EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 04 June 2021

Addressees
Registrant of JS_16470-24-9 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
9 August 2019

Registered substance subject to this decision ("the Substance")
Substance name: Tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate]
EC number: 240-521-2
CAS number: 16470-24-9

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed in D.1 below by the deadline of ***13 December 2021***, and all other information listed below by the deadline of ***9 June 2023***.

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. Spectral data (Annex VI, Section 2.3.5.);
   - Nuclear magnetic resonance (NMR) or mass spectrum
2. Description of the analytical methods (Annex VI, Section 2.3.7.);
   - Identification and quantification of counter-ion.

B. 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) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102;
2. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201).

C. Information required from all the Registrants subject to Annex VIII of REACH

1. If negative results are obtained in tests performed for the information requirement of Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. then: *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490).

D. Information required from all the Registrants subject to Annex IX of REACH

1. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route (gavage), in rats;

2. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211);

3. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: OECD TG 210).

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

- Appendix entitled "Reasons common to several requests";
- Appendices entitled "Reasons to request information required under Annexes VI to IX 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 Annexes VI, VII, VIII and IX to REACH, for registration at 100-1000 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.

The same study as listed in D.1. has already been requested from other registrants (decision CCH-D-2114450733-50-01/F) with the deadline of 4 January 2021. As only one study is to be generated, the deadline for provision this study by you is set to 6 months from the date of this decision.

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

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

In your comments to the draft decision, you clarify that you are seeking to adapt the following 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.)
- In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
- Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

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 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.

Your weight of evidence approach as provided in the comments has deficiencies that are common to all information requirements under consideration and also deficiencies that are specific for these information requirements individually.

A common deficiency is set out here, while the specific ones are set out under the information requirement concerned in the Appendices B and C.

Reliability of the provided information with analogue 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 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 (eco)toxicological properties

In your comments to the draft decision you have provided a justification document entitled [redacted]. For (eco)toxicological properties under the the weight of evidence for the endpoints listed above, 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 substance	Human health information requirements	Environmental information requirements
Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate (EC no 240-245-2; CAS no 16090-02-1)	Sub-chronic toxicity *In vitro* gene mutation in mammalian cells	Growth inhibition study aquatic plants Long term toxicity on aquatic invertebrates (added in the comments) Long term toxicity on fish
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
Hexasodium 4,4'-bis(2-phenoxy-4-(2,5-disulfonatoanilino)-1,3,5-triazine-6-ylamino)stilbene-2,2'-disulfonate (EC 255-284-0; CAS No. 41267-43-0)	*In vitro* gene mutation in bacteria (added in the comments)	Growth inhibition study aquatic plants (added in the comments) Long term toxicity on aquatic invertebrates (added in the comments)
Hexasodium 2,2'-[vinylenebis[(3-sulphonato-4,1-phenylene)imino[6-(diethylamino)-1,3,5-triazine-4,2-diyl]imino]]bis(benzene1,4-	Sub-chronic toxicity (added in the comments)	

[2] ECHA Guidance R.6: QSARs and grouping of Chemicals

disulphonate) (EC no 255-217-5; CAS no 41098-56-0)		
Tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl]sulphonyl] phenyl] azo] naphthalene-2,7-disulphonate (CAS no 17095-24-8)		Long term toxicity on fish (28 days-study 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 (CAS no 70942-01-7; EC no 275-031-8)	*In vitro* gene mutation in bacteria (added in the comments)	
C.I. Fluorescent Brightener 1 (CAS no 15339-39-6)	*In vitro* gene mutation in mammalian cells (added in the comments)	

You indicated that the "*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 further 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*" and indicate that you have selected the 'Scenario 2'' for the analogue approach to justify the read across analogues.

Therefore, ECHA understands that you read-across between CAS no 4193-55-9, CAS no 41267-43-0, CAS no 16090-02-1, CAS no 70942-01-7, CAS no 41098-56-0, and CAS no 17095-24-8 as source substances and the Substance as target substance, and you 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) or based on a worst-case approach.

In addition, in your registration dossier, you have provided information on the in vitro gene mutation in mammalian cells conducted with the source substance tetrasodium 1-acetamido-2-hydroxy-3-(4-((4-sulphonatophenylazo)-7-sulphonato-1-naphthylazo)) naphthalene-4,6-disulphonate (Brilliant black 1), EC no 219-746-5 (CAS No. 2519-30-4).

ECHA notes the following shortcoming with regards to predictions of (eco)toxicological properties based on analogue approach.

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

1.1 Predictions for toxicological properties

1.1.1. Absence of justification for use of information on analogue substance for in vitro gene mutation in mammalian cells

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

For *in vitro* gene mutation in mammalian cells you have provided a source study conducted with source substance CAS No. 2519-30-4 in order to comply with the REACH information requirements. You have not provided documentation as to why this information is reliable and relevant for your Substance to be used as part of weight of evidence adaptation.

In the absence of such documentation, ECHA cannot verify that the properties of your Substance can be predicted from the data on the source substance(s). Therefore, the information from the analogue substances submitted under your weight of evidence adaptation is not considered reliable.

1.1.2. Missing supporting information for in vitro gene mutation

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.

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 the source substances.

In the justification document provided in your comments, you have provided outcomes of the OECD QSAR Toolbox structural alerts for the target and the source substances and indicate that "*According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analogue.*".

The structural profilers for the genetic toxicity showed consistent lack of structural alerts for the target and the source substances. This information could contribute to the conclusion on in vitro gene mutation study in bacteria and in mammalian cells.
However, it is only referred to and discussed in your comments and is not yet included in your registration dossier.

1.1.3. Read-across hypothesis contradicted by existing data for systemic toxicity following repeated exposure

[3] ECHA Guidance R.6

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances. The ECHA Guidance[Error! Bookmark not defined.] indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". 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.

The observation of differences in the toxicological properties between the source substance(s) and the Substance would contradict the hypothesis that the properties of the Substance can be predicted from the data on the source substances. An explanation why such differences do not affect the read-across hypothesis needs to be provided and supported by scientific evidence.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar target and source substances cause the same type of effect(s). Furthermore, for the repeat dose oral toxicity you state that "*overall, it can be inferred that the optical brighteners are comparatively non-toxic when exposed to standard test organisms.*".

In the dossier and in your comments you provided following sources for information for the repeated dose toxicity:

- OECD QSAR Toolbox v 3.4 structural alerts based on repeated dose (HESS) profiler showing no structural alerts for repeated dose toxicity
- Dietary combined chronic toxicity/carcinogenicity study conducted with the Substance showing significant increases in GOT and GPT at 10000 ppm (males) and of GPT at ≥1000 ppm (females) as well as increase in the kidney weights at 10000 ppm of test substance in diet (about 520 and 710 mg/kg bw/d in males and females, respectively).
- Oral gavage 10 weeks repeated dose toxicity study conducted with the Substance showing no signs of toxicity at 500 mg/kg bw/d, the highest dose tested.
- Dietary combined chronic toxicity/carcinogenicity study conducted with the source substance EC no 240-245-2 showing increased absolute liver and kidney weights in males and increased absolute ovary weights in females at the highest dose tested (524 and 709 mg/kg bw/d in males and females, respectively).
- Oral agavage sub-acute (28-day) repeated dose toxicity conducted with the source substance EC no 240-245-2 showing increased relative heart weights in females at 1000 mg/kg bw/d, the highest dose tested. In addition, non-dose dependent increase in relative kidney weight was reported in male rats.
- Dietary sub-chronic (90-day) repeated dose toxicity study conducted with the source substance EC no 255-217-5 showing reduced body weights, reduction in (relative) testis and ovary weights as well as histopathology in testes (atrophy) and kidneys (necrosis swelling, vacuolisation and granular deterioration of tubular cells in the renal cortex) at 10000 ppm of the test substance in diet.

The available set of information on the Substance and source substances indicates that there may be differences in the properties of the substances leading to differences in the type of effects observed following repeated systemic exposures. In particular,

- changes in ovary weights were reported in the chronic and sub-chronic dietary studies with the source substances but not in the chronic dietary study with the target

substance.
- effects on testes and histopathological findings in kidneys were reported only for the source substance EC no 255-217-5, but not for the source substance EC no 240-245-2 or the target substance
- while severe testicular and nephrotoxicity were reported for the source substance EC no 255-217-5, the repeat dose toxicity profiler (HESS) did not identify structural alerts for repeat dose toxicity.

These findings contradict your read-across hypothesis whereby the structurally similar target and source substances cause the same type of effect(s). Most importantly, the studies suggest that the Substance and the source substances may have differences in the type of effects observed following repeted systemic exposures. Therefore you have not demonstrated and justified that the properties of the source substance(s) and of the Substance are likely to be similar despite the observation of these differences.

In the light of the identified differences in the properties of the substances following repeated exposures, the information on the analogue substances cannot contribute to deriving reliable conclusions on the repeated dose toxicity (90-day) properties of the Substance.

1.1.4. Conclusion for prediction of toxicological properties

Based on the information in the dossier and provided in the comments, the information provided with the source substance CAS No. 2519-30-4 on *in vitro* gene mutation in mammalian cells and the information related to the systemic toxicity following repeated exposure from the analogue substances submitted under your weight of evidence adaptation is not considered reliable.

On the other hand, the information on the analogue substances provided for in vitro gene mutation in bacteria and in mammalian cells could be considered as reliable. This information could contribute to the conclusion on *in vitro* gene mutation study in bacteria and in mammalian cells 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.

1.2 Predictions for ecotoxicological properties

1.2.1 Missing of 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 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.

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

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 structural elements. In addition, you have identified one source substance CAS No. 17095-24-8 which does not contain the common [redacted] constituents. With respect to this substance you argue that it shares *"functional group like aryl and sodium sulfonate group common with the target substance"*. However, 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 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 for aquatic toxicity on the Substance and the analogue substances indicated in the table above:

Study	**Target substance (EC 240-521-2/ CAS 16470-24-9)**	**EC 240-245-2 / CAS: 16090-02-1**	**EC 255-284-0/ CAS: 41267-43-0**	**EC 224-073-5 / CAS: 4193-55-9**	**EC 241-164-5 / CAS: 17095-24-8**
Short-term toxicity to fish	Six studies (under weight of eveidence), including one study according to OECD TG 203, one study according to Directive 84/449/EEC, C.1 , and 4 studies (method not specified). EC50>100 mg/L				

Short-term toxicity to invertebrates	- Study (i), OECD TG 202 . EC50-48h >= 1000 mg/L - Study (ii), Directive 92/69/EEC, C.2: EC50=113 mg/L . - Study (iii), OECD TG 202 . EC50-24h >= 1000 mg/				
Toxicity to algae	- OECD TG 201, 72h: EC50 > 1000 mg/L. (nominal)	- OECD TG 201, 72h: EC50 > 100 mg/L (nominal) or > 112 mg/L measured)	- OECD TG 201, 72h: EC50 > 100 mg/L (nominal) or > 23 mg/L (measured)	- OECD TG 201, 72h: NOEC <100 mg/L and EC50 >100 mg/L (measured)	
Long-term toxicity to invertebrates	- OECD TG 211, 21d: NOEC = > 31.6 mg/L to < 100 mg/L. (measured)	- OECD TG 202, 21d: NOEC = 0.75 mg/L and EC50 => 2.4 mg/L (measured)	- OECD TG 211, 21d: NOEC = 17 mg/L and EC50 =26.7 mg/L (measured)		
Long-term toxicity to fish	- UBA procedural proposal "Extended Toxicity", 14d: NOEC=> 859 mg/L (measured)	-Study (ii) - OECD TG 204, 14d: NOEC= 61.8 mg/L and LC50=165mg/L (measured)			- Study OECD TG 204, 28d: NOEC = 10 mg/L (nominal) - OECD TG 204, 14d: LC50 > 100 mg/L (nominal)

We have assessed this information and identified the following issues:

- 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 ecotoxicological properties such as aquatic toxicity (growth inhibition of algae, reproductive toxicity to Daphnia, develpmental toxicity to fish). In fact, the complexity of the aquatictoxicity 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 toxicokinetics behaviour in aquatic

compartment, this information do 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 long-term invertebrate and fish data, as described in the appendices below (sections B.2, D.3 and D.4, respectively), the studies are not considered reliable and therefore they cannot be used to compare the ecotoxicological properties of the substances.

  - Regarding the short-term studies on aquatic toxicity. You have provided aquatic toxicity studies on invertebrate and fish on the Substance.

    For invertebrate, as listed in the table above you have provided three studies. Except for study (ii), for which you have indicated the analytical method, for study (i) and (iii) you have not provided any information on the analytical method nor on the performance parameters, and that for any of the studies. Regarding the number of immobilised daphnids and dissolved oxygen concentrations throughout the test, you have not provided any information for study (i) and (ii). For study iii you have only reported information on the immobility however the test duration was 24h instead of 48 h, which might impact the sensitivity of the test and its reliability.

    For aquatic toxicity on fish, as specified in the table above you have provided six studies including one that was conducted according to OECD 203 (i.e. study i) and another one that was conducted according to Directive 84/449/EEC, C.1 (i.e study ii). Regarding the other studies (i.e. study iii, iv, v and vi) no information has been provided regarding the tested method.
    For study (i) you have provided information on mortality in the control and on the content of the oxygen. However no information was provided for any of the other studies (i.e. study ii, iii, iv,v and vi)
    For study (ii), you have performed the analytical monitoring, however no information was provided for any of the other studies (i.e. study i, iii,iv,v and vi).

    Furthermore, we note that that for short term aquatic toxicity, you have not provided any information on the analogues (EC 240-245-2, 255-284-0, 224-073-5 and 241-164-5). Therefore, no comparison can be made between the Substance and the analogues to support your claim of similarity in ecotoxicological properties.

  Based on the above, the aquatic toxicity studies on the Substance are not considered reliable and therefore they cannot be used to compare the ecotoxicological properties of the substances

1.2.2 Conclusion for prediction of eco toxicological properties

Therefore, 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 for the requests to comply with Annex VI of REACH

Under Article 10(a)(ii) of REACH, each technical dossier must contain information on the identity of the substance as specified in Annex VI, Section 2. In accordance with Annex VI, Section 2 the information provided has to be sufficient to enable each substance to be identified.

1. **Nuclear magnetic resonance (NMR) or mass spectrum (Annex VI, Section 2.3.5)**

   - Nuclear magnetic resonance (NMR) or mass spectrum

Spectral data are a formal information requirement as laid down in Annex VI, Section 2.3.5 of REACH. Adequate information needs to be present in the technical dossier to meet this information requirement.

The registration dossier contains two extracts from a nuclear magnetic resonance (NMR) spectrum and a mass spectrum. The two extracts of the NMR spectrum covers two chemical shift ranges, namely 2.2 ppm to 5.4 ppm and 7.2 ppm to 9.6 ppm. The NMR extracts are incomplete in that they do not show the whole NMR spectrum. Further, the signals observed in the extracts shown do not correspond to the molecular structural features of the registered substance. Likewise, in the mass spectrum, the *m/z* values of the signals observed are not consistent with the proposed molecular structure for the main constituent as to confirm the identity of the substance.

Consequently, the information requirement under Annex VI, Section 2.3.5. is not fulfilled and it is not possible to verify the identity of the substance.

Therefore, you are requested to submit an NMR spectrum, or alternatively, a mass spectrum, generated on your imported/manufactured substance. The resolution and coverage of the NMR spectrum shall be such that all peaks corresponding to the registered substance are displayed and well resolved, to allow verification of the identity of the substance. In addition, the description of the analytical methods used for recording the spectrum must be specified in the dossier in such details to allow the methods to be reproduced in line with the requirements under Annex VI Section 2.3.7 of REACH. The description of the analytical methods must include details of the experimental protocol followed, any calculation made, and the results obtained.

If it is not technically possible to provide such spectra, a scientifically based justification should be given in section 1.4 of your dossier. It should be noted that any justification for waiving will be assessed for its validity and may not be accepted.

The requested spectral data (and relative method descriptions) shall be attached in section 1.4 of your IUCLID dossier.

In your comments to draft decision, you have indicated that "*As soon as we receive the NMR spectra and ICP-OES analysis report from the CRO, we will update the dossier with the required details on priority*". Based on this, ECHA understands that you agree to provide the information requested.

2. **Description of the analytical methods (Annex VI, Section 2.3.7.)**

   - Identification and quantification of counter-ion

According to Annex VI, section 2.3.7 of the REACH Regulation, a registration dossier shall report a description of the analytical methods or the appropriate bibliographic references for the identification of the substance and where appropriate for the identification of impurities and additives. The reporting shall be given in sufficient detail that the methods may be reproduced.

You have identified your substance as "tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate]", which indicates that sodium ions are present in your substance. However, you have not reported the description of the method(s) used to identify and quantify the sodium ions.

Therefore, the information submitted is not sufficient to verify the substance identity reported in sections 1.1 and 1.2 of your dossier.

Consequently, you are requested to report the description of the method(s) used to identify and quantify the sodium ions. The description of the method(s) shall be given in such detail that the method(s) may be reproduced and shall include details of the experimental protocol, any calculations made and the results obtained. The information shall be sufficient to verify the identity of the substance as reported in your dossier.

The requested information shall be attached in section 1.4 of your IUCLID dossier.

In your comments to draft decision, you have indicated that "*As soon as we receive the NMR spectra and ICP-OES analysis report from the CRO, we will update the dossier with the required details on priority*". Based on this, ECHA understands that you agree to provide the information requested.

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 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 the registration dossier you have provided two (2) bacterial reverse mutation studies (similar OECD TG 471; US EPA, 1979) conducted with the Substance (sources of information i and ii).

In your comments to the draft decision, you have provided following sources of information:

iii. bacterial reverse mutation study (OECD TG 471) conducted with the source substance CAS No. 41267-43-0
iv. bacterial reverse mutation study (OECD TG 471) conducted with the source substance CAS No. 70942-01-7

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

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 8.4.1 at Annex VII include:

- 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 and ii) provide information on detection and quantification of gene mutation in 4 bacterial strains (TA1535, TA1537, TA 100 and TA 98). However, the sources of information do not inform on detection and quantification of gene mutation in the 5th bacterial strain (either S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101)). These studies provide partly relevant information on the Substance. The studies are also considered reliable.

Studies (iii and iv) provide relevant information on *in vitro* gene mutation in bacteria. As regards reliability of the sources of information (iii) and (iv), 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 provided and justified in the comments is also included 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 only partly cover the required information for the reasons explained above, the set of information for the Substance and the analogue substances 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, the *in vitro* gene mutation study in bacteria (OECD TG 471) should be performed using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

Possibility for data sharing
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[5].

2. 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.

In your comments to the draft decision you have adapted the standard information requirements mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In your registration dossier you have provided the following studies:

i. Key study, OECD TG 201, (handbook and secondary sources 2008), conducted with the Substance.

ii. Supporting study, OECD TG 201 ([redacted] 2019), conducted with analogue substance (EC no 240-245-2 / CAS No. 16090-02-1).

iii. Supporting study, OECD TG 201 (HPVIS 2019), conducted with analogue substance (EC 224-073-5 / CAS No. 4193-55-9).

In your comments you have provided additionally the following study :

iv. Source study, OECD TG 201, conducted with analogue substance (EC 255-284-0 /CAS No. 41267-43-0).

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

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

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 (ii), (iii) and (iv) is 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 and in your comments to the draft decision you have provided the following information regarding sources of information (i),(ii), (iii) and (iv):

- For study (i) and (iii) no 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 (ii) and (iv), you have specified that the analytical monitoring was performed and the results are reported based on nominal and measured concentrations, respectively. However, you have not provided performance parameters of the analytical method (e.g. LOD, LOQ, recovery) for any of the studies. Furthermore, for study (ii) 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.

- For study (i) you have not provided the data related to the biomass . For study (ii),

(iii) and (iv), in your comments you have provided the initial cell density of the culture (10000 cells/ml , 100000 cell/ml, and 5000 cells/ml, respectively).

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 values of measured concentration for each dose level for study (iv), however not indicating if those measurements reported were taken at 0-h, 24-h, 48-h or at the end of the test. For the study (ii) you did not provide the information on measured exposure concentrations. In addition to these uncertainties, you have not provided performance parameters of the analytical method for neither studies (ii) and (iv). In conclusion, an independent assessment of the information with regards to analytical monitoring is not possible.

Furthermore, regarding the biomass data, as indicated above no data has been provided for study (i) and only the initial density was provided for study (ii), (iii) and (iv). Therefore, the required results of algal biomass determined in each flask at least dailywere not provided. In the absence of these data the validity of the studies cannot be confirmed.

Due to the above deficiencies, the studies (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.

Appendix C: Reasons to request information required under Annex VIII of REACH

1. *In vitro* gene mutation study in mammalian cells

An *in vitro* gene mutation study in mammalian cells is a standard information requirement in Annex VIII to REACH in case of a negative result in the *in vitro* gene mutation test in bacteria and the *in vitro* cytogenicity test.

Your dossier contains (i) a negative result for *in vitro* cytogenicity study in mammalian cells (Annex VII, Section 8.4.2.), and (ii) inadequate data for *in vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.) which is currently rejected for reasons provided in section B.1.

Therefore, the result of the request for information in section B.1 will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

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

In the registration dossier you have provided two (2) *in vitro* mouse lymphoma mutagenicity assays (similar to OECD TG 476) conducted with the following analogue substances (Seifried et al., 2006):

(i) disodium4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate, EC no 240-245-2 (CAS No. 16090-02-1);
(ii) tetrasodium 1-acetamido-2-hydroxy-3-(4-((4-sulphonatophenylazo)-7-sulphonato-1-naphthylazo))naphthalene-4,6-disulphonate (Brilliant black 1), EC no 219-746-5 (CAS No. 2519-30-4).

In your comments to the draft decision, you have provided additionally the following study:
(iii) gene mutation study in mammalian cells conducted with the source substances CAS No. 15339-39-6

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

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 8.4.3 at Annex VIII includes similar information that is produced by the OECD TG 476/490 and OECD TG 488 This includes:

- Detection and quantification of gene mutations (point mutations, frame-shift mutations, small deletions, etc.) including data on the frequency of mutant colonies in cultured mammalian cells (*in vitro*) or mutant frequency for each tissue in mammals (*in vivo*).

The sources of information (i-ii) provide relevant information on detection and quantification of gene mutation in cultured mammalian cells. The source of information (iii), based on the

executive summary included in your comments appears to provide relevant information on detection and quantification of gene mutation in cultured mammalian cells.

The reliability of the source of information (ii) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests and therefore it cannot contribute to the conclusion on *in vitro* gene mutation study in mammalian cells.

As regards reliability of the sources of information (i) and (iii), 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 mammalian cells once these additional information provided and justified in the comments is also included in an updated registration dossier.

Conclusion

Based on the information provided in the dossier and in the comments, ECHA considers that the set of information for the Substance and the analogue substances 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 mammalian cells. 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, both the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) and the thymidine kinase gene (OECD TG 490) are considered suitable.

Possibility for data sharing

The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[5].

Appendix D: Reasons to request information required under Annex IX of REACH

1. Sub-chronic toxicity study (90-day)

A Sub-chronic toxicity study (90 day) is a standard information requirement in Annex IX to REACH.

In your comments to the draft decision, you indicated that you have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. of REACH.

In the registration dossier, you have provided the following studies:

Conducted with the Substance:

i. Non-GLP compliant combined chronic toxicity / carcinogenicity dietary study similar to OECD TG 453 (Key study, US EPA, 1978; US EPA HPVIS, 2006)
ii. Non-GLP compliant sub-chronic (90-day) repeated dose toxicity study (Supporting study; Kimmerle and Lorke, 1967; US EPA HPVIS, 2006)

Conducted with the source substance EC no 240-245-2:

iii. Non-GLP combined chronic toxicity / carcinogenicity dietary study similar to OECD TG 453 (Supporting study; US EPA, 1978)
iv. Subacute (28-day) repeated dose toxicity study (OECD TG 407; GLP not specified) (Supporting study; HPWIS database, 1991; US EPA HPVIS, 2006)

In your comments to the draft decision you have provided information on following study conducted with the source substance EC No. 255-217-5 (CAS no 41098-56-0):

v. A 90-day repeat dose toxicity (guideline not specified) ([redacted] 1969)

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude on the sub-chronic toxicity (90-day) and you request ECHA to remove the request from the decision.

As explained under Appendix on Reasons common to several requests, section 1, 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 8.6.2 at Annex IX includes, at general level, information on systemic toxicity in intact, non-pregnant and young adult males and females from: in-life observations, 2) blood chemistry, 3) organ and tissue toxicity (including histopathology to address relevant physiological systems such as (circulatory, digestive/excretory, endocrine, immune, integumentary, musculoskeletal, nervous, renal/urinary system, reproductive, and respiratory).

This information is covered by information similar to the information obtained from the OECD TG 408.

The source of information (ii) provide relevant information on in-life observations, but does not include full evaluation of blood chemistry or histopathological evaluation of organs and tissues. Thus, the source of information (ii) provides partially relevant information.

The sources of information (i, iii-v) provide information on systemic toxicity, including in-life observations, blood chemistry, organ and tissue toxicity, and therefore cover the relevant information to support the weight of evidence adaptation.

However, these sources of information have deficiencies affecting their contribution to the weight of evidence:

A. *Information from the dietary chronic toxicity/carcinogenicity studies (studies i and iii)*

For chronic toxicity/carcinogenicity studies conducted via dietary route, the OECD TG 453 and the OECD GD 116 on the conduct and design of chronic toxicity and carcinogenicity studies specifies that:

- OECD TG 453; paragraph 31: information should be available on the stability of the test chemical and the homogeneity of dosing solutions or diets (as appropriate) under the conditions of administration (e.g., diet);
- OECD GD 116; paragraph 121: The substance should be stable during the preparation, storage and period of administration of the diet, for example it should not react chemically with dietary constituents, and analytical data must be provided to demonstrate this; and
- OECD GD 116; paragraph 171: The bioavailability of test substance is often very dependent on the matrix it is administered in, e.g., due to the fat content. If this is the feed, there may be an interaction of the test substance with food matrix. The food composition may alter bioaccessibility.

The technical applications of the Substance as fluorescent whitening agent in paper, textile and household detergents is based on their property to bind to organic matter such as cellulose or cotton fibers. As a result of these properties, the Substance may also attach to constituents of the standard diet used in animal testing and/or on the containers and change the proportion of the substance in feed or modify the bioavailability of the substances.

However, you did not provide information on the stability of the test material under the conditions of administration (via diet) or consider the potential interaction of the test substance with food matrix. Therefore, the extent of associations for the Substance with the dietary constituents is currently unknown, and the amount of test item available for absorption, and the actual doses of the Substance that the experimental animals have been exposed may be overestimated.

In your comments to the draft decision, you considered the potential interactions of the test substances with food matrix and bioaccessibility. You indicated that in the 90-day study conducted with the structurally related fluorescent whitening agent CAS number: 41098-56-0 (EC no 255-217-5) concomitant food intake did not inhibit bioaccessibility of the test substance as evident by dose-dependent effects at ≥10000 ppm. In addition, based on a oral gavage study with the analogue substance (EC no 240-245-2; Black et al., 1977) you propose that only little of the Substance may be absorbed when administered via gavage.

However, this information does not provide any further information on the stability of the test material under the conditions of administration, the amount of test item available for absorption in the dietary studies, or the actual doses of the Substance that the experimental animals have been exposed.

To conclude, you have not provided information on the amount of test item available for absorption and the real amount to which the animals' organism was exposed to at the selected

dietary concentration levels. Without additional considerations on this matter, the sources of information (i ,and iii and v) cannot be considered as reliable.

B. Information with the structurally related substances (studies iii-v)

For the reasons explained in the Appendix on Reasons common to several requests, the information on the source substances (studies iii-v) cannot, in the absence of further information, contribute to deriving reliable conclusions in the context of this weight of evidence approach. Therefore, these sources of information do not contribute to the weight of evidence adaptation.

Conclusion

Even though, the sources of information (i, iii-v) as indicated above provide relevant information, their reliability under this weight of evidence is significantly affected due to deficiencies identified in your predictions of toxicological properties of the Substance using source substances (iii-v) or the the route of exposure leading to uncertainty in the findings (i, iii). On the other hand, the source of information (ii) provides only limited information on the organ and tissue toxicity, and there is no reliable information under this weight of evidence adaptation that can contribute to the missing information in this study.

Therefore, 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 sub-chronic toxicity study (90-day).

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

Information on the design of the study to be performed

Referring to the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because there is no evidence that internal exposure would be higher via other routes. The technical applications of the Substance as fluorescent whitening agent in paper, textile and household detergents is based on its property to bind to organic matter such as cellulose or cotton fibers. As a result of these properties, the Substance may also attach to constituents of the standard diet used in animal testing. Therefore, in order to minimise contact of the test material with diet constituents, testing should be done via oral gavage. The schedule described in Appendix E point A.4 must be followed.

Therefore the sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and with oral (gavage) administration of the Substance.

2. Long-term toxicity testing on aquatic invertebrates

Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5) is a standard information requirement in Annex IX to REACH.

In your comments to the draft decision you have adapted the standard information requirements mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In your registration dossier, you have provided the following studies on the Substance :

i. Key study, OECD TG 211 (Karel Verschueren 2008, review article or handbook).

ii. Supporting study, OECD TG 211 (Karel Verschueren 2008, review article or handbook)

In your comments to the draft decision you have provided the following studies:

iii. Source study, OECD TG 202, part 2, conducted with the analogue substance Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate (CAS number: 16090-02-1; EC number: 240-245-2) (Daphnia sp., Reproduction Test, 1993).
iv. Source study, OECD TG 211, conducted with the analogue substabce Hexasodium 4,4'-bis(2-phenoxy-4-(2,5-disulfonatoanilino)-1,3,5-triazine-6-ylamino)stilbene-2,2'-disulfonate (CAS number: 41267-43-0; EC number: 255-284-0)

ECHA has assessed all information provided and identified the following issue(s):

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 9.1.5 at Annex IX includes similar information that is produced by the OECD TG 211. This includes:

1. the reproductive output of Daphnia sp., and
2. the survival of the parent animals during the test, and
3. the time to production of the first brood.

Concerning key investigation (1) the reproductive output of Daphnia sp.

Sources of information (i), (ii), (iii) and (iv) provide relevant information covering this key investigation by reporting the effect values based on reproduction. However, all these sources of information have the following deficiencies affecting their reliability.

A. The reliability of source of information (iii) and (iv) is 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:

B. Testing in accordance with OECD TG 211 requires that the following specifications/conditions must be met:

- The full record of the daily production of living offspring during the test is provided;
- The number of deaths among the parent animals is provided and the day on which they occurred;
- 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 must be available;

- The results can be based on nominal or measured initial concentration only if the concentration of the test material has been maintained within 20 % of the nominal or measured initial concentration throughout the test;

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

- You have not provided information on daily production of living offspring for any of the studies;
- You have reported a mortality rate of 10% for the control and for the 10mg/L test concentration for study (iv) but for none of the studies you provided the number of deaths among the parent animals and the day on which they occurred;
- You have not provided details on analytical methods used, including specificity, recovery efficiency, precision, limits of determination, for any of the studies;
- For studies (ii), (iii) and (iv) you have specified that the analytical monitoring was performed and the results are reported based on measured concentrations. For study (i) you have specified that the analytical monitoring was performed revealing that test concentration was 66.3-66.8% of nominal concentration after 72h and you have reported the results based on nominal concentration.

The absence of information on living offspring and number of deaths among the parent animals does not allow an independent assessment of the validity criteria. Furthermore, you have not provided performance parameters of the analytical methods nor the measured concentrations for any of the studies, hence no independent assessment can be made with regards to stability of the test item in the test solutions. Finally, for study (i) you have reported that measured concentration of test material decreased more than 20% of the nominal concentration nevertheless, you have reported the results based on nominal concentration. Lacking all these information, sources (i), (ii), (iii) and (iv) cannot be considered as reliable/or have low reliability.

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 of the reproductive output of Daphnia sp.

Concerning key investigation (2) *survival of parent animal during the test.*

Studies (i), (ii), (iii) and (iv) do provide relevant information covering this key investigation however, as explained under point (1) (A-B) above, the reliability of the sources of information is significantly affected. Therefore, sources of information (i), (ii), (iii) and (iv) cannot contribute to the conclusion on this key investigation.

Concerning key investigation (3) *the time to produe the first brood.*

Sources of information (i), (ii), (iii) and (iv) do not provide any information covering this key investigation therefore, they do not provide information that would contribute to the conclusion on this key investigation.

Taken together, sources of information as indicated above, provide information on reproductive output of Daphnia sp. and survival of parental animals but information on time of production of first brood is not provided. Furthermore, even the information provided on reproduction and survival is not reliable.

Conclusion

Accordingly, 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 211 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled.

3. Long-term toxicity testing on fish

Long-term toxicity testing on fish (Annex IX, Section 9.1.6) is a standard information requirement in Annex IX to REACH.

In your comments to the draft decision you have adapted the standard information requirements mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In your registration dossier, you have provided the following studies:

i. Key study, according to UBA procedural proposal "Extended Toxicity Test in Zebra fish (*Brachydanio rerio*)" (from handbook and secondary sources), on the Substance;
ii. Supporting study, OECD TG 204 (Secondary source), on an analogue substance (EC no 240-245-2 / CAS No. 16090-02-1);
iii. Supporting study, OECD TG 204 (authoritative database, 2019), on analogue substance (EC no 241-164-5 / CAS No.17095-24-8)

In your comments to the draft decision you have provided the following study:

iv. an OECD TG 204 study on analogue substance 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)

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude on long-term toxicity to fish.

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 9.1.6 at Annex IX includes similar information that is produced by the OECD TG 210. This includes:

1. the stage of embryonic development at the start of the test, and
2. hatching of fertilized eggs and survival of embryos, larvae and juvenile fish, and
3. the appearance and behaviour of larvae and juvenile fish, and
4. the weight and length of fish at the end of the test.

Concerning key investigations (1) *the stage of embryonic development at the start of the test* and (4) *the weight and length of fish at the end of the test.*

Sources of information (i), (ii), (iii) and (iv) do not provide any information covering these key investigations therefore, they do not provide information that would contribute to the conclusion on these key investigations.

Concerning key investigation (2) *hatching of fertilized eggs and survival of embryos, larvae and juvenile fish.*

All sources of information (i, ii, iii and iv) provide partial information on this key investigation as only survival of juvenile fish is reported. Information on hatching of fertilized eggs and survival of embryos, larvae is not provided.

However, the reliability of these sources of information is significantly affected by the following deficiencies:

A. The reliability of sources of information (ii), (iii) and (iv) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests.
In addition, the following endpoint-specific deficiency has been identified in your read-across prediction:
Whenever the Substance and/or the source substance(s) are UVCB (Unknown or Variable composition, Complex reaction products or of Biological materials) substances, qualitative compositional information of the individual constituents of the substances needs to be provided; as well as quantitative characterisation in the form of information on the concentration of the individual constituents of these substances; to the extent that this is measurable. Where the composition of two, or more, complex substances is similar (within boundaries defined by the category description) qualitative properties can be established and data gaps filled. [6]

   In your read-across justification document you indicate that the target chemical and the analogue substances EC 255-284-0, EC 240-245-2, EC 255-217-5, EC 275-031-8 and EC 224-073-5 are monoconstituent substances while analogue substance EC 241-164-5 is a UVCB. No compositional information is provided for the UVCB analogue substance, and no information on the individual constituents of the UVCB source substance is provided.
   Therefore no qualitative or quantitative comparative assessment of the compositions of the Substance and of the source substance EC 241-164-5 can be completed. Therefore, ECHA considers that it is not possible to assess whether the attempted predictions are not compromised by the composition of the source substance.

B. The conditions of exposure in OECD TG 210 specifies that the test should start as soon as possible after the eggs have been fertilised and continue until species-specific time period that is necessary for the control fish to reach a juvenile life-stage (28-60-d post-hatch, according to Annex 2 of OECD TG 210).

   However, the studies (i), (ii) and (iii) have a duration of 14 days and are perfomed with developed fish. For study (iv) you reported study duration of 28-d while 30-d post hatch is recommended for *Danio rerio*. You did not report that the test started after the eggs have been fertilised and covered a species-specific time period that is necessary for the control fish to reach a juvenile life-stage.

   Therefore, the study duration is shorter than indicated in the OECD TG 210. This condition of exposure is essential because the effects observed in a long-term study might be considerably more pronounced than over a shorter study duration.

[6] ECHA Guidance R.6: QSARs and grouping of Chemicals, Section R.6.2.5.5

Altogether, the provided studies cannot be considered a reliable source of information that could contribute to the conclusion on this key parameter investigated by the required study.

Concerning key investigation (3) *the appearance and behaviour of larvae and juvenile fish.*

Sources of information (i), (ii), (iii) and (iv) provide partial information on this key investigation as only abnormal behaviour of developed fish is reported. No information regarding larvae and appearance is provided.

However, as explained under point (2) (A-B) above, the reliability of the source of information (i), (ii), (iii) and (iv) are significantly affected. Therefore, source of information (i), (ii), (iii) and (iv) cannot contribute to the conclusion on this key investigation.

Taken together, sources of information as indicated above, provide information on long-term toxicity to fish but essential parts of information of the dangerous property is lacking (stage of embryonic development at the start of the test, hatching of fertilized eggs and survival of embryos and larvae, appearance of larvae and juvenile fish, behaviour of larvae, weight and length of fish at the end of the test). Furthermore, even the information provided on survival and behaviour of juvenile fish is not reliable.

Conclusion

Accordingly, 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 210 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled.

Appendix E: 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].

4. Specific precautions must be taken to ensure that the test material(s) used in the studies requested above is/are sufficiently characterised by analytical controls. The manufactured substances may photoconvert in solution from the trans-conformation to the cis-conformation, and photodegradation in aquatic solutions may follow the isomerisation of the substances. The analytical control of the dosing solutions therefore must be able to determine the test substance in cis- and trans-conformations. Furthermore, the test substances may associate to the test equipment and may also attach to constituents of the standard diet used in animal testing. The extent of such association for the test substance is currently unknown.

   It is therefore necessary to minimize the contact of the test material with diet constituents. In the future studies conducted by oral gavage as administration route, this must be achieved by removing the access to the diet 2 hours prior to the gavage administration for rats. Access to the diet must be given again earliest 2 hours after the gavage administration for rats. The determination of an appropriate fasting time before and after gavage administration takes into account the provisions of Directive 2010/63/EU. The time period for fasting was determined based on the gastric emptying times of rats. These are not fixed values but rather ranges varying depending on the diet, stress level, age and other factors. For rats, the passage of the majority of food through the stomach is estimated to be 2 hours.[8]

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 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.

[7] https://echa.europa.eu/practical-guides
[8] R.A. Purdon and P. Bass (1973), Gastroenterology 64: 968-976

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[9].

[9] https://echa.europa.eu/manuals

Appendix F: 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 20 September 2019.

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

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

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

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

Appendix G: List of references - ECHA Guidance[10] 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)[11]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[11]

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[12]

[10] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[11] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[12] 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 H: 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.