~~Confidential~~

Helsinki, 18 February 2021

Addressees
Registrant(s) of JS_67786-25-8 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
2 November 2019

Registered substance subject to this decision ("the Substance")
Substance name: Tetrasodium 4,4'-bis[[4-[bis(2-hydroxypropyl)amino]-6-[(4-sulphonatophenyl)amino]-1,3,5-triazin-2-yl]amino]-stilbene-2,2'-disulphonate
EC number: 267-097-1
CAS number: 67786-25-8

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 ***25 August 2021***.

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

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

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

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

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

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

- Appendix entitled "Reasons common to several requests";
- Appendix entitled "Reasons to request information required under Annex VII of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

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

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

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix on Reasons common to several requests

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

You seek to adapt the following standard information requirements by applying weight of evidence approaches in accordance with Annex XI, Section 1.2:

- *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- 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(es) 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.

You have provided summaries in separate endpoint study records for *in vitro* genotoxicity, short-term toxicity testing on aquatic invertebrates and growth inhibition of aquatic plants. In those summaries you briefly present each of the sources of information, describe the results and conclude that this information can be used as WoE to predict the (eco)toxicological properties of the Substance for the above-mentioned endpoints.

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 approach 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 information provided with analogues substances

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

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 (eco)toxicological properties

For (eco)toxicological properties you read-across between the following substances, reported in your dossier and in the comments on the draft decision, as source substances and the Substance as target substance:

Source/analogue	Human health endpoints	Environmental endpoints
disodium 4,4'-bis[6-anilino-[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate, EC No. 224-073-5 (CAS No. 4193-55-9);		Growth inhibition study aquatic plants Short-term toxicity testing on aquatic invertebrates
disodium4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate, (EC: 240-245-2; CAS: 16090-02-1)	*In vitro* gene mutation study in bacteria	Growth inhibition study aquatic plants Short-term toxicity testing on aquatic invertebrates
Hexasodium 2,2'-[vinylenebis[(3-sulphonato-4,1-phenylene)imino(6-phenoxy-1,3,5-triazine-4,2-diyl)imino]]bis(benzene-1,4-disulphonate) (EC: 255-284-0; CAS: 41267-43-0)	*In vitro* gene mutation study in bacteria (added in the comments)	Growth inhibition study aquatic plants (added in the comments) Short-term toxicity testing on aquatic invertebrates(added in the comments)
Tetrasodium 2,2'-ethene-1,2-diylbis[5-({4-[bis(2-	*In vitro* gene mutation study in	Growth inhibition study aquatic plants

[2] ECHA Guidance R.6

hydroxyethyl)amino]-6-[(4-sulfonatophenyl)amino]-1,3,5-triazin-2-yl}amino)benzenesulfonate] (EC: 240-521-2; CAS: 16470-24-9)	bacteria (added in the comments)	Short-term toxicity testing on aquatic invertebrates(added in the comments)
Tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-sulphonatooxy)ethyl]sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate (EC: 241-164-5; CAS: 17095-24-8	*In vitro* gene mutation study in bacteria (added in the comments)	
Potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate (EC 275-031-8; CAS 70942-01-7)	*In vitro* gene mutation study in bacteria	

In your comments to the draft decision you have addressed the deficiency identified in the initial draft decision, as regards absence of justification for use of information on analogue substances, and provided a document entitled "[redacted]. With this document you intend to justify the use of information obtained on the aforementioned analogue 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 (eco)toxicological 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 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 (eco)toxicological properties.

The common deficiencies are set out here, while the specific ones, which also add to the overall conclusion, are set out under Appendix A. sections 2 and 3 below.

Supporting information for (eco)toxicological properties

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.

You have provided the following information to support your hypothesis:
- Structural information on the Substances and analogues
- Information on structural alerts
- Information on physicochemical, degradation and bioaccumulation properties
- Bridging data to compare the ecotoxicological properties of the substances

ECHA has assessed the provided supporting information below and identified the following issues:

1. Predictions for toxicological properties

1.1 Supporting information indicating an impact of structural differences on predictions

According to Annex XI, Section 1.5 there needs to be structural similarity between substances resulting in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties. 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 the Substance and of the source substance(s) are likely to have similar properties.

You have provided target and source substances which have [redacted] as common structural elements . However, the substances have variations in the amino aniline moiety ([redacted]) as well as in the amino alkylderivative moieties ([redacted] or include no amino but a phenyl ether moeity (CAS No. 41267-43-0). In addition, you have identified one source substance CAS No. 17095-24-8 which does not contain the common [redacted] constituents. This source substance also has an azo functional group that is not shared by the target substance.

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

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

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 show presence of nearly similar functional groups, different structural activity amongst the various read across substances is hardly expected. As per the analysis conducted with the OECD (Q)SAR Toolbox v.3.4, it revealed that target and the read across analogues share similar structural alerts*". Furthermore, you state that "*structural alerts for toxicological endpoints are consistent between the target substance and the read-across analogues*" and that "*the overall common alerts in predicted structure activity confirm the hypothesis that target and read across analogues are having similar reactivity towards biological targets*".

The profiles of structural alerts for the analogue substances CAS 16090-02-1, CAS 16470-24-9 and CAS 70942-01-7 and the Substance are consistent. However, the structural alerts for DNA binding and *in vivo* mutagenicity (Micronucleus) or protein binding for chromosomal aberration are different between the Substance and the analogue substances CAS No. 41267-43-0 and 17095-24-8 (either no alert or different structural alerts, pointing at different chemical mechanisms). This is related to the structural differences of the phenyl ether moiety istead of an amino alkylderivative (CAS No. 41267-43-0) and the azo functional groups instead of [redacted] constituents (CAS No. 17095-24-8). These differences in the structural alerts indicate that the substances may have differences in the reactivity. You have not explained why these differences in the alerts profiled do not influence the toxicological properties.

The available set of structural alerts on the target and source analogues CAS No. 41267-43-0 and 17095-24-8 indicates that there may be differences in the reactivity of the substances. Therefore you have not demonstrated and justified that the toxicological properties of the source substances CAS No. 41267-43-0 and 17095-24-8 and of the Substance are likely to be similar despite the observation of these differences.

1.2 Conclusion for prediction of toxicological properties

Therefore, the information from the analogue substances CAS No. 41267-43-0 and 17095-24-8 submitted under your weight of evidence adaptation is not considered reliable. On the other hand, the information on the analogue substances CAS 16090-02-1, CAS 16470-24-9 and CAS 70942-01-7 could be considered as reliable . This information could contribute to the conclusion on *in vitro* gene mutation study in bacteria once this data and the justification for their use as part of a weight of evidence approach as included in your comments is provided in an updated registration dossier.
Additional issues related to weight of evidence are addressed under the corresponding information requirement.

2. Predictions for ecotoxicological properties

2.1 Missing supporting information

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*"[4]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and

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

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

You have provided target and source substances which have [redacted] as common constituents (except for source substance CAS No. 17095-24-8). However, the substances have structural differences as described under point 1.1 above.

You have assessed the impact of the 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 show presence of nearly similar functional groups, different structural activity amongst the various read across substances is hardly expected. As per the analysis conducted with the OECD (Q)SAR Toolbox v.3.4, it revealed that target and the read across analogues share similar structural alerts*".

- Experimental studies

In the read-across justification you argue that the target and source substances have similar ecotoxicity values. 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 analogue substances indicated in the table above:

Study	**Target substance (EC 267-097-1 / 67786-25-8)**	**EC 224-073-5 / CAS 4193-55-9**	**EC 240-245-2 / CAS: 16090-02-1**	**EC 255-284-0/ CAS: 41267-43-0**	**EC 240-521-2 / CAS: 16470-24-9**
Short-term toxicity to invertebrates	- EU method C.2, 48h: EC50>100 mg/L (nominal)	- method not specified 48h: EC50>100 mg/L (nominal)	- OECD TG 202, 48h: EC50>50 mg/L (nominal)	- OECD TG 202, 48h: EC50>100 mg/L (measured)	- OECD TG 202, 48h: EC50>1000 mg/L (nominal)
Toxicity to algae		- OECD TG 201, 72h: NOEC <100 mg/L and EC50 >100 mg/L (measured)	- OECD TG 201, 72h: EC50 > 100 mg/L (nominal)	- OECD TG 201, 72h: EC50 > 100 mg/L (nominal)	- OECD TG 201, 96h: EC50 > 1000 mg/L (nominal)

Furthermore, in your comments to the draft decision you reported that "acute toxicity values

(L(E)C50) for the three trophic levels are > 100 mg/L" and that, based on the available chronic studies for the source substances, for long-term toxicity to aquatic invertebrates the 21d NOEC values are in the range 0.75 to 17 mg/L and for long-term toxicity to fish the 14d NOEC values are in the range 14 to > 859 mg/L and 28d NOEC value is 10 mg/L.

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

- 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, this information does not confirm, on its own, that the Substance and the source substances have similar (eco)toxicological properties such as aquatic toxicity (growth inhibition of algae, mobility of *Daphnia*). In fact, the complexity of the systemic interactions and the large number of targets/mechanisms associated with those broad areas of toxicity is 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 your 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 toxicokinetics and 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.

- Experimental studies

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

Regarding algae and short-term invertebrate data, as described in the appendices below (sections A.3 and A.2, respectively), the studies are not considered reliable and therefore they cannot be used to compare the ecotoxicological properties of the substances.

Regarding the information on short-term toxicity on fish and on long-term toxicity on aquatic invertebrates and fish, the studies are not provided in the dossier nor in the comments to the draft decision, thus their reliability cannot be assessed.

On the basis of the above, based on the information provided no reliable comparison of the properties of the Substance and the analogues can be made.

Overall, the data set reported in your dossier and in your comments to the draft decision does not include relevant, reliable and adequate information for the Substance and of the source substance(s) to support your your claim of similarity in ecotoxicological properties.

2.2 Conclusion for prediction of ecotoxicological properties

Based on the information in the dossier and provided in the comments, the information from the analogue 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 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 a standard information requirement in Annex VII to REACH.

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In support of your adaptation, you have provided two bacterial reverse mutation studies, similar to OECD TG 471:

(i) Seifried 2006, conducted with analogue substance disodium4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate, EC no 240-245-2 (CAS 16090-02-1);
(ii) OECD SIDS 2005, conducted with analogue substance potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate, EC No. 275-031-8 (CAS No. 70942-01-7).

In your comments to the draft decision you have provided additional studies in support of your adaptation:

(iii) Japan Existing Chemical Database (JECDB), conducted with analogue substance Hexasodium 2,2'-[vinylenebis[(3-sulphonato-4,1-phenylene)imino(6-phenoxy-1,3,5-triazine-4,2-diyl)imino]]bis(benzene-1,4-disulphonate), EC no 255-284-0 (CAS 41267-43-0)
(iv) Bakshi and Sharma 2003, conducted with analogue substance Tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]phenyl]azo] naphthalene-2,7-disulphonate, EC no 241-164-5, (CAS 17095-24-8)
(v) two OECD SIDS studies, conducted with analogue substance Tetrasodium 2,2'-ethene-1,2-diylbis[5-({4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfonatophenyl)amino]-1,3,5-triazin-2-yl}amino)benzenesulfonate], EC no 240-521-2 (CAS 16470-24-9).

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude that the substance does not induce gene mutations in bacteria.

As explained under 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 8.4.1 at Annex VII includes similar information that is produced by the OECD TG 471. It includes:

- Detection and quantification of gene mutations (base pairs, substitution or frame shift) in cultured bacteria including data on the number of revertant colonies; and
- Data provided on 5 bacterial 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 sources of information (i) (Seifried 2006) and (v) (OECD SIDS), as well as (iv) (Bakshi and Sharma 2003) provide partially relevant information, as each of these sources is missing information on detection and quantification of gene mutation, respectively, in a bacterial strain of S. typhimurium TA102, E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101)), or in a bacterial strain of TA1537 or TA97a or TA97.

The sources of information (ii) (OECDS SIDS 2005) and (iii) (JECDB) provide all relevant information on detection and quantification of gene mutation in the five relevant bacterial strains (TA 98, TA 100, TA1535, TA1537 and E. coli WP2 uvr A).

The reliability of the sources of information (iii) and (iv) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests and therefore they cannot contribute to the conclusion on *in vitro* gene mutation study in bacteria .
As regards reliability of the sources of information (i), (ii) and (v), based on the information provided in the comments, as explained under Appendix on Reasons common to several requests, these sources could be considered as reliable and contribute to the conclusion on *in vitro* gene mutation study in bacteria once these additional information is provided in an updated registration dossier.

Conclusion

Based on the information provided in the dossier and in the comments, ECHA considers that, even though some sources of information cannot reliably contribute to the weight of evidence or only partly cover the required information for the reasons explained above, the set of information for the analogue substances (i), (ii) and (v) taken together could allow the conclusion whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an *in vitro* gene mutation study in bacteria. However, as the additional information provided in your comments is currently not available in your registration dossier, the adaptation cannot be considered yet as valid and the data gap remains. You should therefore submit this information in an updated registration dossier by the deadline set out in the decision.

Information on the study design

To fulfil the information requirement for the Substance, if the data gap is not addressed by updating your dossier in line with your comments, the *in vitro* gene mutation study in bacteria (OECD TG 471) must be performed.

2. Short-term toxicity testing on aquatic invertebrates

Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.) is a standard information requirement in Annex VII to REACH.

You have adapted the standard information requirements mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In support of your adaptation, you have provided the following study records:

<u>With the Substance:</u>

i. Aquatic invertebrates, acute toxicity test (according to EU Method C.2, no GLP, secondary source (U.S. Environmental Protection Agency, 2005 and 2018))

With analogue substance(s):

ii. Aquatic invertebrates, acute toxicity test (according to OECD TG 202, GLP not specified, authoritative database (National Institute of Technology and Evaluation, 2019)) with EC No. 240-245-2
iii. "*Determination of short term toxicity of test material on the growth of daphnia magna.*" (TG and GLP not specified, secondary source (United Nations Environmental Programme (UNEP), 2005)) with EC No. 224-073-5

In your comments to the draft decision, you have additionally provided, in support of your adaptation, the following study records:

iv. Aquatic invertebrates, acute toxicity test (according to OECD TG 202, GLP not specified, authoritative database (National Institute of Technology and Evaluation, 2019)) with EC No. 255-284-0
v. Aquatic invertebrates, acute toxicity test (according to OECD TG 202, GLP not specified, authoritative database (National Institute of Technology and Evaluation, 2019)) with EC No. 240-521-2

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude on the toxicity to aquatic invertebrates.

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. Therefore the following requirements must be met: the concentration of the test material leading to the immobilisation of 50% of daphnids at the end of the test is estimated.

All sources of information (i, ii, iii, iv and v) provide relevant information on concentration of the test material leading to the immobilisation of 50% of daphnids. However, these sources of information have the following deficiencies affecting their reliability.

The reliability of source of information (ii), (iii), (iv) and (v) are significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests.

In addition, the reliability of source of information (i), (ii), (iii), (iv) and (v) is also affected by the following issue:

Testing in accordance with OECD TG 202 requires that the following specifications/conditions must be met:

- Use of a reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range, when available.

- 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.

In your dossier you have provided the following information regarding sources of information (i), (ii), (iii), (iv) and (v):

- For studies (i) and (iii) no analytical monitoring of exposure was conducted and for studies (ii) and (v) it is not specified if analytical monitoring of exposure was conducted. Therefore you have provided no evidence that exposure concentrations were maintained within 20 % of the nominal concentration throughout the test.
- For study (iv) you specify that the analytical monitoring was performed and the results are reported based on nominal concentrations. However, you have not provided performance parameters of the analytical method (e.g. LOD, LOQ, recovery). Furthermore, although you have specified that measured exposure concentrations were maintained within ± 20 % of the nominal concentration throughout the test, you have not provided any evidence to support this (e.g. lack of adequate information on analytical method and results of analytical determinations, as explained above). Therefore you have provided no evidence that results can be expressed based on nominal concentrations.Tabulated data on the number of immobilised daphnids after 24 and 48 hours for each treatment group and control are not reported for studies (i), (ii), (iv) and (v).

Without performance of analytical monitoring it is not possible to conclude if the daphnids were exposed to the Substance or analogue substance nor what was the real exposure concentration. In your comments to the draft decision you have provided measured concentration for study (iv), however you have not provided performance parameters of the analytical method in order to allow an independent assessment of the information.

Furthermore, in the absence of data related to daphnids immobilization the validity of the studies cannot be confirmed. Therefore, without these critical information 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 the immobilisation of 50% of daphnids.

Conclusion

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 aquatic invertebrates acute toxicity test. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

Information on the study design

Available information on the Substance points to high adsorptive and ionisable properties.[5] The Substance is therefore considered difficult to test. OECD TG 202 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve

[5] According to disseminated data for the Substance available at https://echa.europa.eu/home

and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 202. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

3. Growth inhibition study aquatic plants

Growth inhibition study aquatic plants (Annex VII, Section 9.1.2) is a standard information requirement in Annex VII to REACH.

You have adapted the standard information requirements mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In support of your adaptation, you have provided the following study records with analogue substances:

i. Algae, growth inhibition test (according to OECD TG 201, no GLP, [redacted] 2019) with EC No. 240-245-2
ii. Algae, growth inhibition test (according to OECD TG 201, GLP not specified, secondaty source (United States environment protection agency (USEPA), 2017)) with EC No. 240-521-2
iii. Algae, growth inhibition test (according to OECD TG 201 / EU Method C.3, GLP not specified, secondary source (United States environment protection agency (USEPA), 2017)) with EC No. 224-073-5

In your comments to the draft decision, you have additionally provided, in support of your adaptation, the following study records:

iv. Algae, growth inhibition test (according to OECD TG 201, GLP not specified) with EC No. 255-284-0

Based on the presented sources of information, you argue that the available data gives sufficient information to conlude on the toxicity to algae.

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) and (iv) 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.

The reliability of source of information (i), (ii), (iii) and (iv) are significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests.

In addition, the reliability of source of information (i), (ii), (iii) and (iv) is also affected by the following issue:

Testing in accordance with OECD TG 201 requires that the following specifications/conditions must be met:

- Use of a reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range, when available;
- The results can be based on nominal or measured initial concentration only if evidence is provided that the concentration of the test material has been maintained within 20 % of the nominal or measured initial concentration throughout the test;
- The results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form.

In your dossier you have provided the following information regarding sources of information (i), (ii), (iii) and (iv):

- For study (iii) no analytical monitoring of exposure was conducted and for study (ii) it is not specified if analytical monitoring of exposure was conducted. Therefore you have provided no evidence for both studies that exposure concentrations were maintained within 20 % of the nominal concentration throughout the test.

- For study (i) and (iv), you have specified that the analytical monitoring was performed and the results are reported based on nominal concentrations. However, you have not provided performance parameters of the analytical method (e.g. LOD, LOQ, recovery). Furthermore, although you have specified that measured exposure concentrations were maintained within ± 20 % of the nominal concentration throughout the test you have not provided any evidence to support this (e.g. lack of adequate information on analytical method and results of analytical determinations, as explained above). Therefore you have provided no evidence that results can be expressed based on nominal concentrations.

You have not provided the data related to the biomass for any of the studies. Without performance of analytical monitoring it is not possible to conclude if the algae were exposed to the Substance or analogue substance nor what was the real exposure concentration. In your comments to the draft decision you have provided measured concentrations for studies (i) and (iv), however you have not provided performance parameters of the analytical method in order to allow an independent assessment of the information.

Furthermore, in the absence of data related to biomass the validity of the studies cannot be confirmed. Therefore, without these critical information study (i), (ii), (iii) and (iv) cannot be considered as reliable.

Taken together, even though, the sources of information (i), (ii), (iii) and (iv) 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

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 algae growth inhibition study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

Information on the study design

OECD TG 201 specifies that for difficult to test substances OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in '*Information on the study design*' under Section A.2.

Possibility for data sharing for studies not involving vertebrate animals

The jointly submitted registration 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(s) and then make every effort to reach an agreement on the sharing of data and costs[6].

[6] https://echa.europa.eu/regulations/reach/registration/data-sharing

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

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

2. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[7].

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 variation in compositions reported by all members of the joint submission,
   - 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 and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[8].

[7] https://echa.europa.eu/practical-guides
[8] https://echa.europa.eu/manuals

Appendix C: 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 5 December 2019.

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

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

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix D: List of references - ECHA Guidance[9] 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)[10]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[10]

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[11]

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

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

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

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

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

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.