~~Confidential~~

Helsinki, 11 June 2021

Addressees
Registrant(s) of JS_526-75-0 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
08/02/2019

Registered substance subject to this decision ("the Substance")
Substance name: 2,3-xylenol
EC number: 208-395-3
CAS number: 526-75-0

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 listed below, by the deadline of ***20 December 2021***.

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

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

1. Apply the harmonised classification and labelling on the Substance for acute oral toxicity, skin corrosion/irritation and serious eye damage/eye irritation and for long-term (chronic) aquatic hazard (Annex VI, Section 4.);

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

2. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: EU B.13/14. / OECD TG 471)

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

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

5. Ready biodegrability (Annex VII, Section 9.2.1.1.; test method: OECD TG 301A/B/C/D/E/F or OECD TG 310)

Reasons for the request(s) are explained in the following appendices:

- Appendix entitled "Reasons common to several requests";
- Appendix entitled "Reasons for the requests to comply with Annex VI of REACH"
- Appendix entitled "Reasons to request information required under Annex 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 VI, 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 on Reasons common to several requests

Assessment of your weight of evidence adaptation under Annex XI, Section 1.2.

In you registration dossier you originally provided a key study and supporting studies for the endpoints listed below. On this basis ECHA assessed this information on the basis of the corresponding test guideline requirements (or Annex XI, Section 1.1.2., if applicable). In your comments, in your comment to the draft decision you clarified that you have adapted the following standard information requirements by applying weight of evidence approaches in accordance with Annex XI, Section 1.2:

- Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

ECHA has considered the scientific and regulatory validity of your weight of evidence approach in general before assessing the specific standard information requirements in the following appendices.

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

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach.

In your comments to the draft decision, you have summarised the sources of information for each endpoint, in relation to the reliability, coverage of key parameters, consistency and results and conclude that as a weight of evidence based on the available sources of information, no further studies are needed.

ECHA has assessed the validity of your adaptation and identified the following issues:

Your weight of evidence adaptation has deficiencies that are common to all information requirements under consideration and also deficiencies that are specific for these information requirements individually. The common deficiencies are set out here, while the specific ones are set out under the information requirement concerned in the Appendices below.

Reliability of the provided information with analogue substances

ECHA understands that you intend to predict the ecotoxicological properties of the Substance for the listed above endpoints, from data obtained with analogue substances in a read-across approach as part of your weight of evidence adaptation.

Grouping of substances and read-across approach

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which

results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (addressed under 'Assessment of prediction(s)').

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance[2].

Predictions for ecotoxicological properties

For ecotoxicological properties you read-across between the following substances: Phenol, EC No. 203-632-7 (CAS No. 108-95-2), 2,4-xylenol EC No. 203-321-6 (CAS No. 105-67-9), 2,6-Dimethylaniline EC No. 201-758-7 (CAS No. 87-62-7) as source substances and the Substance as target substance.

In your comments to the draft decision you have provided a document entitled "[redacted]"

With this document you intend to justify the use of information obtained on the aforementioned source substances in your weight of evidence adaptation.

In your justification document you have indicated that 'Scenario 2' was selected for the analogue approach. You provided the following reasoning for the prediction of ecotoxicological properties:

"*read-across of environmental fate, ecotoxicological and toxicological data from an analogue may be justified on the basis of:*

- *Identifying the read across substances based on common functional groups and further filled with relate mechanistic approaches and finally fine-tuned with structural similarity using the QSAR Toolbox Version 3.4*
- *Common structural alerts or reactivity*
- *Common physico-chemical properties*
- *Likelihood of common breakdown products via biological/degradation processes*"

You conclude that "*the descriptors, various alerts and scenario (for analogue approach) which were taken into consideration for ecotoxicological and toxicological assessment as reported in this RA justification document obtained by using OECD QSAR toolbox v.3.4 of the target substance and source substances (i.e., read across analogues) were evaluated to be similar and therefore justified and appropriate"*

Based on the above, ECHA understands that you used the QSAR Toolbox for the identification of analogues and use information on these analogues to predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substance(s).

ECHA notes the following deficiencies with regards to predictions of ecotoxicological properties.

Missing supporting information

[2] ECHA Guidance R.6

Annex XI, Section 1.5 of the REACH Regulation states that "*physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)*". For this purpose "*it is important to provide supporting information to strengthen the rationale for the read-across*"[3]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

Supporting information must include information to confirm that the Substance and the source substances have similar (eco)toxicological properties and that the structural differences would not affect the predicted properties of the substances.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substances cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm that both substances cause the same type of effects. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

In order to support your read-across hypothesis, you have provided the following information:

- Alert profiles using the QSAR Toolbox

In your justification you indicated structural similarities and differences between the target and source substances, as follows: they have phenol as a common basic moiety in their structure, with the exception of the source substance 2,6-Dimethylaniline EC No. 201-758-7 (CAS No. 87-62-7). Furthermore, source and target substances have in common an aromatic ring, but have variations in the type and position substituents, i.e. amino substituent that is present only in 2,6-Dimethylaniline (EC no. 201-758-7, CAS no. 87-62-7), alkyl substituents that you indicate are common functional groups for the target and two source substances (i.e. 2,4-xylenol (EC No. 203-321-6, CAS No. 105-67-9) and 2,6-Dimethylaniline (EC no. 201-758-7, CAS no. 87-62-7)).

You have assessed the impact of these structural differences using a set of physico-chemical and (abiotic and biotic) degradation properties, structural characteristics and mechanistic alerts obtained from the QSAR Toolbox v3.4 for the Substance and for each of the source substances.

You indicate that "*As the target and read across analogues contain nearly similar functional groups, different structural activity amongst the various read across substances is not expected. As per the analysis conducted with the OECD (Q)SAR Toolbox v.3.4 , it is indicated that target and the read across analogues share similar structural alerts*".

- Experimental studies

In your dossier and/or in your comments to the draft decision, you have provided the following information on experimental data for aquatic toxicity on the Substance and the source substances:

<u>*Short-term toxicity testing on aquatic invertebrates*</u>

i. key and supporting studies taken from study report and different published literature with the Substance 2,3-xylenol (EC no. 208-395-3, CAS no. 526-75-0).

[3] ECHA Guidance R.6: Section R.6.2.2.1.f

ii. OECD TG 202 study, equivalent or comparable with the OECD TG 202 on the source substance Phenol (EC no. 203-632-7, CAS no. 108-95-2) taken from the different published literature, journal ([redacted], 1991 and secondary sources).
iii. OECD TG 202 Study, according to U. S. EPA method performed on the source substance 2,4-xylenol (EC No. 203-321-6, CAS No. 105-67-9) taken from the published literature/journal ([redacted], 1980).

Growth inhibition of algae

iv. key and supporting studies taken from study report and different published literature with the Substance 2,3-xylenol (EC no. 208-395-3, CAS no. 526-75-0).
v. OECD TG 201 study taken from the published literature (secondary source, 2012) with the source substance 2,6-Dimethylaniline (EC no. 201-758-7, CAS no. 87-62-7).
vi. OECD TG 201 study taken from the published literature (J-CHECK) with the source substance 2,4-xylenol (EC No. 203-321-6, CAS No. 105-67-9).

We have assessed the supporting information provided and identified the following issues:

- Regarding alerts obtained from the QSAR toolbox

There are structural differences between the target and source substances. While the similarity in presence or absence of structural alerts may indicate that the differences do not influence the reactivity of the substance e.g. on the protein or DNA binding, this information does not confirm, on its own, that the Substance and the source substances have similar ecotoxicological properties such as aquatic toxicity (growth inhibition of algae, toxicity to *Daphnia*). In fact, the complexity of the aquatic toxicity and the mechanisms associated are not covered by computational tools. Therefore, the structural alerts reported in the justification document do not represent adequate information on the above mentioned properties of the Substance and the source substances, e.g. bridging studies of comparable design and duration.

Similarly regarding the predicted physicochemical and degradation properties, while this information might be relevant to support similarity in behaviour in aquatic compartment, this information does not allow the prediction of complex information requirements that you intend to cover with your adaptation, as indicated above.

- Regarding experimental studies

ECHA has identified shortcomings with the reliability of the experimental studies provided as supporting information:

Regarding the short term toxicity to invertebrates and the algae growth inhibition studies with the Substance and with the source substances, as described in the appendices below (sections B.3 and B.4, respectively), the studies are not considered reliable and therefore they cannot be used to compare the ecotoxicological properties of the substances.

Conclusion for prediction of ecotoxicological properties

Based on the information provided, no reliable comparison of the properties of the Substance and the source substances can be made.

Therefore, based on the information in the dossier and provided in the comments, the information from the source substances submitted under your weight of evidence adaptation is not considered reliable. Additional issues related to weight of evidence are addressed under the corresponding information requirement.

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

Under Article 10(a) of REACH, a technical dossier must contain information specified in Annex VI to REACH.

1. **Apply the harmonised classification and labelling on the Substance for acute oral toxicity, skin corrosion/irritation and serious eye damage/eye irritation and long-term (chronic) aquatic hazard (Annex VI, Section 4.)**

Classification and labelling of the substance, resulting from the application of Title I, II and III of Regulation (EC) No 1272/2008 (CLP), is an information requirement as specified in Annex VI to REACH.

To fulfil the information requirement, the classification and labelling of the substance subject to harmonised classification and labelling through an entry in Part 3 of Annex VI to CLP must be done in accordance with that entry (Annex VI, Section 4 in conjunction with Article 4(3) and Article 21(3) of CLP).

The Substance has following harmonised classification and labelling entry in Part 3 of Annex VI to CLP

- for acute oral toxicity: Acute Tox. 3 with hazard statement 'Toxic if swallowed' (H301)
- for skin corrosion/irritation and serious eye damage/eye irritation: Skin Corr. 1B with hazard statement 'Causes severe skin burns and eye damage' (H314)
- for long-term (chronic) aquatic hazard: Aquatic Chronic 2 with hazard statement 'Toxic to aquatic life with long lasing effects' (H411).

However, you have not applied it.

To fulfil the information requirement for the Substance, the harmonised classification and labelling must be applied.

In your comments to the draft decision you agreed with the request.

Appendix B: Reasons to request information required under Annex VII of REACH

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is an information requirement under Annex VII to REACH (Section 8.4.1.).

You have provided a key study and supporting studies with the Substance in your dossier:

i. Epler et al (1979), a bacterial gene mutation toxicty study with the following strains, TA 98, TA 100, which all gave negative results (key study)
ii. Florin et al (1980), a bacterial gene mutation toxicty study with the following strains, TA 98, TA 100, TA 1535, and TA 1537 which all gave negative results (supporting study)
iii. Jansson et al (1986), an *in vitro* sister-chromatid exchange assay in human lymphocytes, with a negative result (supporting study).

Although you do not explicitly claim an adaptation, ECHA understands that the information provided was submitted in order to meet the required information by way of adaptation under Annex, Section XI 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular:

- Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case OECD TG 471[4] (1997).

The key parameters of this test guideline include:

- The study must be an *in vitro* gene mutation study in bacterial cells, and include information on detecton and quantification of gene mutations (base pairs, substitution or frame shift) in cultured bacteria
- The test must be performed with 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)
- Two separate test conditions must be assessed: in absence of metabolic activation and in presence of metabolic activation
- The maximum dose tested must induce a reduction in the number of revertant colonies per plate compared to the negative control, or the precipitation of the tested substance. ~~I~~If no precipitate or limiting cytotoxicity is observed, the highest test dose must correspond to 5 mg/plate or 5 ml/plate
- One positive control must be included in the study. The positive control substance must produce a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control
- The number of revertant colonies per plate for the concurrent negative control must be inside the historical control range of the laboratory.

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

The reported data for the provided key study (i) and the supporting study (ii) did not include:

- results for the appropriate 5 strains, that is in TA1535, TA1537 or TA97a or TA97, *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101) (study (i))
- results for the appropriate 5 strains, that is in the required fifth strain, *S. typhimurium*

[4] ECHA Guidance R.7a, Table R.7.7–2, p.557

TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101) (study (ii))
- a maximum dose of 5 mg/plate or 5 ml/plate or that induced a reduction in the number of revertant colonies per plate compared to the negative control, or the precipitation of the tested substance (study (ii))
- information on the nature and validity of a positive control (a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control) (study (i))
- information on the validity of the negative control (a number of revertant colonies per plate inside the historical control range of the laboratory) (study (ii)).

Study (iii) relates to an *in vitro* sister-chromatid exchange (SCE) assay in human lymphocytes, which provides an indication of induced damage to DNA, but does not provide direct evidence of mutation. Therefore, the information provided (study (iii)) is not an *in vitro* gene mutation study in bacterial cells and does not provide information on detection and quantification of gene mutations (base pairs, substitution or frame shift) in cultured bacteria.

Based on the above, the studies (i), (ii) and (iii) do not provide adequate and reliable coverage of the key parameters foreseen to be investigated in the OECD TG 471.

Therefore, your adaptation is rejected and the information requirement is not fulfilled.

Study design

To fulfil the information requirement for the Substance, the *in vitro* gene mutation study in bacteria (OECD TG 471) is considered suitable.

Possibility for data sharing:

The other registrant of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information.

In your comments to the draft decision you agreed to perform the test requested.

2. 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.).

While in the draft decision sent to the registrant ECHA had assessed the key study (i.e. study "i") on the basis of the corresponding test guideline requirements, and the supporting studies as an adaptation under Annex XI, Section 1.1.2. (i.e. studies ii-v). In your comments to the draft decision you clarified that you have adapted this standard information requirement according to Annex XI, Section 1.2. of REACH (weight of evidence).

In your dossier you have provided the following information with the Substance:

i. a key study according to OECD TG 202 (2017, Testing facility: [redacted], Sponsor: [redacted]);
ii. a supporting study, publication (2000, Kahru *et al.*);
iii. a supporting study, publication (1988, Devillers);
iv. a supporting study, publication (2000, Kahru *et al.*);
v. a supporting study, secondary literature (2011, U.S. National Library of Medicine).

In your comments to the draft decision you have provided additionally the following studies with source substances:

vi. OECD TG 202 study, equivalent or comparable with the OECD TG 202 on the source substance Phenol (EC no. 203-632-7, CAS no. 108-95-2) taken from the different published literature, journal ([redacted], 1991 and secondary sources).

vii. OECD TG 202 study, according to U.S. EPA method performed on the source substance 2,4-xylenol (EC No. 203-321-6, CAS No. 105-67-9) taken from the published literature/journal ([redacted], 1980).

We have assessed this information and identified the following issues:

As explained under Appendix on Reasons common to several requests, the weight of evidence must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 9.1.1. at Annex VII includes similar information that is produced by the OECD TG 202. The OECD TG 202 requires the study to investigate the following key parameter:

- The concentration leading to 50% immobilisation of daphnids.

The sources of information listed above from (i) to (vii) provide relevant information on this key parameter. However the reliability of these sources of information is significantly affected by the following issues:

A. The reliability of sources of information (vi) and (vii) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests.

B. The reliability of sources of information from (i) to (vii) is also affected by the following issue:

For a study conducted according to OECD TG 202, the following specifications must be met:

Reporting of the methodology and results

- the test design is reported (*e.g.* static or semi-static test, number of replicates, number of test concentrations);
- the test procedure is reported (*e.g.* composition of the test medium, loading in number of *Daphnia* per test vessel);
- the number of immobilised daphnids is determined at 24 and 48 hours. Data are summarised in tabular form, showing for each treatment group and control, the number of daphnids used, and immobilisation at each observation;
- the dissolved oxygen measured at least at the beginning and end of the test are reported;
- the EC50 at 48h for immobilisation is reported;
- 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;

Characterisation of the exposure:

- the concentrations of the test material are measured at least at the highest and lowest test concentration, at the beginning and end of the test;

Technical specifications impacting the sensitivity/reliability of the test

- the test duration is 48 hours or longer;
- young daphnids aged less than 24 hours at the start of the test, are used;
- at least 20 animals are used at each test concentration and for the controls;

Validity criteria

- validity criteria specified in the test guideline must be met:
  - the percentage of immobilised daphnids is ≤ 10% at the end of the test in the controls (including the solvent control, if applicable);
  - the dissolved oxygen concentration is ≥ 3 mg/L in all test vessels at the end of the test;

In your dossier and in your comments to the draft decision you have provided the following information:

Reporting of the methodology and results

- on the test design, you have not specified number of replicates and the number of tested concentrations for studies (ii), (iv) and (v).
- on the test procedure, you have not specified the composition of the test medium for the studies (ii-vii), and for studies (ii), (iv) and (v) there is no information for number of organisms per test vessel.
- tabulated data on the number of immobilised daphnids after 24 and 48 hours for each treatment group and control are not reported for any of the studies. For studies (vi) and (vii), you only report that immobilised daphnids in the controls were below 10%.for studies (ii), (iii), (iv), (v) and (vi) the dissolved oxygen measured at least at the beginning and end of the test are not reported.
- for studies (vi) and (vii) you have not reported the EC50 values at 48h.
- for study (vi) analytical monitoring was performed, but you have not reported the performance parameters of the method and the results of the analytical determination of exposure concentrations.

Characterisation of the exposure:

- No analytical monitoring was conducted for studies (i) to (v) and for study (vii).

Technical specifications impacting the sensitivity/reliability of the test

- the test duration was 24 hours for for studies (iii) and (iv).
- for studies (ii) and (iv) the age of test organisms is not reported and for study (iii) the daphnids were <72h old.
- the number of animals used at each test concentration and for the controls is 15 for studies (iii) and (vii) and 10 for study (vi).

Validity criteria

- you indicate that the validity criteria were met for study (i). However you have not provided the number of immobilised daphnids in the controls for studies i-v.
- You have not provided information on dissolved oxygen for studies ii- vi.

On this basis there are major deficiencies impacting all sources of evidence provided in support of your weight-of-evidence adaptation, including the following:

- *Reporting of the methodology and results:* In absence of information detailed above on the study design and procedure for any of the studies, it is not possible to verify if the test design and test conditions followed the requirements of OECD TG 202.
- *Characterisation of the exposure:* in the absence of analytical monitoring for studies (i) to (v) and for study (vii), and in the absence of results of the analytical determination for study (vi), you have not demonstrated the stability of the test

substances for any of the studies.

- *Technical specifications impacting the sensitivity/reliability of the test:* First, the exposure duration was shorter than 48 hours (i.e. 24 hours) for studies (iii) and (iv). Shorter test duration generally leads to higher effect values and hence to an underestimation of the toxicity (ECHA Guidance R.7b). Second, there is no information on the age of test organisms for studies (ii) and (iv) and for study (iii) the daphnids were <72h old. This may underestimate the toxicity, because the sensitivity of test organisms may be lower if they are aged more than 24h at test start (ECHA Guidance R.7b). Third, the number of test animals was lower than 20 for studies (iii), (vi) and (vii), which may influence the statistical power of the test. For all these reasons, there is significant uncertainty with regard to the reliability of these studies (ii-vii).
- *Validity criteria*: as you have not provided information on dissolved oxygen (studies ii-vi) and the number of immobilised daphnids in the controls (studies i-v), it is not possible to verify that the validity criteria are met for these studies (i-vi).

Due to the above deficiencies, the studies (i), (ii), (iii), (iv), (v), (vi) and (vii) cannot be considered as reliable.

Taken together, even though the sources of information (i), (ii), (iii), (iv), (v), (vi) and (vii) as indicated above may provide relevant information, their reliability is affected significantly, therefore, they cannot contribute to the conclusion on the concentration of the Substance leading to the immobilisation of 50% of daphnids.

Conclusion
On this basis, 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 properties foreseen to be investigated in an OECD TG 202 study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

3. Growth inhibition study aquatic plants

Growth inhibition study aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

While in the draft decision sent to the registrant ECHA had assessed the key study (i.e. study "i") on the basis of the corresponding test guideline requirements, and the supporting studies as an adaptation under Annex XI, Section 1.1.2. (i.e. studies ii-iii). In your comments to the draft decision you clarified that you have adapted this standard information requirement according to Annex XI, Section 1.2. of REACH (weight of evidence).

In your dossier you have provided the following information with the Substance:

i. a key study according to OECD TG 201 (2017, Testing facility: [redacted], Sponsor: [redacted])- *Chlorella vulgaris;*
ii. a supporting study according to OECD TG 201 (2017, Testing facility: [redacted], Sponsor: [redacted]) - *Desmodesmus subspicatus;*
iii. a supporting study, publication (2000, Kahru *et al.*).

In your comments to the draft decision, you have provided additionally the following studieswith source substances:

iv. OECD TG 201 taken from the published literature (secondary source, 2012) with the source substance 2,6-Dimethylaniline (EC no. 201-758-7, CAS no. 87-62-7).
v. OECD TG 201 taken from the published literature (J-CHECK) with the source substance 2,4-xylenol (EC No. 203-321-6, CAS No. 105-67-9).

We have assessed this information and identified the following issues:

As explained under Appendix on Reasons common to several requests, the weight of evidence must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 9.1.2 at Annex VII includes similar information that is produced by the OECD TG 201. Therefore, the following requirements must be met:

- the concentrations of the test material leading to a 50 % and 0% (or 10%) inhibition of growth at the end of the test are estimated.

The sources of information (i), (ii), (iii), (iv) and (v) provide relevant information on concentrations of test material leading to a 50% and 0% (or 10%) inhibition of algae growth. However, these sources of information have the following deficiencies affecting their reliability:

A. The reliability of sources of information (iv) and (v) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests.

B. The reliability of source of information from (i) to (v) is also affected by the following issue:

For a study conducted according to OECD TG 201, the following specifications must be met:

Reporting of the methodology and results
- the test design (*e.g.*, number of replicates, number of test concentrations and geometric progression used) and the test conditions are reported (*e.g.*, composition of the test medium, test temperature, test species, biomass density at the beginning of the test);
- 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;

Characterisation of the exposure:
- the concentrations of the test material are measured at least at the beginning and end of the test.

Validity criteria:
- validity criteria specified in the test guideline must be met:
  - exponential growth in the control cultures is observed over the entire duration of the test;
  - at least 16-fold increase in biomass is observed in the control cultures by the end of the test;
  - the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is ≤ 35%;
  - the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is ≤ 7% in tests with *Pseudokirchneriella*

subcapitata or *Desmodesmus subspicatus*. For other less frequently tested species, the value is ≤ 10%;

In your dossier and in your comments to the draft decision you have provided the following information:

Reporting of the methodology and results:

- For studies (iii) and (v), you have not provided information on the test design and procedure, e.g. on number or replicates, concentrations used, spacing factor used, pH measurements, composition of the test media.
- tabulated data on the algal biomass determined daily for each treatment group and control are not reported for the studies (ii), (iii), (iv), and (v).
- for study (iv), analytical monitoring was performed and you have specified that the source substance (2,6-Dimethylaniline, EC no. 201-758-7) is stable, but you have not reported the performance parameters of the method and the results of the analytical determination of exposure concentrations.

Characterisation of the exposure:

- No analytical monitoring of exposure concentration was conducted for studies (i) and (ii), For studies (iii) and (v) you do not specify if analytical monitoring was performed.

Validity criteria:

- for study (i), the initial biomass and the biomass at the end of the test was approximately 10,000 cells/mL and 25,000 cell/mL, respectively. This correspond to a 2.5-fold increase when it should be >16;
- for study (iv) you have provided an initial cell density of algae and indicated that the validity criteria are met, but you have not provided the biomass data at the end of the test.
- for studies (ii), (iii) and (v) you have not specified if the validity criteria were met.

On this basis there are major deficiencies impacting all sources of evidence provided in support of your weight-of-evidence adaptation, including the following:

- *Characterisation of the exposure:* in the absence of analytical monitoring for studies (i), (ii), (iii) and (v), and in the absence of results of the analytical determination for study (iv), you have not demonstrated the stability of the test substances for any of the studies;
- *Reporting of the methodology and results:* The reporting of the studies (iii) and (v) is not sufficient to conduct an independent assessment of its reliability.
- *Validity criteria:* the validity criteria of OECD TG 201 are not met for study (i). Furthermore, in the absence of tabulated data on the algal biomass determined daily for studies (ii-v), it is not possible to verify that the validity criteria were met.

Due to the above deficiencies, the studies (i), (ii), (iii), (iv) and (v) cannot be considered as reliable.

Taken together, even though the sources of information (i), (ii), (iii), (iv) and (v) as indicated above may provide relevant information, their reliability is affected significantly, therefore, they cannot contribute to the conclusion on the concentrations of the test material leading to a 50 % and 0% (or 10%) inhibition of algae growth.

Conclusion

On this basis, 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 properties foreseen to be investigated in an OECD TG 201 study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

On this basis, the information requirement is not fulfilled.

Possibility for data sharing for studies not involving vertebrate animals

The opt-out registrant's dossier for the Substance contains data which is relevant for this endpoint. In accordance with Title III of the REACH Regulation, you may request it from the other registrant and then make every effort to reach an agreement on the sharing of data and costs.

4. Ready biodegradability

Ready biodegradability is an information requirement in Annex VII to REACH (Section 9.2.1.1.).

You have provided the following information:

i. a key study, publication (1982, Snider & Manning);
ii. a supporting study, from database (2016, U. S. National Library of Medicine, and 2017, SRC PhysProp database);
iii. a supporting study, publication (1991, Mueller *et al*.);
iv. a supporting study, secondary source (1978, P.C. Singer *et al*.);
v. a supporting study according to OECD TG 301 C, publication (1988, Kondo *et al*.)

We have assessed this information and identified the following issues:

A. Although you do not explicitly claim an adaptation, ECHA understands that the studies (i) to (iv) were submitted in order to meet the required information by way of adaptation under Annex, Section XI 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular:
   - Adequacy for the purpose of classification and labelling and/or risk assessment;
   - Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
   - Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3).

OECD TG 301 or 310 are the preferred guideline to fulfil this information requirement. The OECD TG 301 requires that the following specifications must be met:

- the ultimate aerobic biodegradation (as measured by parameters such as DOC removal, CO_2 production and oxygen uptake) of the test material under low inoculum concentration is measured at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation;
- The test duration is normally 28 days. The duration of the test may only be shortened if the biodegradation curve has reached a plateau for at least three consecutive determinations;
- Determination is carried out at least in duplicate;
- The dilution water is checked by DOC analysis prior to use;

- The test temperature is 22°C ± 1°C (OECD 301A, 301B, 301D, 301E, 301F) or 25°C ± 1°C (OECD 301C);
- The source of the inoculum, its concentration in the test and any pre-conditioning treatment are reported;
- The inoculum is not be pre-adapted to the test material;
- A reference compound, which meets the criteria for ready biodegradability, is tested in parallel in all tests. Appropriate reference compounds include aniline (freshly distilled), sodium acetate and sodium benzoate;
- The results of measurements at each sampling point in each replicate is reported in a tabular form;
- validity criteria specified in the test guideline must be met:
  - The degradation of the reference compound has reached the pass level by day 14;
  - The difference of extremes of replicate values of the removal of the test material at the plateau, at the end of the test or, if appropriate, at the end of the 10-d window is ≤ 20%;
  - In the toxicity control, the degradation of the reference substance has reached ≥ 35% (based on DOC) or ≥ 25% (based on ThOD or $ThCO_2$) by day 14;
  - The test material is the sole source of added organic carbon;

Your registration dossier provides information from secondary literature and publications which do not follow OECD TG 301 or 310 (studies i-iv), showing the following:

- in studies (i) and (iii) the % of removal was determined by gas chromatography/mass spectrometry. Thus, studies (i) and (iii) cannot be considered to provide information on ultimate aerobic biodegradation;
- The test duration is 4 days for the key study (i), 5 days for study (ii), 14 days for study (iii), 20 days for study (iv). There is no information provided if the biodegradation curve has reached a plateau for at least three consecutive determinations;
- Determination is reported to be carried out in duplicate only in studies (iii) and (iv), and there is no information on replication for the studies (i), (ii);
- The dilution water is not checked by DOC analysis prior to use by any study;
- The test temperature is not reported for study (i), is 20°C in study (ii), 30°C in study (iii), and 18°C in study (iv);
- The source of the inoculum, its concentration in the test and any pre-conditioning treatment are not reported for the key study (i) and study (ii);
- In study (iii) the test inoculum was isolated from surface soil which was freshly obtained from the American Creosote Works site and you indicate that the inoculum was "creosote-adapted" microorganisms;
- You do not report if a reference compound, which meets the criteria for ready biodegradability, is tested in parallel in the tests;
- You do not provide the results of measurements at each sampling point in each replicate in a tabular form;
- For none of the studies you reported if the studies meets the validity criteria.

Based on the above,

- the key parameters of a OECD TG 301 study are not met by the provided studies (i) and (iii), because they did not measure ultimate degradation;
- the study duration in all studies was less than required in OECD TG 301;
- specifications of the OECD TG 301 were not followed, which may influence the reliability of the information. Specifically the inoculum was pre-adapted and the test temperature was higher in study (iii);
- the reporting of the studies is not sufficient to conduct an independent assessment of their reliability. More specifically, information is lacking on source of inoculum and pre-conditioning (studies i-ii), as well as on degaradation of a reference compound and data on degradation in tabular form (studies i-iv), which would allow to verify if the

validity criteria of the OECD TG 301 were met.

For the reasons noted above, the studies do not provide adequate and reliable coverage of the key parameters foreseen to be investigated in a study according to OECD TG 301, and do not have a comparable exposure duration. Therefore, they are not adequate for the purpose of classification and labelling and/or risk assessment.

Therefore, your adaptation is rejected.

B. To fulfil the information requirement, a study must comply with the OECD TG 301 or 310 (Article 13(3) of REACH). For a study according to OECD TG 301, certain requirements must be met, as already listed in point A above.

Your registration dossier provides information from a publication which is claimed to follow OECD TG 301 C (study v), showing the following:

- The test duration is 3 days. There is no information provided if the biodegradation curve has reached a plateau for at least three consecutive determinations;
- there is no information on replication;
- The dilution water is not checked by DOC analysis prior to use;
- The test temperature is 30°;
- You do not report if a reference compound, which meets the criteria for ready biodegradability, is tested in parallel;
- You do not report the results of measurements at each sampling point in each replicate in a tabular form; and
- You do not specify if the study meets the validity criteria.

Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically, study duration was less than 28 days required in the OECD TG 301, and the test temperature was higher than 25°C ± 1°C required in the OECD TG 301C;
- the reporting of the study is not sufficient to conduct an independent assessment of the reliability and if it would fulfil the performance criteria of OECD TG 301. More specifically, information is lacking on degaradation of a reference compound and data on degradation in tabular form, which would allow to verify if the validity criteria were met.

On this basis, the information requirement is not fulfilled.

In your comments to the draft decision, you agree to perform the requested study.

Appendix C: 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[5].

B. Test material

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[6].

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

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 28 July 2020.

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

ECHA took into account your comments and did not amend the request(s).

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[7] 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)[8]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[8]

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[9]
Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

[7] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[8] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[9] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

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.