EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 25 May 2021

Addressees
Registrant(s) of JS_4193-55-9 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: Disodium 4,4'-bis[6-anilino-[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate
EC number: 224-073-5
CAS number: 4193-55-9

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 A.1, B.3, C.3 and C.4. below by the deadline of ***2 December 2021***, and all other information listed below by the deadline of ***30 August 2023.***

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

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

1. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.; test method: OECD TG 473) or In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487);

2. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490);

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.64/OECD TG 422) by oral route, in rats.

C. 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, in rats;

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit);

3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test

method: EU C.20./OECD TG 211);

4. 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 VII 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 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.

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".

The same studies as listed in A.1, B.3, C.3 and C.4. have already been requested from other registrants (decision CCH-D-2114450737-42-01/F) with the deadline of 4 January 2021. As only one set of data is to be generated, the deadline for provision of these studies by you is set to 6 months from the date of this decision.

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* cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

In the comments to the draft decision you have provided information seeking to adapt, by applying weight of evidence approaches, also the following standard information requirements:

- Sub-chronic toxicity (Annex IX, Section 8.6.2.)
- Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.).

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

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

According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the (dangerous) property investigated by the required study.

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

You have provided summaries in separate endpoint study records for genotoxicity, reproductive and developmental toxicity, toxicity to algae and long-term toxicity to fish. 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, including the newly added endpoints sub-chronic toxicity and long term toxicity to aquatic invertebrates, in relation to the reliability, coverage of key parameters, consistency and results and conclude that as a weight of evidence based on the available sources of

information, no further studies are needed.

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

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

Reliability of the provided information with analogue substances

ECHA understands that you intend to predict the (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

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
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 Sub-chronic toxicity study (added in the comments) Pre-natal developmental toxicity (additional study added in the comments)	Growth inhibition study aquatic plants Long-term toxicity testing on fish Long-term toxicity testing on aquatic invertebrates (added in the comments)
[redacted]	In vitro gene mutation	
Hexasodium 2,2'-[vinylenebis[(3-	Combined repeated dose and reproductive	Growth inhibition study aquatic plants

[2] ECHA Guidance R.6

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;	toxicity study (added in the comments) Pre-natal developmental toxicity (added in the comments)	(added in the comments) Long-term toxicity testing on aquatic invertebrates (added in the comments)
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: 240-521-2; CAS: 16470-24-9)	Combined repeated dose and reproductive toxicity study (added in the comments) Sub-chronic toxicity study (added in the comments) Pre-natal developmental toxicity (additional study added in the comments)	Long term toxicity on aquatic invertebrates (added in the comments) Long term toxicity on fish (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		Long term toxicity on fish (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(benzene-1,4-disulphonate) (EC: 255-217-5; CAS: 41098-56-0)	Sub-chronic toxicity study (added in the comments)	

In your comments to the draft decision you have provided a document entitled [redacted]. With this document you intend to justify the use of information obtained on the aforementioned 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 and toxicological assessment as reported in this RA justification document obtained by using OECD QSAR toolbox v.3.4 of the target substance and source substances (i.e., read across analogues) were evaluated to be similar and therefore justified and appropriate*

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

ECHA notes the following deficiencies with regards to predictions of (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 B. section 2 and Appendix C, sections 3 and 4 below.

I.1 Predictions for toxicological properties

I.1.1 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*"[3]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

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

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

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

- Alert profiles using the QSAR Toolbox

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

You have provided target and source substances which have stilbene and triazine (melamine) as common constituents. However, the substances have variations in the amino aniline moiety (mono- or di-sulphonated or unsulphonated aniline) as well as in the amino alkylderivative moieties (morpholino or bis(2-hydroxyethyl)amino) include no amino but a phenyl ether moeity (CAS No. 41267-43-0).

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 order to support your claim that the Substance and source substance have similar properties for the endpoints under consideration in the read-across approach, you referred to their repeated dose toxicity properties as well as acute toxicity, irritation, skin sensitisation, and *in vitro* genotoxicity properties.

In the dossier and in your comments you provided information for the following repeat dose toxicity studies:

(i) Two year dietary toxicity study in rats (no guideline provided, no GLP), performed with the Substance
(ii) Three generation reproductive toxicity study in rat via diet (no guideline, no GLP, textbook information, 1977).
(iii) Three-generation reproductive toxicity study in rat via diet (publication Lyman et al., (1975) Long-term toxicity of the test chemical in dogs and rats, Food and Cosmetics Technology.
(iv) Sub-chronic (90-day) dietary toxicity study in rats (no guideline reported, pre-GLP, [redacted], 1969), performed with analogue substance EC: 255-217-5 (CAS: 41098-56-0)

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 repeated dose toxicity, reproductive and developmental toxicity. In fact, the complexity of the systemic interactions and the reproductive process 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 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, this information do not allow the prediction of complex information requirements that you intend to cover with your adaptation, as indicated above.

- Experimental studies

First, you consider that the above mentioned studies allow to compare the systemic and reproductive properties of the Substance with the relevant studies provided for the source substance(s). However, as explained in Appendix B, section 3 and Appendix C, section 1, the dietary route is not considered suitable for the Substance and therefore, dietary studies do not provide reliable information to assess toxicological properties for the Substance.

Second, the two-year repeated dose toxicity study conducted with the Substance has significant deficiencies in the relevant information provided. Importantly, among others, testicular toxicity was not evaluated in this study. This is a critical deficiency considering that testicular toxicity including reduced testis weight and testicular atrophy were reported in a repeated dose toxicity study for one of the source substances (EC: 255-217-5, see Appendix C, section 1, study vii).

Third, while the information on acute toxicity, irritation, skin sensitisation, and in vitro genotoxicity of the substances may provide support that the substances have similar properties for these toxicological properties, these studies do not inform on the sexual function, fertility and developmental properties of the target and source substances. Therefore, this information does not provide relevant information for the Substance and of the source substance(s) to support your read-across hypothesis.

Based on above, the available data set do not provide reliable supporting information to support your claim of similarity in toxicological properties. 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.

I.1.2 Conclusion for prediction of toxicological 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.

I.2 Predictions for ecotoxicological properties

I.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 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

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

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 stilbene and triazine (melamine) as common structural elements. In addition, you have identified one source substance CAS No. 17095-24-8 which does not contain the common stilbene and triazine 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 on experimental data for aquatic toxicity on the Substance and the analogue substances indicated in the table above:

Study	**Target substance (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**	**EC 241-164-5 / CAS: 17095-24-8**
Short-term toxicity to fish		- OECD TG 203, 96h: LC50> 59.79mg/L (measured)			
Short-term toxicity to invertebrates	- Study 1, method not specified 48h: EC50>100 mg/L (nominal) - Study 2, method not specified 48h:				

	EC50>100 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) or > 112 mg/L measured)	- OECD TG 201, 72h: EC50 > 100 mg/L (nominal) or > 23 mg/L (measured)		
Long-term toxicity to invertebrates	- ECOSAR, 21d: NOEC= 3.002 mg/L	- 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)	- OECD TG 202, 21d: NOEC = 10 mg/L and EC50 => 31.6<100 mg/L (nominal)	
Long-term toxicity to fish	- NOEC-14d= 14 mg/L. OECD 204	-Study 1 - OECD TG 204, 14d: NOEC= 61.8 mg/L and LC50=165mg/L (measured) - Study 2 - OECD TG 204, 14d: NOEC = 14mg/L and EC50= 40mg/L (nominal)		- UBA procedural proposal "Extended Toxicity", 14d: NOEC=> 859 mg/L (measured)	- OECD TG 214, 28d: NOEC = 10 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 A.1, C.3 and C.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 invertebrates on the Substance, you indicate that no test guideline was followed.The robust study summaries do not contain enough information to make an independent assessment of their validity (e.g. information to assess if the validity criteria were met) . You indicate that analytical monitoring of the test concentration were either not performed (second study) or "not specified" (first study). The Substance is considered to be adsorptive (log Koc = 10.195) and therefore analytical monitoring is required to verify if the daphnids were effectively exposed to the tested substance. Therefore, the studies are not considered reliable and therefore they cannot be used to compare the ecotoxicological properties of the substances.

Furthermore we note that for short term toxicity on fish you have provided information on a single analogue and no information on the Substance. On short term toxicity on invertebrates you have not provided any information on any analogue (i.e EC 255-284-0; 240-521-2 and 241-164-5).

Based on the above, the short-term studies are not considered reliable and therefore they cannot be used to compare the ecotoxicological properties of the substances.

I.2.2 Conclusion for prediction of ecotoxicological properties

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

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 to request information required under Annex VII of REACH

1. 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 the Substance:

i. Alga, 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 analogue substance(s):

ii. Alga, growth inhibition test (according to OECD TG 201, no GLP, [redacted], 2019) with EC No. 240-245-2

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

iii. Alga, growth inhibition test (according to OECD TG 201) with EC No. 255-284-0

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude 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) and (iii) 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) and (iii) 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) and (iii) 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) and (iii):

- For study (i) 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 (iii), 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, although you have specified that measured exposure concentrations were maintained within ± 20 % of the nominal (i.e. study ii) and/or measured (i.e. study iii) 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) and (iii), in your comments you have provided the initial cell density of the culture (5000 to 1000 cells/ml and 5000 cells/ml, respectively), and for study (iii) you have also mentioned that additionals details relating to biomass of the test organism are planned to be provided in the updated dossier.

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 a value of measured concentration for studies (ii) and (iii), howeveryou have not provided performance parameters of the analytical method nor the measured concentrations in order to allow an independent assessment of the information.

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) and (iii), however the results of algal biomass determined in each flask at least daily were not provided. In the absence of these data the validity of the studies cannot be confirmed.

On this basis the studies (i), (ii) and (iii) cannot be considered as reliable.

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

The Substance is difficult to test due to the adsorptive properties: log Koc = 10.195. OECD TG 201 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 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 201. 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.

Appendix B. Reasons to request information required under Annex VIII of REACH

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is a standard information requirement in Annex VIII 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 the following study records with analogue substances:

(i) *In vitro* chromosomal aberration test (no guideline, no GLP, data from handbook, 1977) with EC: 235-422-6
(ii) *In vitro* chromosomal aberration (according to OECD TG 473, GLP not specified) with EC: 240-245-2

As explained under Appendix on Reasons common to several requests, the weight of evidence adaptation 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 8.4.2. at Annex VIII includes:

- Detection and quantification of cytotoxicity and the frequency of cells with structural chromosomal aberration(s) or the frequency of micronuclei in cultured mammalian cells *(in vitro)* or in mammals (*in vivo*).

A level of information on these aspects similar to that obtained from *in vitro/in vivo* chromosomal aberration tests (OECD TG 473/OECD TG 474) or *in vitro/in vivo* micronucleus tests (OECD TG 487/OECD TG 475) is required.

The sources of information provide relevant information on detection and quantification of gene mutation in cultured mammalian cells. However, these sources of information have the following deficiencies affecting their reliability.

The sources of information (i) and (ii) have the following deficiencies:

Testing in accordance with OECD TG 473 or OECD TG 487, respectively[5], requires that the following specifications/conditions have to be met:

- Two separate test conditions must be assessed: in absence of metabolic activation and in presence of metabolic activation.
- One positive control must be included in the study. The positive control substance must produce a statistically significant increase in the response compared with the concurrent negative control.
- Data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures must be reported.

[5] ECHA Guidance R.7a, Table R.7.7-2, p.557

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

a) Study (ii) has been performed under two separate conditions (with and without metabolic activation), however for study (i) the information about the metabolic activation is identified as "*not specified*". Without such information it is not clear which effect was measured – those of the parent compound (without metabolic activation) or those of the metabolite (with metabolic actiovation).
b) There is no positive control in both studies. Therefore, there is no information on the quality of the experiment because there is no basis to measure the differences in severity of the effects among the experimental groups.
c) There is no data on cytotoxicity and/or the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures in both studies. Without data it is not possible to conclude if the cells were exposed to the analogue substance nor what was the real exposure concentration.

In the absence of such information on those critical aspects of the specification/conditions of the provided studies, ECHA cannot evaluate the reliability of the conclusions on cytotoxicity and the frequency of cells with structural chromosomal aberration(s).

In summary, the sources of information (i) and (ii) have significant reliability issues and cannot contribute to the conclusion on the potential of the Substance to cause cytotoxicity and cannot provide information on the frequency of cells with structural chromosomal aberration(s) or the frequency of micronuclei in cultured mammalian cells.

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 vitro* cytotoxicity study in mammalian cells or in vitro micronucleus study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

In your comments to the draft decision you agree to perform the test.

Information on the study design

To fulfil the information requirement for the Substance, either *in vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) or *in vitro* micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487) 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[6].

2. 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.

You have adapted the standard information requirement mentioned above according to Annex

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

XI, Section 1.2. of REACH (weight of evidence) and provided the following study records with analogue substances:

(i) *In vitro* gene mutation in mammalian cells (no guideline, GLP not specified, Seifried et al 2006) performed with EC: 240-245-2, giving negative results.

(ii) *In vitro* gene mutation in mammalian cells no guideline, GLP not specified; Cameron et al. 1987) performed with CAS: 15339-39-6, giving negative results.

In addition, you have provided two *in vivo* studies with the Substance:

(i) *In vivo* dominant lethal mutagenicity test (key study, no guideline, no GLP, Burg et al. 1977).
(ii) *In vivo* dominant lethal mutagenicity test (supporting study, no guideline, no GLP, Burg et al. 1977).

Although you do not explicitly claim an adaptation, ECHA understands that the information provided was submitted in order to meet the required information by way of adaptation under Annex, Section XI 1.1.2. (existing information) and has assessed it accordingly.

ECHA has assessed the available information for weight of evidence (sub-section 1 below) and for existing information (sub-section 2 below) and has identified the following issue(s):

1. Annex XI, Section 1.2. of REACH (weight of evidence).

Based on the presented sources of information (i) and (ii), you argue that the available data gives sufficient information to conlude 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 adaptation 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) and (ii) provide relevant information on detection and quantification of gene mutation in cultured mammalian cells.
However, the reliability of these sources of information is significantly affected by the following endpoint-specific deficiency has been identified in your read-across prediction:

A. Regarding the source of information (i)

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*"[7]. 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).

Adequate supporting information to compare properties of the substance(s)

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 order to support your claim that your Substance and source substance(s) have similar properties for genotoxicity, in your read-across justification document attached to your comments you have provided mechanistic and structural alerts, generated by QSAR Toolbox v. 3.4. (e.g. DNA alerts for AMES by OASIS v.1.4, DNA alerts for CA and MNT by OASIS v.1.1, in vivo mutagenicity (Micronucleus) alerts by ISS, Protein binding alerts for Chromosomal aberration by OASIS v.1.2). You conclude that "*the (Q)SAR analysis and the experimental data indicate that the test substance and the read-across analogue members are not likely to cause mutagenicity*".

ECHA notes that the structural alerts provided to support your conclusion on lack of mutagenicity are based on bacterial reverse mutation and chromosomal aberration. Alerts on the potential of a substance to cause reverse mutation in bacteria does not allow on its own to conclude on the potential of a substance to cause gene mutation in mammalian cells. Alerts on the potential of a substance to cause chromosomal aberration may inform on the cytogenicity of a substance but does not inform on the properties of a substance to cause gene mutation in mammalian cells. Hence, these structural alerts are not all relevant supporting information on the mutagenicity in mammalian cells, therefore cannot be used to compare the the properties under consideration for the Substance and the source substance.

B. Regarding the source of information (ii)

Absence of justification for use of information on analogue substances

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).[8]

In your comments to the draft decision you have provided documentation to support the read-across from other analogue substances but no such documentation was provided for the source substance CAS: 15339-39-6.

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 this analogue substance submitted under your weight of evidence adaptation is not considered reliable.

In summary, even though the sources of information (i) and (ii) provide relevant information, they have a significant reliability issue and cannot contribute to the conclusion on the potential

[7] ECHA Guidance R.6: Section R.6.2.2.1.f
[8] ECHA Guidance R.6

of the Substance to cause gene mutations.

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 vitro* gene mutation study in mammalian cells. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

2. Annex. XI, Section XI 1.1.2.

Adaptation under Annex, Section XI 1.1.2. enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case detection and quantification of gene mutations (point mutations, frame-shift mutations, small deletions, etc.) including data on mutant frequency for each tissue.

You have provided *in vivo* dominant lethal mutagenicity tests from 1977, not conducted according to any test guideline, The test was conducted with only one dose level and does not investigate the required key parameter for mammalian gene mutation (mutation frequency for each tissue) following the specifications/conditions described above. Therefore, the provided information does not cover the key parameters and specifications/conditions investigated under the OECD TG 488 study. Therefore these studies cannot be considered adequate for the purpose of classification and labelling as required by Annex XI, Section 1.1.2.Therefore, your adaptation is rejected and the information requirement is not fulfilled.

Information on the study design

To fulfil the information requirement for the Substance, either the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) or 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[9].

3. Screening for reproductive/developmental toxicity

A Screening for reproductive/developmental toxicity study (test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) is a standard information requirement under Annex VIII to REACH, if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant. There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

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 the following study records:

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

With the Substance:

(i) In vivo dominant lethal mutagenicity test (no guideline, no GLP, Burg et al. 1977).
(ii) Three generation reproductive toxicity study in rat via diet (no guideline, no GLP, textbook information, 1977).
(iii) Three-generation reproductive toxicity study in rat via diet (publication Lyman et al., (1975) Long-term toxicity of the test chemical in dogs and rats, Food and Cosmetics Technology.

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

(iv) Two year dietary toxicity study in rats (no guideline provided, GLP not specified)

With analogue substances:

(v) Combined repeated dose and reproductive toxicity study in rat, oral-gavage (according to OECD TG 422, no GLP compliant, study report, 2004) with EC: 255-284-0
(vi) Two generation reproduction and fertility study in rats, oral gavage (no guideline, no GLP compliant, [redacted], 2001) with EC: 240-521-2

Based on the presented sources of information, you argue that the available data gives sufficient information to conlcude that the substance does not induce reproductive toxicity.

As explained under Appendix on Reasons common to several requests, the weight of evidence adaptation 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 8.7.3 at Annex VIII includes similar information that is produced by the EU B.63/OECD TG 421 or EU B.64/OECD TG 422. At general level, it includes information on the following key elements: 1) sexual function and fertility, 2) toxicity to offspring, and 3) systemic toxicity.

Sexual function and fertility

Sexual function and fertility on both sexes must include information on mating, fertility, gestation (length), maintenance of pregnancy (abortions, total resorptions), parturition, lactation, organ weights and histopathology of reproductive organs and tissues, litter sizes, nursing performance and other potential aspects of sexual function and fertility.

The source of information (i) provides information on fertility (number of corpora lutea, litter sizes) and maintenance of pregnancy. However, it does not inform on parturition, lactation, organ weights and histopathology of reproductive organs and tissues, litter sizes, nursing performance as forseen to be investigated in EU B.63/OECD TG 421 or EU B.64/OECD TG 422. Therefore, it only provides limited information on this key element.

The new source of information (iv) reported in your comments, provides relevant information on organ weights and histopathology of reproductive organs, however, it does not provide information on the sexual function and fertility. Therefore, it only provides limited information on this key element.

The sources of information (ii-iii and v-vi) provide relevant information on all aspects of the sexual function and fertility.

However, the sources of information (ii-vi) have deficiencies affecting their reliability as follow:

A. The reliability of sources of information (v) and (vi) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests, and cannot contribute to the conclusion on this key element.

B. Regarding the method of administration (dietary) for the sources of information (ii), (iii) and (iv):

In accordance with the relevant guidelines for reproductive toxicity (OECD TG 421/422/416), "*the test chemical is usually administered by gavage; however, alternatively, test chemicals may also be administered via the diet or drinking water*". In all cases, the stability and homogeneity of the test chemical in the vehicle should be determined in order to ensure that it does not influence on the absorption, distribution, metabolism, or retention of the test substance.

You have applied the dietary method of administration for the sources of information (ii), (iii) and (iv). The Substance has properties that determine its attachment to organic matter. Indeed, the technical applications of the Substance as fluorescent whitening agent in paper, textile and household detergents are based on its properties to bind to organic matter such as cellulose or cotton fibres. As a result of its 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 substance.
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. The extent of associations for the Substance with the dietary constituents is currently unknown.

In your comments to the draft decision, you refer to the study provided for the sub-chronic toxicity (source of information (vii)) described in Appendix C, section 1 below), and conclude that "the advantage of delivering the registered substance by oral gavage as opposed to feed, in terms of bioaccessibility, is most likely negligible in this case". For the reasons explained in Appendix C, section 1. the information provided is still missing what is the amount of test item available for absorption and the real amount to which the animals' organism was exposed to, based on the selected concentration levels. Without such information, the dose of the test item that the animals have been exposed to may be over estimated and the sources of information (ii, iii and iv) cannot be considered as reliable to provide information on sexual function and fertility.

C. Regarding dose-level setting, according to ECHA Guidance[10] the highest dose level should be intended to produce some toxicity (or to reach the oral limit dose of 1000 mg/kg bw/day) to provide adequate information on reproductive toxicity for the purpose of both classification (including categorisation within the Reproductive toxicity hazard class) and risk assessment. Dose level selection (and vehicle used) must be justified and documented to allow independent evaluation of the choice made.

The highest dose level in the sources of information (ii), (iii) and (v) is below the limit dose and did not induce any toxicity and you have not shown that the aim was to induce toxicity. Furthermore, as explained for studies ((ii) and (iii) in sub-section B. there is uncertainty on the real dose levels available for absorption. Therefore, the dose level selection was too low

[10] ECHA Guidance R.7, section R.7.6.2.3.2

for (ii), (iii) and (v), and the studies do not fulfil the criterion set in EU B.63/OECD TG 421 or EU B.64/OECD TG 422.

Taken together, there is only limited information provided that covers some but not all information on this key element by source study (i) and (iv), and sources of information (ii-vi) cannot contribute to the conclusion on this key element due to the significant reliability issues.

Toxicity to offspring

Information on pre- and perinatal developmental toxicity reflected by litter sizes, postimplantation loss (resorptions and dead foetuses), stillborns, and external malformations, postnatal developmental toxicity reflected by survival, clinical signs and body weights of the pups (or litters), and other potential aspects related to pre-, peri- and postnatal developmental toxicity observed up to postnatal day 13.

The source of information (i) provides information on litter sizes/post-implantation loss only. Therefore, it provides very limited information on this key element.

The sources of information (ii-vi) provide relevant information on toxicity to the offspring, however they are affected by significant reliability issues as explained above under A-C. Therefore, they cannot contribute to the conclusion on this key element.

Systemic toxicity

Information on systemic toxicity include clinical signs, survival, body weights, food consumption, haematology, clinical chemistry, organ weights and histopathology of non-reproductive organs and other potential aspects of systemic toxicity in the parental generation up to postnatal day 13.

The source of information (i) provides information on clinical signs, survival, body weights, food consumption. However, it does not provide information on clinical biochemistry as desribed in EU B.63/OECD TG 421 or on haematology, clinical biochemistry, specific observations, organ weights and histopathology of non-reproductive organs and other potential aspects of systemic toxicity in the parental generation up to postnatal day 13 as forseen to be investigated in EU B.64/OECD TG 422. Therefore, it only provides limited iinformation on this key element.

The sources (ii-vi) provide relevant information on systemic toxicity. However they are affected by significant reliability issues as explained above under A-C. Therefore, they cannot contribute to the conclusion on this key element.

Taken together, the key elements: sexual function and fertility, toxicity to offspring and systemic toxicity are covered by sources of information (ii.-vi), however, due to significant reliability issues, they cannot contribute to the conclusion on the potential of the Substance to cause reproductive toxicity. The source of information (i) provides some relevant information on fertility, maintenance of pregnancy, toxicity to offspring (litter sizes/post-implantation loss only) and systemic toxicity (clinical signs, survival, body weights, food consumption) but not on all aspects that has to be covered, as defined above.

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 OECD TG 421/422. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

Information on study design

A study according to the test method EU B.63/OECD TG 421 or EU B.64/OECD TG 422 must be performed in rats with oral[11] administration of the Substance. 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 coton 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. To minimise contact of the test material with the diet, the schedule described in Appendix C point 4 must be followed.

[11] ECHA Guidance R.7a, Section R.7.6.2.3.2.

Appendix C: 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.

You have provided the following studies, conducted with the Substance:

(i) 2-year chronic toxicity study in rat via oral-diet (supporting study, no guideline, no GLP, 1992).
(ii) Chronic (2-year) toxicity study in rats via diet (no guideline, no GLP, publication Lyman et al., (1975) Long-term toxicity of the test chemical in dogs and rats, Food and Cosmetics Technology).
(iii) Chronic (2-year) toxicity study in dog via diet (no guideline, no GLP, publication Lyman et al., (1975) Long-term toxicity of the test chemical in dogs and rats, Food and Cosmetics Technology).

Although you do not explicitly claim an adaptation, ECHA understands that the information provided was submitted in order to meet the required information by way of adaptation under Annex, Section XI 1.1.2., and assessed it accordingly below (section A).

In addition, in your comments you have claimed an adaptation 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 the Substance:

(iv) Two year dietary toxicity study in rats (no guideline provided, no GLP), performed with the Substance

With the analogue substances:

(v) Short-term (28-day) toxicity study, oral-gavage (OECD TG 407, GLP, published in the US EPA HPVIS, 2006), performed with analogue substance EC: 240-245-2 (CAS: 16090-02-1)
(vi) Sub-chronic (10 weeks) toxicity study in rats, oral-gavage (no guideline reported, pre-GLP, published in the US EPA HPVIS, 2006), performed with analogue substance EC: 240-521-2 (CAS: 16470-24-9)
(vii) Sub-chronic (90-day) dietary toxicity study in rats (no guideline reported, pre-GLP, [redacted], 1969), performed with analogue substance EC: 255-217-5 (CAS: 41098-56-0)

ECHA has assessed your new adaptation accordingly below (section B).

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

A. Adaptation under Annex, Section XI 1.1.2. enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular adequacy for the purpose of classification and labelling and/or risk assessment.

To be adequate for the purpose of classification and labelling and/or risk assessment, the study must enable concluding whether the Substance has dangerous properties and supports the determination of the No-Observed Adverse Effect Level (NOAEL).

You have provided three chronic toxicity/carcinogenicity studies conducted with the Substance via dietary route. For chronic toxicity/carcinogenicity studies conducted via dietary route, the OECD TG 453 and OECD GD 116 on the conduct and design of chronic toxicity and carcinogenicity studies specify that:

- OECD TG 453 [para 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 [para 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 [para 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;

ECHA notes that in all three studies the test Substance is administered via diet. 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 fibres. As a result of its 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.

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. The extent of associations for the Substance with the dietary constituents is currently unknown.

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 number: 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, you propose that only little of the Substance may be absorbed when administered via gavage, based on literature data ([redacted], 1977) for analogue substance EC: 240-245-2.

This information does not provide any further information on the amount of test item available for absorption. Furthermore, even though information on a structurally related substance indicates that only little may be absorbed in the gut when administered via gavage, it does not provide information on the absorption of that substance when provided in diet.

To conclude, you have not provided information what is the amount of test item available for absorption and the real amount to which the animals' organism was exposed to, based on the selected concentration levels. Without such information, the dose of the test item that the animals have been exposed to may be over estimated, so no reliable NOAEL and LOAEL can be identified from these studies. Therefore these studies cannot be considered adequate for the purpose of risk assessment and classification and labelling as required by Annex XI, Section 1.1.2

B. As explained under Appendix on Reasons common to several requests, the weight of evidence adaptation 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: 1) in-life observations, 2) blood chemistry, 3) organ and tissue toxicity. Information should address effects on the following physiological systems: circulatory system, digestive/excretory system, endocrine system, immune system, integumentary system, musculoskeletal system, nervous system, renal/urinary system, reproductive system, and respiratory system. This information is covered by information similar to OECD TG 408.

In-life observations

In-life observations must include information on survival, body weight development, clinical signs, functional observations, food/water consumption and other potential aspects of in life observations on the relevant physiological systems (circulatory, digestive/excretory, integumentary, musculoskeletal, nervous, renal/urinary, and respiratory).

All new sources of information (iv - vii) provide relevant information on survival, body weight development, clinical signs, food/water consumption. However, they do not inform on functional observations. Any other potential aspects of in life observations on the relevant physiological systems (circulatory, digestive/excretory, integumentary, musculoskeletal, nervous, renal/urinary, and respiratory) was not reported. Therefore, these sources of information provides limited information on this key element.

Additionally, the reliability of sources of information (iv) to (vii) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests, and cannot contribute to the conclusion on this key element. Furthermore, the reliability of source of information (iv) and (vii) is affected by the same reasons as already explained above under point A.

Blood chemistry

Information on blood chemistry must include haematological (full-scale) and clinical chemistry analysis (full-scale), and other potential aspects related to blood chemistry to address relevant physiological systems (circulatory digestive/excretory, endocrine, immune, musculoskeletal, and renal/urinary)

All new sources of information (iv-vii) provide relevant information on some haematological (haemoglobin, haematocrit, total and differential leukocyte counts) and clinical-chemistry (blood sugar, blood urea nitrogen, and serum glutamic oxaloacetic transaminase) parameters, however, not a full-scale. Any other potential aspects related to blood chemistry to address relevant physiological systems (circulatory digestive/excretory, endocrine, immune, musculoskeletal, and renal/urinary) was not reported. Therefore, these sources of information provides limited information on this key element.

Additionally, the reliability of sources of information (iv) to (vii) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests (read-across), and cannot contribute to the conclusion on this key element. Furthermore, the

reliability of source of information (iv) and (vii) is affected by the same reasons as already explained above under point A.

Organ and tissue toxicity

Organ and tissue toxicity must include information on terminal observations on organ weights, gross pathology and histopathology (full-scale and other potential aspects related to organ and tissue toxicity to address relevant physiological systems (circulatory, digestive/excretory, endocrine, immune, integumentary, musculoskeletal, nervous, renal/urinary system, reproductive, and respiratory).

All new sources of information (iv-vii) provide relevant information on organ weights and gross pathology. Sources of information (iv), (v) and (vii) provide some relevant information on histopathology, but not full scale and the source of information (vi) does not provide any information on histopathology. Specifically, the source of information (iv) does not include evaluation on testes (both organ weight and histopathology). This is a serious limitation of this source of information, since testicular toxiicty is reported in source of information (vii). Any other potential aspects related to blood chemistry to address relevant physiological systems (circulatoy, digestive/excretory, endocrine, immune, musculoskeletal, and renal/urinary) was not reported.

Therefore, these sources of information provides limited information on this key element.

Additionally, the reliability of sources of information (iv) to (vii) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests (read-across), and cannot contribute to the conclusion on this key element. Furthermore, the reliability of source of information (iv) and (vii) is affected by the same reasons as already explained above under point A.

Taken together, the key elements: in-life observation, blood chemistry and organ and tissue toxicity are only partialy covered by the sources of information (iv) – (vii). Information on important aspects related to blood chemistry, organ and tissue toxicity to address relevant physiological systems (circulatoy, digestive/excretory, endocrine, immune, musculoskeletal, and renal/urinary) have not been provided. In addition, none of the sources of information are considered as reliable.

Conclusion

It is not possible to conclude, based on any of the new sources of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in OECD TG 408.

Based on the above, the adaptation is rejected and the information requirement is not fulfilled.

Information on the study design

According to the OECD TG 408 rat is the preferred species.

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.

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

As discussed above, the Substance has properties to bind to organic matter and 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 route of administration. To minimise contact of the test material with the diet, the schedule described in Appendix C point 4 must be followed.

2. Pre-natal developmental toxicity study in one species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX 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 the following study records:

With the Substance:

(i) Three generation reproductive toxicity study in rat via diet (no guideline, no GLP, textbook information, 1977).
(ii) Three-generation reproductive toxicity study in rat via diet (publication Lyman et al., (1975) Long-term toxicity of the test chemical in dogs and rats, Food and Cosmetics Technology.

With analogue substances:

(iii) Pre-natal developmental toxicity study in rabbit via oral-gavage (equivalent to OECD TG 414, GLP not specified, publication, 1999) with EC: 240-245-2
(iv) Pre-natal developmental toxicity study in rabbit via oral-gavage (equivalent to OECD TG 414, GLP not specified, HPVIS database 1999) with EC: 240-521-2

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

(v) Combined repeated dose and reproductive toxicity study in rat, oral-gavage (according to OECD TG 422, no GLP compliant, study report, 2004), performed with EC: 255-284-0 (CAS: 41267-43-0)

(vi) Pre-natal developmental toxicity study in rat via oral-gavage (according to OPPTS Test Guidelines 870.3700, published in the US EPA HPVIS), performed with EC: 240-521-2 (CAS: 16470-24-9)

(vii) Pre-natal developmental toxicity study in rat via diet (no guideline available, no GLP, abstract, 1976), performed with EC: 240-245-2 (CAS: 16090-02-1)

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude on the 1st species prenatal developmental toxicity.

As explained under Appendix on Reasons common to several requests the weight of evidence adaptation 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 8.7.2 at Annex IX includes similar information that is produced by the OECD TG 414 on one species. The following aspects are covered: 1) prenatal developmental toxicity, 2) maternal toxicity, and 3) maintenance of pregnancy.

Prenatal developmental toxicity

Prenatal developmental toxicity includes information after prenatal exposure on embryonic/foetal survivial (number of live foetuses; number of resorptions and dead foetuses, postimplantation loss), growth (body weights and size) and structural malformations and variations (external, visceral and skeletal).

Sources of information (i), (ii) and (v) provide information on some of the elements of developmental toxicity, such as litter sizes, postnatal survival and growth of pups. However, they do not inform on structural malformations and variations (external, visceral and skeletal) as foreseen to be investigated in OECD TG 414. Therefore, they only provide limited iinformation on this key element in general.

The sources of information (iii., iv., vi. and vii) cover all relevant aspects of prenatal developmental toxicity key element. However, the reliability of these sources of information is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests, and cannot contribute to the conclusion on this key element.

Maternal toxicity

Maternal toxicity includes information after gestational exposure on maternal survival, body weight and clinical signs and other potential aspects of maternal toxicity in dams.

All sources of information provide relevant information on maternal toxicity. However, the reliability of the sources of information (iii - vii) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests, and cannot contribute to the conclusion on this key element.

Maintenance of pregnancy
Maintenance of pregnancy includes information on abortions and/or early delivery as a consequence of gestational exposure and other potential aspects of maintenance of pregnancy.

All sources of information provide relevant information on maintenance of pregnancy. However, the reliability of the sources of information (iii - vii) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests, and cannot contribute to the conclusion on this key element.

Taken together, the sources of information as indicated above provide information on maternal toxicity and maintenance of pregnancy, but only limited information on the key elements of (prenatal) developmental toxicity. Specifically, no information is provided on structural malformations and variations (external, visceral and skeletal) as sources of information (i), (ii) and (v) do not investigate these, and information from sources of information (iii), (iv), (vi) and (vii) is not considered reliable.

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 OECD TG 414, prenatal developmental toxicity study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[12] administration of the Substance.

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 coton 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. To minimise contact of the test material with the diet, the schedule described in Appendix C point 4 must be followed.

3. 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 IXAnnex IX to REACH.

You have adapted the standard information requirement by using a Qualitative or Quantitative structure-activity relationship ((Q)SAR) under Annex XI, Section 1.3. Your adaptation is based on the following study record in your dossier:

i. QSAR calculation "Long-term toxicity to aquatic invertebrates by ECOSAR Version 1.11".

In addition, in your comments you have claimed an adaptation 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 the analogue substances:

ii. an OECD TG 211 study on analogue substance Hexasodium 4,4'-bis(2-phenoxy-4-(2,5disulfonatoanilino)-1,3,5-triazine-6-ylamino)stilbene-2,2'-disulfonate, CAS: 41267-43-0 (EC: 255-284-0)
iii. an OECD TG 202, part 2 study on analogue substance 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
iv. an OECD TG 202, part 2 study on 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: 240-521-2; CAS: 16470-24-9)

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

A. Annex XI, Section 1.3. states that the results obtained from valid QSAR models may be used instead of testing when the following cumulative conditions are met, in particular:

1. results are derived from a QSAR model whose scientific validity has been established;
2. the substance falls within the applicability domain of the QSAR model;
3. adequate and reliable documentation of the applied method is provided; and

[12] ECHA Guidance R.7a, Section R.7.6.2.3.2.

4. the results are adequate for classification and labelling and/or risk assessment.

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

However, you have not provided any documentation for the QSAR prediction. In particular, you have not included a QMRF and/or a QPRF in your technical dossier.

Therefore, ECHA cannot establish whether the model is scientifically valid, whether the Substance falls within the applicability domain of the model, and whether the results are adequate for classification and labelling and/or risk assessment.
Therefore, your adaptations do not fulfil the criteria specified in Annex XI, Section 1.3. and is rejected.

B. 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 investigations (1) the reproductive output of Daphnia sp.

Sources of information (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.

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 (ii), (iii) and (iv) is also affected by the following issue:

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 (ii) 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, such as LOQ and LOD, for any of the studies;
- For studies (ii) and (iii) you have specified that the analytical monitoring was performed and the results are reported based on measured concentrations. For study (iv) you have specified that that 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, although for studies (ii) and (iii) you have reported results based on measured concentrations, 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. Finally, for study (iv) 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 (ii), (iii) and (iv) cannot be considered as reliable/or have low reliability.

Taken together, even though the sources of information (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 (ii), (iii) and (iv) do provide relevant information covering this key investigation however, as explained under point (1) above, the reliability of the sources of information is significantly affected. Therefore, sources of information (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 (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 these 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.

Information on study design

OECD TG 211 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.1.

4. 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

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 substance (EC No. 240-245-2):

i. Fish, Prolonged Toxicity Test: 14-day study (according to OECD TG 204, GLP not specified, authoritative database (Japan chemicals collaborative knowledge database (J-check), 2019)
ii. Fish, Prolonged Toxicity Test: 14-day study (according to OECD TG 204, secondary sources (United Nations Environmental Programme (UNEP), 2005; U. S. Environmental Protection Agency, 2005 and Human & Environmental Risk Assessment [HERA], 2004))

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

iii. a study following the "UBA procedural proposal" on analogue substance Tetrasodium 4,4'-bis((4-(bis(2-hydroxyethyl)amino)-6-(4sulphonatoanilino)-1,3,5-triazin-2-yl)amino)stilbene-2,2'-disulphonate) (CAS: 16470-24-9; EC No. 240-521-2)
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 (i), (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. [13]

   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 240-521-2, EC 255-217-5, EC 275-031-8 and EC 235-422-6 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

[13] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.5.5

might be considerably more pronounced than over a shorter study duration.

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.*

Source of information (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) above, the reliability of the source of information (ii), (iii) and (iv) are significantly affected. Therefore, source of information (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.

Information on the study design

OECD TG 210 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.1.

Appendix D: 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[14].

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 substance 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 substance 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 each 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 and 3 hours prior to the gavage administration for rabbits. Access to the diet must be given again earliest 2 hours after the gavage administration for rats and earliest 3 hours after the administration for rabbits. 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 and rabbits. 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[15]. For rabbits, the passage of food through the stomach is estimated to be 3 – 6 hours[16].

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,

[14] https://echa.europa.eu/practical-guides
[15] R.A. Purdon and P. Bass (1973), Gastroenterology 64: 968-976
[16] R. R. Davies et al. (2003), Vet Clin Exot Anim 6: 139–153

- 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[17].

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

Appendix E. 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 7 October 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).

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 F. List of references - ECHA Guidance[18] 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)[19]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[19]

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[20]

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