ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 4 November 2020

**Addressee**
Registrant of [redacted] listed in the last Appendix of this decision

**Date of submission for the jointly submitted dossier subject of a decision**
16/09/2019

**Registered substance subject to this decision, hereafter 'the Substance'**
Substance name: [redacted]
List number: [redacted]
CAS number: NS

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

## DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***11 May 2022*** *from the date of the decision*.

### A. Requirements applicable to all the Registrants subject to Annex IX of REACH

1. Dissociation constant (Annex IX, Section 7.16.; test method: OECD TG 112) with the Substance;

2. *In vivo* mammalian alkaline comet assay (Annex IX, 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 Substance. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

3. Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.; test method: EU C.25./OECD TG 309) at a temperature of 12 °C with the Substance;

4. Identification of degradation products (Annex IX, Section 9.2.3.) using an appropriate test method;

5. Short-term toxicity to terrestrial plants (Annex IX, Section 9.4.3.; test method: OECD TG 208) with at least three species tested (with as a minimum one monocotyledonous species and two dicotyledonous species) with the Substance;

6. Effects on soil micro-organisms (Annex IX, Section 9.4.2.; nitrogen transformation test, test method: EU C.21/OECD TG 216 and carbon transformation test, test method: EU C.22/OECD TG 217) with the Substance.

**Conditions to comply with the requests**

You are bound by the requests for information corresponding to the REACH Annexes applicable to your own registered tonnage of the Substance at the time of evaluation. Therefore, you have to comply with the requirements of Annexes VII to IX of REACH, as you have registered a substance at 100-1000 tpa.

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

**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 for the requirements applicable to all the Registrants subject to Annex IX of REACH**

This decision is based on the examination of the testing proposals you submitted.

**1. Dissociation constant (Annex IX, Section 7.16.)**

Dissociation constant is a standard information requirement in Annex IX to REACH.

Your technical dossier does not include information on dissociation constant for the Substance. You have submitted a testing proposal for Dissociation constant in water (test method: OECD TG 112) with the Substance.

ECHA considers that the proposed study is appropriate to fulfil this information requirement.

Under Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed test with the Substance.

In your comments on the draft decision, you agreed to conduct the requested study on the Substance.

**2. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2)**

Under Annex IX, Section 8.4, column 2 of REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if:
1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and
2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

Your dossier contains positive results for the *in vitro* cytogenicity test and *in vitro* gene mutation study in mammalian cells which raise the concerns for both gene mutations and chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

Therefore, you submitted a testing proposal for an *in vivo* mammalian erythrocyte micronucleus test to be performed with the Substance.

ECHA requested your considerations for alternative methods to fulfil the information requirement for *Genetic toxicity in vivo*. ECHA notes that 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 agrees that an appropriate *in vivo* follow up genotoxicity study is necessary to address the concern identified *in vitro.*

*a) Test selection*

The positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation. You proposed to perform the *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474). ECHA notes that the proposed test is appropriate to

investigate effects on chromosomal aberrations *in vivo,* however it is not suitable to follow up the concern for gene mutation.

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.

Therefore, as also indicated in the ECHA Guidance, it is possible to combine the comet assay and the MN test into a single study. The combined study can help reduce the number of tests performed and the number of animals used while addressing both chromosomal aberration and gene mutation.

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

*b) Test design*

You did not specify the species and the route to be used for testing.

According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combined test (OECD TG 489 and OECD TG 474) 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.

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[2]).

*c) Germ cells*

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.

You may consider to collect the male gonadal cells collected from the seminiferous tubules

[2] Bowen D.E. 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. Mutation Research 722 7–19

(as described by e.g. O'Brien *et al.*[3]), at the same time as the other tissues, 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 accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells, using the comet assay. 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.

*d) Third Party consultation*

ECHA has not received third party information concerning the testing proposal during the third party consultation.

*e) Outcome*

Under Article 40(3)(b) of REACH, you are requested to carry out the proposed test under modified conditions, as explained above with the Substance.

## 3. Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.)

Simulation testing on ultimate degradation in surface water is a standard information requirement at Annex IX to REACH.

Your technical dossier does not include information on simulation testing on ultimate degradation in surface water for the Substance. You have submitted a testing proposal for simulation testing on ultimate degradation in surface water (test method: OECD TG 309) with the Substance.

ECHA considers that the proposed study is appropriate to fulfil this information requirement.

Under Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed test with the Substance.

In your comments on the draft decision, you agreed to conduct the requested study on the Substance.

*Study design*

Under Annex XIII, the information must be based on data obtained under conditions relevant for the PBT/vPvB assessment. Therefore:

- You must perform the OECD TG 309 test, by following the pelagic test option with natural surface water containing approximately 15 mg dw/L of suspended solids (acceptable concentration between 10 and 20 mg dw/L) (ECHA Guidance R.11).

- You must perform the test at the temperature of 12°C, the average environmental temperature for the EU (ECHA Guidance R.16, Table R.16-8). Performing the test at this temperature is in line with the applicable test conditions of the OECD TG 309.

[3] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

- The reference temperature for providing results is 12°C for surface water environment and 9°C for marine environment. Therefore, the degradation half-life for the Substance, even if measured in any other temperature, would still need to be corrected to the temperature of 12°C using Arrhenius equation (ECHA Guidance R.11 and R.7b, Section R.7.9.4.1).

Non-extractable residues (NER) must be quantified in all simulation studies. The reporting of results must include a scientific justification of the used extraction procedures and solvents. By default, total NER is regarded as non-degraded Substance. However, if reasonably justified and analytically demonstrated a certain part of NER may be differentiated and quantified as irreversibly bound or as degraded to biogenic NER. Such fractions could be regarded as removed when calculating the degradation half-life(s) (ECHA Guidance R.11).

## 4. Identification of degradation products (Annex IX, 9.2.3.)

Identification of the degradation products is a standard information requirement at Annex IX of REACH. According to Annex IX, Section 9.3.2, Column 2 this information does not need to be provided if the substance is readily biodegradable.

In your technical dossier, you have provided:

- a ready biodegradability study according to OECD TG 301B with the Substance showing 29% biodegradation after 28 days. You conclude that the Substance is not readily biodegradable.

However, you have not provided information on the identity of the degradation products of the Substance.

Under Article 40(3)(c) of the REACH Regulation, you are requested to provide this information for the Substance using an appropriate method.

In your comments on the draft decision, you agreed to conduct the requested study on the Substance.

*Study design*

Identity, stability, behaviour, and molar quantity of the degradation/transformation products relative to the Substance must be evaluated and reported in biodegradation simulation studies, when analytically possible. Therefore, you may obtain this information from the Simulation testing on ultimate degradation in surface water requested above under A.3 above. In addition, degradation half-life, potential for bioaccumulation and toxicity of the transformation/degradation product may be investigated. If any other method than the method requested under A.3 is used for identification of the transformation/degradation products, you must provide a scientifically valid justification for the chosen method.

## 5. Short-term toxicity to terrestrial plants (Annex IX, Section 9.4.3.)

Short-term toxicity to terrestrial plants is a standard information requirement at Annex IX to REACH.

Your technical dossier does not include information on effects on short-term toxicity to terrestrial plants for the Substance. You have submitted a testing proposal for a Short-term toxicity test to terrestrial plants (test method: OECD TG 208) with the Substance.

ECHA agrees that the proposed test is appropriate to fulfil the information requirement of

Annex IX, Section 9.4.3.

Under Article 40(3)(c) of the REACH Regulation, you are requested to provide this information for the Substance using an appropriate method.

In your comments on the draft decision, you agreed to conduct the requested study on the Substance.

*Study design*

OECD guideline 208 (Terrestrial plants, growth test) considers the need to select the number of test species according to relevant regulatory requirements, and the need for a reasonably broad selection of species to account for interspecies sensitivity distribution. For short-term toxicity testing, ECHA considers three species as the minimum to achieve a reasonably broad selection. Testing shall be conducted with species from different families, as a minimum with one monocotyledonous species and two dicotyledonous species, selected according to the criteria indicated in the OECD TG 208 guideline. You should consider if testing on additional species is required to cover the information requirement.

## 6. Effects on soil micro-organisms (Annex IX, Section 9.4.2.)

Effects on terrestrial micro-organisms is a standard information requirement at Annex IX to REACH.

Your technical dossier does not include information on effects on terrestrial micro-organisms for the Substance. You have submitted a testing proposal for a Soil microorganisms: nitrogen transformation test (test method: OECD TG 216) with the Substance.

According to ECHA Guidance R.7c, Section R.7.11.3.1 the nitrogen transformation test (EU C.21/OECD TG 216) is suitable for most non-agrochemicals however, for agrochemicals the carbon transformation test (EU C.22/OECD TG 217) must also be conducted. Considering that the Substance is used as a fertilizer in soil amendments, this additional test is required.

In your comments on the draft decision, you agreed to conduct the requested nitrogen transformation test (C.21/OECD TG 216) on the Substance. However, you explain that you disagree to conduct the carbon transformation test (EU C.22/OECD TG 217) for the following reasons:

1. you consider the carbon transformation process to be less sensitive to stress and toxicity due to higher functional redundancy of the carbon transformation pathways within soil microbial communities;
2. you consider that ECHA Guidance R.7.11 does not require to conduct both tests for agrochemicals;
3. you point out that the carbon transformation test (EU C.22/OECD TG 217) is not an information requirement under the old fertilizer regulation (EC 2003/2003) and new fertilizer regulation (EU 2019/1009); under the plant protection products regulation (EC 1107/2009); or under the regulation on active substances in plant protection products (EU 283/2013).

On point 1 above, while it can be assumed that the functional redundancy of carbon transformation pathways in the soil microbial community is higher compared to the nitrogen transformation processes, this does not constitute a definitive proof of the higher sensitivity of the nitrogen transformation test (EUC.21/OECD TG 216). Furthermore, the tests differs in the nature of the substrate used to determine nitrogen transformation (*i.e.* a complex powdered plant meal) and carbon transformation (*i.e.* glucose amendment) which may lead

to differences in the bioavailability of some test materials and hence in the overall sensitivity of the tests (*e.g.* Hunde-Rinke & Schlich, 2010, doi: 10.1186/s12302-014-0028-z).

On point 2 above, ECHA Guidance R.7.11.3.1 specifies that for most non-agrochemicals the nitrogen transformation test is considered sufficient. Conversely, it is understood that both the nitrogen transformation test and the carbon transformation test must be conducted for agrochemicals. This is in line with the requirements of both OECD TG 216 and 217 in which it is stated that "*If agrochemicals (e.g. crop protection products, fertilisers, forestry chemicals) are tested, both carbon transformation and nitrogen transformation tests are conducted*".

On point 3 above, we point out that the regulations on fertilizers and plant protection products you refer to are without prejudice to the information required under the REACH Regulation for uses of the Substance others than fertilizer and plant protection.

Therefore under Article 40(3)(a) of the REACH Regulation, you are requested to carry out the following proposed test: Soil microorganisms: nitrogen transformation test (EU C.21/OECD TG 216). In addition, under Article 40(3)(c) to REACH, you are requested to carry out the following additional test: Soil microorganisms: carbon transformation test (EU C.22/OECD TG 217), both with the Substance.

## Appendix B: Procedural history

ECHA received your registration containing the testing proposals for examination on 17 September 2019.

ECHA held a third party consultation for the testing proposals from 17 December 2019 until 31 January 2020. 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.

ECHA took into account your comments and did not amend the requests.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-71 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

## Appendix C: Observations and technical guidance

1. This testing proposal examination decision does not prevent ECHA from initiating compliance checks at a later stage on the registrations present.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State(s).

3. Test guidelines, GLP requirements and reporting

   Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.

   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.

   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: 'How to report robust study summaries'[4].

4. Test material

   *Selection of the test material(s)*

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/impurity is known to have or could have 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.

   *Technical reporting of the test material*

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

   Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[5].

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

5. Testing strategy

ECHA notes that, under Article 41 of the REACH Regulation, you are also requested to conduct toxicity tests on fish, aquatic invertebrates and algae and the results of this test may subsequently allow the derivation of PNECwater. If the results of the requested toxicity test on fish, aquatic invertebrates and algae allow the subsequent derivation of a PNECwater, you may consider the ITS as recommended in section R.7.11.6., Chapter R.7c of the ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, June 2017), and determine the need for further testing on terrestrial plants.

ECHA emphasises that the intrinsic properties of soil microbial communities are not addressed through the EPM extrapolation method and therefore the potential adaptation possibility does not apply.

6. List of references of the ECHA Guidance and other guidance/ reference documents[6]

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 in this decision.

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

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.

OECD Guidance documents

[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

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

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

**Appendix D: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them**

| Registrant Name | Registration number | (Highest) Data requirements to be fulfilled |
|---|---|---|
| [redacted] | [redacted] | [redacted] |

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.