for the separation technique);
- prepare WAFs separately for each dose level (i.e. loading rate) and in a consistent manner.

## 6. Growth inhibition study aquatic plants

Growth inhibition study aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).
You have provided the following information:

i. OECD TG 201, key study (study report, [redacted]: Growth Inhibition Study with the Green Alga, Pseudokircltneriel/a subcapitata,2016)

We have assessed this information and identified the following issues:

To fulfil the information requirement, a study must comply with OECD TG 201 (Article 13(3) of REACH). Therefore, the following specifications must be met:

- the results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form;
- adequate information on the analytical method (including performance parameters of the method) and on the results of the analytical determination of exposure concentrations is provided;

Your registration dossier provides a study conducted according to the OECD TG 201 showing the following:

- Tabulated data on the algal biomass determined daily for each treatment group and control are not reported
- No analytical monitoring of exposure was conducted;

Based on the above, the reporting of the study is not sufficient to conduct an independent assessment of its reliability. For instance, you mentioned that the validity criteria were met. However, as you have not provided data on the algal biomass. ECHA cannot verify if the validity criteria were met. Furthermore, as the analysis of the test solution was not performed, ECHA is not in a position to verify wether the tested organisms (i.e. *Pseudokirchneriella subcapitata*) have been exposed to the Substance.

Therefore, the requirements of the OECD TG 201 are not met.

*Study design*

The OECD TG 201 specifies that for difficult to test substances the OECD GD 23 must be followed. As already explained above under Section 1. of this Appendix, if the results will show that the water solubility is below 1 mg/L, the Substance will be considered as difficult to test. In that case, you must fulfil the requirements described in 'Study design' under Appendix A.5.

## 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[3].

### B. 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 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[4].

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

## Appendix C: General recommendations when conducting and reporting new tests for REACH purposes

### A. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in ECHA Guidance R.11 (Section R.11.4.2.2), you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the "known constituents approach" (by assessing specific constituents), or
- the "fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the "whole substance approach", or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

**Appendix D: Procedure**

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 14 October 2020.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA did not receive any comments within the commenting period.

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 E: List of references - ECHA Guidance[5] 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)[6]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[7]

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.

OECD Guidance documents[8]

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

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.

**Appendix F: Addressees of this decision and their corresponding information requirements**

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.