EUROPEAN CHEMICALS AGENCY

Helsinki, 9 June 2020

**Addressees**
Registrants of Sodium 4-oxovalerate listed in the last Appendix of this decision

**Date of submission for the jointly submitted dossier subject of this decision**
12/04/2018

**Registered substance subject to this decision, hereafter 'the Substance'**
Substance name: Sodium 4-oxovalerate
EC number: 243-378-4
CAS number: 19856-23-6

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

## DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***16 March 2021***.

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

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method EU B.13/14. / OECD TG 471) with the Substance or with levulinic acid (EC No 204-649-2, CAS No 123-76-2) ('the analogue substance');

1. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method EU C.2./OECD TG 202) with the Substance or with the analogue substance;

2. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method EU C.3./OECD TG 201) with the Substance or with the analogue substance.

### Conditions to comply with the requests

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.

To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa;
- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if you have registered a substance at 100-1000 tpa;

Registrants are only required to share the costs of information that they are must submit to fulfil the information requirements for their registration.

The Appendix A state the reasons for the requests for information to fulfil the requirements set out in the respective Annex of REACH.

The Appendix C 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. The timeline has been set to allow for sequential testing where relevant.

**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.

EUROPEAN CHEMICALS AGENCY

**Appendix A: Reasons for the requests to comply with Annex VII of REACH**

Under articles 10(a) and 12(1) of REACH, a technical dossier registered at 1 to 10 tonnes or more per year must contain, as a minimum, the information specified in Annex VII to REACH.

## 1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

An *In vitro* gene mutation study in bacteria is a standard information requirement in Annex VII to REACH.

You have provided a key study in your dossier: In vitro gene mutation study in bacteria (1997) with the following strains, TA 97, TA 98, TA 100, TA 102, TA 104, TA 1535, TA 1537, and TA 1538 which all gave negative results.

ECHA has assessed this information and identified the following issues:

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471 (1997).

The key parameters of this test guideline include specifications for the concentrations of the test substance. Amongst the criteria to be taken into consideration when determining the highest concentration of test substance is cytotoxicity. The recommended maximum test concentration for soluble non-cytotoxic substances is 5 mg/plate or 5 µl/plate. Test substances that are cytotoxic already below 5 mg/plate or 5 µl/plate should be tested up to a cytotoxic concentration.

The reported data for the study you have provided indicates that instead of testing the Substance, you have tested a mixure containing an ingredient with antibacterial activity [redacted]. The highest tested concentration of the mixture was [redacted] µg/plate.

The tested mixture contained only [redacted]% of the Substance, i.e. its maximum tested concentration was [redacted] µg/plate [redacted] mg/plate).

In your comments to the draft decision, you refer to the read-across justification submitted for the registration of sodium 4-oxovalerate and argue that the purity of the Substance is actually the sum of sodium 4-oxovalerate [redacted]%) and levulinic acid ([redacted]%), i.e. [redacted]%, as these two substances can be considered to have comparable toxicological properties. In addition, you point out that ECHA has agreed in a decision on a testing proposal on the Substance that levulinic acid can be used to predict the toxicological properties of the Substance.

You state that the highest tested concentration of the mixture was selected on the basis of a preliminary cytotoxicity test. ECHA notes however that the presence of an antibacterial substance in the tested mixture could have confounded the test. Cytotoxicity observed in the preliminary test may be due to the effects of [redacted] and not caused by the Substance.

In your comments, you agree that [redacted] can have an antibacterial acitivity and cytotoxicity observed during the preliminary study might be due to the presence of alpha-terpineol and not due to the intrinsic cytotoxic properties of the Substance. However, you consider that the purity of the Substance in the mixture ([redacted]%) is actually high enough in order to be used in the *in vitro* gene mutation study in bacteria.

ECHA notes that even if [redacted] mg/plate would be regarded as the maximum tested concentration of the Substance, it is still considered to be very low when compared to the maximum test concentration of 5 mg/plate recommended in OECD TG 471 for soluble non-

cytotoxic substances. The information you have provided does not enable to conclude whether the Substance causes cytotoxicity or not in the bacterial strains tested. If it does, you have not provided any evidence on whether the Substance has been tested up to the cytotoxic concentration as required in OECD TG 471 for substances that causes cytotoxicity below 5 mg/plate.

Therefore, ECHA concludes that the reported data does not provide any conclusive information on the cytotoxicity of the Substance and whether the Substance has indeed been tested up to its cytotoxic concentration.

Further, in your comments, you claim that the *in vitro* gene mutation study in bacteria is not required due to the available negative higher-tier *in vitro* studies. You also state that the available data provided in your comments, i.e. experimental data from higher tier studies, literature data and QSAR predictions, is considered as enough and is scientifically robust to assess the mutagenic potential of the Substance.

We have assessed this information and identified the following issues:

a. As pointed out above, to fulfil the information requirement for this endpoint, the study has to meet the requirements of OECD TG 471 (1997). One of the key parameters of this test guideline is that the gene mutation study in bacteria must be performed with the following 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

In your comments, you refer to the following in vitro studies provided in the dossier:

i. *In vitro* mammalian chromosome aberration study with levulinic acid, negative result, key study (2016).
ii. *In vitro* gene mutation study with the Substance in mammalian cells with levulinic acid, negative result, key study (2017).

More specifically you indicate the following in your comments:

- According to the bacterial reverse mutation test guideline (OECD TG 471): "*in case bacterial reverse mutation test is not appropriate for the evaluation of certain classes of chemicals, for example highly bactericidal compounds (e.g. certain antibiotics) mammalian mutation tests may be more appropriate*". You therefore consider the *in vitro* gene mutation study in mammalian cells as "*enough*" to evaluate the gene mutation induction potential of the Substance that would had been screened otherwise in the Ames test.
- Since the Ames test is "*only a screening*" study, even if the reported data does not provide any conclusive information on the cytotoxicity of the Substance and "*therefore the available Ames test is not considered as completely performed according to the OECD TG 471*", the negative outcome is confirmed by the rest of *in vitro* tests on levulinic acid. According to your comments, the absence of point mutations is evaluated and confirmed in the higher-tier *in vitro* gene mutation study in mammalian cells.
- Even if the Ames test results to be positive, according to the ECHA Guidance R.7a "*when different results between test systems are obtained, they should be evaluated with respect to their individual significance*". You state that "*the results of mammalian test might be of higher significance*".

ECHA notes that the information from the higher-tier *in vitro* studies (i) and (ii) relates to different tests from the OECD TG 471. Therefore, this information does not cover key parameters required by the OECD TG 471.

Further, as regards to your comments above, ECHA notes that you did not provide any data indicating that a gene mutation test in bacteria would be inappropriate with the Substance. The *in vitro* gene mutation study in bacteria is a standard information requirement at Annex VII. As explained in the ECHA Guidance R.7a[2], the study is required as part of the testing strategy for mutagenicity. Therefore, the higher-tier *in vitro* mutagenicity studies in mammalian cells, required at Annex VIII (section 8.4.2. and 8.4.3.), cannot be used to waive the bacterial test required at Annex VII. According to the ECHA Guidance R.7a the results of mammalian tests may be considered of higher significance. However, it is also stated in this guidance that "additional data may be needed to explain possible differences" such as differences in substance uptake and metabolism, or in genetic material organisation and ability to repair. ECHA notes that you did not provide any "additional data" to support this statement for your Substance. Therefore, in case of a positive Ames test an in vivo testing may be required to reach a clear conclusion on mutagenicity, even if the in vitro gene mutation study in mammalian cells is negative.

b. Further, in your comments to the draft decision, you state that the available data provided in your comments is considered as enough and is scientifically robust to assess the mutagenic potential of the Substance.

In addition to the higher tier in vitro studies (i) and (ii) in mammalian cells, you have provided the following information in your comments:

iii. Literature data: No evidence of mutagenic effects of aminolevulinic acid in *in vitro* gene mutation study in mammalian cells or *in vivo* micronucleus study in mouse.
iv. Literature data: At least one report in the literature has noted genotoxic effects in cultured rat hepatocytes exposed to aminolevulinic acid. Other studies have documented oxidative DNA damage in vivo and in vitro as a result of aminolevulinic acid exposure.
v. Literature data: No evidence of mutagenic effects of aminolevulinic acid in 2 *in vitro* Salmonella-Escherichia coli/mammalian microsome reverse mutation assay (Ames mutagenicity assay).
vi. Literature data: *In vitro* gene mutation study in bacteria with mixtures did not indicate any differences in the revertants when samples with or without 5.65% of levulinic acid were tested.
vii. QSAR prediction: The OECD QSAR Toolbox profiling (v.4.2.) has not identified a structural alert regarding the in vitro gene mutation assay in bacteria (Ames) for the Substance. Evaluation on the structural alerts of the Substance by VEGA (v.1.1.4) with different models predicted that the Substance is non-mutagenic.

You also indicate in your comments that levulinic acid and levulinate salts are widely used and already recognised that they do not present any potential for genotoxicity. You state that levulinic acid has been granted GRAS (generally recognized as safe) status by FEMA (1965) and it is listed as an approved food additive by the FDA (21 CFR 5172.515). Therefore, no safety concern is identified for levulinic acid.

While an adaptation was not specifically indicated by you, ECHA has evaluated the provided information according to Annex XI, Section 1.2.

[2] ECHA Guidance R.7a, Section R.7.7.6.3

Annex XI, Section 1.2 states that there may be sufficient weight of evidence (WoE) from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance of the information for the given regulatory endpoint. Subsequently, relevance, reliability, consistency and results of these lines of evidence must be balanced in order to decide whether they provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

To fulfil the information requirement, normally a study according to OECD TG 471 must be provided. OECD TG 471 requires the study to investigate gene mutations in bacteria as a key parameter by using 5 different bacterial strains.

- For (i) and (ii), as already indicated above, and also for (iii) and (iv), ECHA notes that these studies do not provide relevant information as they do not investigate gene mutations in bacteria as required in OECD TG 471. Therefore, (i) to (iv) do not provide information that would contribute to the conclusion on this key parameter.

- In (v), you refer to literature data on in vitro gene mutations studies in bacteria performed with aminolevulinic acid. This source of information may provide relevant information on gene mutations in bacteria.

  However, the reliability of this source information is significantly affected by the following deficiences:

  Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide a justification for the read-across including a hypothesis, explanation of the rationale for the prediction of properties and robust study summary(ies) of the source study(ies).[3]

  You have provided studies conducted with other substance (aminolevulinic acid) than your Substance. You have not provided documentation as to why this information is relevant for your Substance.

  In addition, OECD TG 471 requires the study to investigate gene mutations in bacteria with e.g. the following test conditions:
  - The test must be performed using the following 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101)
  - The maximum dose tested must induce a reduction in the number of revertant colonies per plate compared to the negative control (testing up to a cytotoxic concentration), or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test dose must correspond to 5 mg/plate or 5 ml/plate.
  - Two separate test conditions must be assessed: in absence of metabolic activation and in presence of metabolic activation.
  - At least 5 doses must be evaluated, in each test condition
  - One positive control must be included in the study. The positive control substance

[3] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.6.1

must produce a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control.

ECHA notes that you did not provide any information whether the test was performed in all required 5 strains of bacteria with and without metabolic activation, how many doses were evaluated, no information on cytotoxicity and whether positive control was included in the tests. Based on these deficiencies, ECHA cannot assess the validity of the studies.

Therefore, ECHA considers that this source of information (v) is not reliable.

- In (vi), you refer to literature data on *in vitro* gene mutation study in bacteria (Ames test) performed with mixtures. Four samples were tested; two of the samples contained [redacted]% of levulinic acid. There were no differences detected in the number of revertants among these tested samples. This source of information (vi) may provide relevant information on gene mutations in bacteria.

  However, the reliability of this source information is significantly affected by the following deficiency:

  As indicated above for the information under (v), OECD TG 471 requires the study to investigate gene mutations in bacteria with the abovementioned test conditions.

  For (vi), you indicated that only 1 dose of levulinic acid was tested. In addition, ECHA notes that the tested sample contained only a very low concentration of levulinic acid and no information is provided whether it was tested up to a cytotoxic concentration. Furthermore, you did not provide any information whether the study was performed in all required strains with and without metabolic activation, and whether positive control was included in the study.

  Therefore, ECHA considers that this source of information (v) is not reliable.

- For (vii), you indicate in the QSAR predictions that the OECD QSAR Toolbox profiling (v.4.2.) has not identified a structural alert regarding the in vitro gene mutation assay in bacteria (Ames) for the Substance or levulinic acid. Furthermore, evaluation on the structural alerts of the Substance and sodium 4-oxovalerate by VEGA (v.1.1.4) with different models predicted that the Substance is non-mutagenic. This source of information (vii) may provide relevant information on gene mutations in bacteria.

  However, the reliability of this source information is significantly affected by the following deficiency:

  According to the ECHA guidance R.7a for substances where testing data exists this kind of non-test information can be used in a weight of evidence approach to help confirm results obtained in specific tests.

  ECHA notes that there is not any adequate in vitro gene mutation study in bacteria available with the Substance or levulinic acid. Furthermore, none of the predictions provided by you can address the 5 strains of Ames test as required by OECD TG 471.

  Although these predictions can be useful for screening, they cannot be accepted as the only data for standard information requirement of in vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.).

Finally, with reference to your comment regarding the scientific opinions drawn by other regulatory bodies and committees concluding on the safety of levulinic acid, ECHA notes that

these scientific opinions have been drawn for purposes other than those envisaged by ECHA. Consequently, they do not necessarily call into question the findings made by ECHA in the specific context of the assessment of the compliance of a registration for the Substance against the applicable information requirements laid down by the REACH Regulation.

As a conclusion, sources of information (i) to (iv) are not relevant as they do not investigate gene mutations in bacteria as required in OECD TG 471. Sources of information (v) to (vii) provide information on gene mutation in bacteria but they are not reliable as indicated above.

Accordingly, it is not possible to to conclude, based on any source information alone or considered together, whether your Substance has or has not the particular dangerous property forseen to be investigated in an OECD TG 471 study. Therefore, your adaptation is rejected.

The information provided does not enable appropriate evaluation of mutagenicity of the Substance in bacteria as required by the OECD TG 471. Therefore, the information requirement is not fulfilled.

**2. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)**

Short-term toxicity testing on aquatic invertebrates is a standard information requirement in Annex VII to REACH.

You have adapted the standard information requirement according to Annex XI, Section 1.2. Weight of evidence of REACH.

In support of your adaptation, you have provided the following sources of information:

(i) an experimental study, according to guideline OECD TG 202, from the Lomba et al. publication, 2014 with Klimisch reliability 4;
(ii) a QSAR prediction ECOSAR v1.10, 2011, also with Klimisch reliability 4;

We have assessed this information and identified the following issues:

Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance of the information for the given regulatory endpoint. Subsequently, the lines of evidence should be integrated considering their relative values or weights in order to draw an assumption/conclusion.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach, the assessment of relative weights of individual pieces of information and the subsequent conclusions drawn.

However, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the assumption that the substance has or has not a particular dangerous property.

In spite of this deficiency, ECHA has also assessed to what extent the sources of information submitted enables a conclusion on the dangerous property in question *i.e.* acute aquatic invertebrates test EC50 estimation.

A weight of evidence adaptation may allow a conclusion on the EC50 or acute toxicity for aquatic invertebrates if the sources of information provide sufficient information on the dangerous properties foreseen to be investigated in an OECD TG 202 study.

While the sources of information (i) publication by Lomba et al. and (ii) the QSAR prediction do provide relevant information on EC50 for aquatic invertebrates acute toxicity, these sources of information have the following deficiencies affecting their reliability.

*Publication by Lomba et al.*

Tests on substances must be conducted in accordance with the OECD test guidelines or other internationally recognised test method (Article 13(3) of REACH).

OECD TG 202 requires that the following conditions are met (among others):

- adequate exposure duration of the test (i.e. 48 hours),
- in the control (including the solvent control, if appropriate), no more than 10% of daphnids should be immobilised or show signs of disease or stress by the end of the test,
- dissolved oxygen concentrations at the end of the test should be ≥3 mg/l in all control and test vessels,
- analytical monitoring of exposure concentrations
- effect concentrations based on the measured values rather than nominal values unless the test concentrations are maintained within 20% of the measured initial concentrations throughout testing.

Regarding (i), the *Lomba et al.* publication, this piece of information has limitations and does not address, the key parameters foreseen to be investigated in an OECD TG 202 study. Specifically, in the publication the test conditions are different from TG 202 with regard to the following parameters:

- the exposure duration is 24h instead of 48h.
- you did not provide information on the physiological state of the organisms stemming from the ToxKit for the control or any testing conditions.
- you did not provide data on the monitoring of oxygen concentrations in test vessels or control.
- no monitoring of the test substance concentration was reported.
- you did not demonstrate that the test substance concentration during the test was maintained within the required 20% of the measured initial concentrations.

Therefore, the provided piece of information is considered unreliable with regard to the estimation of EC50 and it cannot taken into account for the weight of evidence approach.

*Non-acceptable QSAR prediction(s)*

Regarding (ii), a QSAR prediction can be used to adapt the standard information requirement, provided that the cumulative criteria from Annex XI, Section 1.3. are met, these criteria include that:

1. the substance falls within the applicability domain of the QSAR model;
2. adequate and reliable documentation of the applied method is provided; and
3. the results are adequate for classification and labelling and/or risk assessment.

According to ECHA's Practical guide "How to use and report (Q)SARs", section 3.4, a QSAR Model Reporting Format (QMRF) and a QSAR Prediction Reporting Format (QPRF) are required to establish the scientific validity of the model, to verify that the Substance falls within the applicability domain of the model, and to assess the adequacy of the prediction for the purposes of classification and labelling.

You have not provided sufficient documentation for the QSAR prediction. In particular, you have not included a QMRF and a QPRF in your technical dossier.
Therefore, ECHA cannot establish, whether the Substance falls within the applicability domain of the model, and whether the results are adequate for classification and labelling and/or risk assessment.

As explained above, the reported QSAR prediction is not considered sufficiently reliable and does not fulfil the criteria in Annex XI, Section 1.3. Therefore, it cannot be used as part of weight of evidence adaptation according to Annex XI, Section 1.2.

Based on our assessment the sources of information you provided are not reliable.

Following the assessment above, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 202 study.

In your comments to the draft decision you acknowledge the deficiencies of the provided information and agreed to perform the test using the analogue substance Levulinic acid (EC No 204-649-2). ECHA acknowledges that the analogue substance may be used as the test substance.

Consequently the information requirement is not fulfilled and a Short-term toxicity testing on aquatic invertebrates must be performed on the Substance or with the analogue substance.

### 3. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

Growth inhibition study aquatic plants is a standard information requirement in Annex VII to REACH.

You have adapted the standard information requirement according to Annex XI, Section 1.2. Weight of evidence of REACH.

In support of your adaptation, you have provided the following sources of information for the analogue substance 4-oxovaleric acid (EC: 204-649-2):

(i) an experimental study (Lomba et al.) according to guideline OECD 201, from the Lomba et al. publication, 2014, with Klimisch reliability 2;
(ii) a QSAR prediction ECOSAR v1.10, 2011, also Klimisch reliability 4;

We have assessed this information and identified the following issues:

Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted based on the reliability of the data, consistency of results/data, nature and severity of effects, and

relevance of the information for the given regulatory endpoint. Subsequently, the lines of evidence should be integrated considering their relative values or weights in order to draw an assumption/conclusion.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach, the assessment of relative weights of individual pieces of information and the subsequent conclusions drawn.

However, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the assumption that the substance has or has not a particular dangerous property.

In spite of this deficiency, ECHA has also assessed to what extent the sources of information submitted enables a conclusion on the dangerous properties in question *i.e.* Algal growth inhibition test expressed as ErC50 and NOEC.

A weight of evidence adaptation may allow a conclusion on Algal growth inhibition test expressed as ErC50 and NOEC if the sources of information provide sufficient information on the dangerous properties foreseen to be investigated in an OECD TG 201 study.

The dangerous properties investigated by this test guideline include :

1) algal growth inhibition ErC50 and additionally
2) the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC), plus any observations of potential adverse effects for Algal populations.

While the sources of information (i) publication by Lomba et al. and (ii) the QSAR prediction do provide relevant information on ErC50 as inhibition of growth, these sources of information have the following deficiencies affecting their reliability.

*Publication by Lomba et al.*

Tests on substances must be conducted in accordance with the OECD test guidelines or other internationally recognised test method (Article 13(3) of REACH).

OECD TG 201 requires that the following conditions are met (among others):

- adequate exposure duration of the test (i.e. 72 hours),
- fulfilment of validity criteria as set out in the test guideline including: biomass exponential and unrestricted growth observed for the algal poplulations in the control,
- use of an appropriate species as detailed in the Annexes to the test guideline,
- use of appropriate apparatus to determine algal biomass. If biomass surrogates are measured via e.g. a fluorimeter, quantification of biomass with conversion to dry weight is needed,
- analytical monitoring of exposure concentrations,
- effect concentrations based on the measured values rather than nominal values unless the test concentrations are maintained within 20% of the measured initial concentrations throughout testing.

Regarding (i), the Lomba et al. publication, this piece of information has limitations and does not address, the key parameters foreseen to be investigated in an OECD TG 201 study.
Specifically, in the publication results, the test conditions are different from TG 201 with regard to the following parameters:

- the exposure duration is not sufficient (only 2 hours of exposure),
- the initial algae cell density was at too high which did not allow for exponential growth and fulfilment of the validity criteria,
- the tested species *Chlamydomonas rheinardii* is not recognised as an appropriate species by OECD TG 201,
- measurement of biomass growth inhibition using fluorescence measurements was provided but without conversion to dry weight so quantification of biomass is not possible.
- no monitoring of the test substance concentration was reported and
- you did not demonstrate that the test substance concentration during the test was maintained within the required 20% of the measured initial concentrations.

The aforementioned conditions of the guidelines are not met, therefore the information provided does not fulfil the information requirement.

The provided piece of information is considered unreliable with regard to the estimation of ErC50. Neither does it provide a reliable estimate of NOEC and LOEC or reliable observations on potential adverse effects for Algal populations. Consequently, it cannot taken into account for the weight of evidence approach.

*Non-acceptable QSAR prediction*

Regarding (ii), a QSAR prediction can be used to adapt the standard information requirement, provided that the cumulative criteria from Annex XI, Section 1.3. are met, these criteria include that:

1. the substance falls within the applicability domain of the QSAR model;
2. adequate and reliable documentation of the applied method is provided; and
3. the results are adequate for classification and labelling and/or risk assessment.

According to ECHA's Practical guide "How to use and report (Q)SARs", section 3.4, a QSAR Model Reporting Format (QMRF) and a QSAR Prediction Reporting Format (QPRF) are required to establish the scientific validity of the model, to verify that the Substance falls within the applicability domain of the model, and to assess the adequacy of the prediction for the purposes of classification and labelling.

You have not provided sufficient documentation for the QSAR prediction. In particular, you have not included a QMRF and a QPRF in your technical dossier.
Therefore, ECHA cannot establish, whether the Substance falls within the applicability domain of the model, and whether the results are adequate for classification and labelling and/or risk assessment.

As explained above, the reported QSAR prediction is not considered sufficiently reliable and does not fulfil the criteria in Annex XI, Section 1.3. Therefore, it cannot be used as part of weight of evidence adaptation according to Annex XI, Section 1.2.

Based on our assessment the sources of information you provided are not reliable.

Following the assessment above, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 201 study.

In your comments you acknowledge the deficiencies of the provided information and agreed to perform the test using the analogue substance Levulinic acid (EC No 204-649-2). ECHA acknowledges that the analogue susbsance may be used as the test substance.

Consequently the information requirement is not fulfilled and a Growth inhibition study aquatic plants using Algae must be performed on the Substance or with the analogue substance.

## Appendix B : Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The compliance check was initiated on 07 March 2019.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

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

**Deadline to submit the requested information in this decision**

In your comments on the draft decision, you requested an extension of the timeline to provide the information requested from 6 to 18 months. You justified your request stating that you would like to "*update and submit the dossier with the requested ecotoxicological studies once, following the deadline (to be set) for the OECD 408 and OECD 414*", which studies are being requested in a separate decision on a testing proposal, on the same substance, with a deadline of 18 months.

ECHA notes that the studies requested in this decision can be performed in parallel to the studies requested in the decision on a testing proposal. Therefore, ECHA has not modified the deadline of the decision.

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 C: Observations and technical guidance

1. The information requirement under Section 8.7.3. of Annex IXto REACH (Extended one-generation reproductive toxicity study, EOGRTS) is not addressed in this decision, because the information from the Sub-chronic toxicity study (90-day), requested in the a TPE decision as a testing proposal for the 90-day study has been submitted, is relevant for the design of the EOGRTS.

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

3. 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 the Member States.

4. 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 shall 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]'.

5. Test material

   *Selection of the test material*

   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 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

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

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].

6. List of references of the ECHA Guidance documents[6]

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 in this decision.

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]

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.

OECD Guidance documents[8]

[5] https://echa.europa.eu/manuals
[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
[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 GD23.
Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

**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 fufilled |
|---|---|---|
| [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.