Helsinki, 18 February 2021

Addressees
Registrant of JS_41098-56-0 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: 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 number: 255-217-5
CAS number: 41098-56-0

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed in A.2 below by **26 June 2023** and all other information listed below by the deadline of ***23 November 2022***.

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

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

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

2. Extended one-generation reproductive toxicity study (triggered by Annex IX, Section 8.7.3., column 1; test method: OECD TG 443) by oral route (gavage), in rats, specified as follows:

   - At least two weeks premating exposure duration for the parental (P0) generation;
   - Dose level setting shall aim to induce systemic toxicity at the highest dose level;
   - Cohort 1A (Reproductive toxicity);
   - Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation which shall be followed to weaning.

   You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

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 appendix:

- Appendix entitled "Reasons common to several requests";
- Appendix entitled "Reasons to request information required under Annex 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

How to comply with your information requirements

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

The study listed in A.2. has already been requested from other registrants (decision CCH-D-2114450731-54-01/F) and the deadline has been aligned.

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

Appeal

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

Failure to comply

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

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

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

Appendix on Reasons common to several requests

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

In your comments to the draft decision you clarify that you are seeking to adapt the following standard information requirements by applying weight of evidence approaches in accordance with Annex XI, Section 1.2:

- Pre-natal developmental toxicity study (PNDT; Annex IX, Section 8.7.2.)
- Toxicity to reproduction (Annex IX, Section 8.7.3.)
- Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

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

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

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

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

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

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

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

Reliability of the provided information with analogue substances

ECHA understands that you intend to predict the (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

You have provided the following read-across approaches as part of your weight of evidence adaptation:

- Analogue approach (for all (eco)toxicity information requirements listed above) where you read-across between structurally related analogue substances as source substances and the Substance as a target substance; and

- Category approach (endpoint dependent) where you predict the pre-natal developmental and reproductive toxicity of the Substance from category members using read-across based on 5 values from 5 nearest neighbours compared by prediction descriptors.

I. Analogue approach:

In addition to the justification provided in the registration dossier, in your comments to the draft decision you have provided a justification document entitled "[redacted] to further support the analogue approach. Under the weight of evidence for (eco)toxicological properties of the endpoints listed above, you read-across between the following substances, reported in your dossier and in the comments on the draft decision, as source substances and the Substance as target substance:

Source substance	Human health information requirements	Environmental information requirements
Hexasodium 4,4'-bis(2-phenoxy-4-(2,5-disulfonatoanilino)-1,3,5-triazine-6-ylamino)stilbene-2,2'-disulfonate (EC 255-284-0; CAS No. 41267-43-0)	Pre-natal developmental toxicity Extended one-generation reproductive toxicity study	Long-term toxicity testing on aquatic invertebrates (added in the comments)
Tetrasodium2,2'-ethene-1,2-diylbis[5-({4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfonatophenyl)amino]-1,3,5-triazin-2-yl}amino) benzenesulfonate] (EC no 240-521-2; CAS no 16470-24-9)	Pre-natal developmental toxicity (added in the comments) Extended one-generation reproductive toxicity (added in the comments)	Long-term toxicity testing on aquatic invertebrates (added in the comments) Long term toxicity on fish (added in the comments)

[2] ECHA Guidance R.6

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)	Pre-natal developmental toxicity (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 no 17095-24-8)		Long term toxicity on fish (added in the comments)

You state that "*the physico-chemical parameters, various toxicological and ecotoxicological endpoint-specific alerts, other mechanistic alerts and scenarios (for analogue approach) which were taken into consideration for assessment of the target chemical along with read across analogues, as reported in this justification document as obtained by using OECD QSAR toolbox v3.4., were evaluated to be similar and therefore concluded to be appropriate*" and indicate that you have selected the 'Scenario 2" for the analogue approach to justify the read across analogues.

Therefore, ECHA understands that you read-across between CAS No 41267-43-0, CAS No. 16470-24-9, CAS No. 16090-02-1 and CAS No. 17095-24-8 as source substances and the Substance as target substance, and you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substance(s).

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

The common deficiencies are set out here, while the specific ones, which also add to the overall conclusion, are set out under Appendix A. section 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).

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

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

- Alert profiles using the QSAR Toolbox

You have provided target and source substances which have [redacted] as common structural elements. However, the substances have variations in the amino aniline moiety ([redacted]) as well as in the amino alkylderivative moieties ([redacted]) or include no amino but a phenyl ether moiety.

In addition, you have identified one source substance CAS no 17095-24-8 which does not contain the common [redacted] constituents. With respect to this substance you argue that "*Target and read across analogue (CAS no 17095-24-8) share aryl and sodium sulfonate groups in common*". 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*".

- Information from 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 following information for the following repeat dose toxicity studies:

- A sub-chronic (90-day) oral toxicity study conducted with the Substance. The no observed adverse effect level (NOAEL) in this study was reported as 100 mg/kg diet (about 120 mg/kg bw/d) based on the observation of severe testicular toxicity reported at 500 mg/kg diet (estimated as 723 mg/kg bw/d in males based on food consumption and body weights)
- A combined repeated-dose and reproductive/developmental toxicity screening test (28-day) conducted with the source substance CI Fluorescent Brightener 271 (EC no 255-284-0). A NOAEL of 20 mg/kg bw/d or less was set based on the observation of vacuolar degeneration (specified in comments) and nephrotoxicity (specified in the dossier), respectively. Highest dose tested was 200 mg/kg bw/d

In addition, you have provided predicted NO(A)EL values for the reproductive and developmental toxicity of the Substance based on OECD QSAR Toolbox category approach.

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 toxicological properties such as 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.

- Information from experimental studies

First, difference in toxicological properties have been identified in the repeated dose toxicity studies above. Testicular toxicity including reduced weight (relative) and atrophy of the testes with histopathological changes were reported in the sub-chronic study conducted with the Substance. On the other hand, the source substance CI Fluorescent Brightener 271 (EC No. 255-284-0) increased relative weight of the testes and did not cause histopathological changes of the testis in the conditions of the combined repeated-dose and reproductive/developmental toxicity screening test. However, due to the differences in dose-selection and the duration of the studies, these two studies do not constitute a reliable experimental basis for establishing that the toxicological properties of the source substance and the Substance are comparable.

Second, due to the deficiencies as noted below (see *II. Category approach*), the OECD QSAR Toolbox category approach for NO(A)EL predictions cannot be used to predict properties of the Substance. For the same reasons, they do not constitute a reliable basis for comparing the properties of the target and source substances for the reproductive toxicity or for the developmental toxicity.

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. Read-across hypothesis contradicted by existing data

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances. The ECHA Guidance[3] indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". The set of supporting information should allow to verify the crucial aspects of the read-

across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance.

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

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar target and source substances cause the same type of effect(s).

In your dossier you have provided a sub-chronic toxicity study conducted with the Substance, where severe testicular toxicity was reported at estimated dose of 723 mg/kg bw/d in males, as indicated already in point I.1.1. above. In your comments to the draft decision, you have provided a two-generation reproductive toxicity study conducted with the source substance EC no 240-521-2 (CAS no 16470-24-9). No testicular toxicity was reported in the parental males in the two-generation toxicity study conducted with the source substance up to the dose 1000 mg/kg bw/d. You have not explained why these differences do not affect the read-across hypothesis.

The available set of data on the target and source substance indicates that there may be differences in the toxicological properties of the substances. This contradicts your read-across hypothesis whereby the structurally similar target and source substances cause the same type of effect(s). Therefore you have not demonstrated and justified that the properties of the source substance(s) and of the Substance are likely to be similar despite the observation of these differences.

I.1.3. 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 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

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

substance(s) is necessary to confirm that both substances cause the same type of effects. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

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

- Alert profiles using the QSAR Toolbox

You have provided target and source substances which have [redacted] as common structural elements , except for source substance CAS No. 17095-24-8. 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 255-217-5/ CAS 41098-56-0)**	**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, LC50 > 100 mg/L.		- OECD TG 203, 96h: LC50> 100 mg/L		
Short-term toxicity to invertebrates			- OECD TG 202: EC50>97 mg/L		
Long-term toxicity to invertebrates			- 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		-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=		- UBA procedural proposal "Extended Toxicity", 14d: NOEC=> 859 mg/L (measured)	- OECD TG 204, 28d: NOEC = 10 mg/L (nominal)

		40mg/L (nominal)			

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 ecotoxicological properties such as aquatic toxicity (reproductive toxicity to *Daphnia*, developmental 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 and 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 the long-term invertebrate and fish data, as described in the appendices below (sections A.3 and A.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 data on the Substance for acute toxicity on fish you have provided an OECD TG 203 study on the Substance. While you report an LC50 of 100 mg/L, the tabulated data on mortality indicates 100% mortality in 96-hours already at nominal concentration of 25 mg/L. This data however has deficiencies. You have indicated that the analytical monitoring was performed, however you have not provided information on the analytical method nor on the performance parameters. Therefore you have not demonstrated that the organisms were effectively exposed to the tested substance.

- Regarding the short-term data on analogue substances, you have provided acute aquatic toxicity studies on *Daphnia* and fish for one analogue (i.e. EC 255-284-0). As regards the OECD TG 202 study on *Daphnia*, you have not provided any information on analytical monitoring, on the number of immobilised daphnids during the test or dissolved oxygen concentrations throughout the test.
  As regards the OECD TG 203 study on fish, you have not provided any information on analytical monitoring data, on the mortality in the control or dissolved oxygen concentrations throughout the test.
  In the absence of these data, you have neither demonstrated that the daphnids and/or fish were effectively exposed to the tested substance nor that validity criteria were met.
  Furthermore, we note that for short term toxicity to fish you have provided information on the Substance and for short-term toxicity to fish and to *Daphnia* you have provided information on a single analogue (i.e. EC 255-284-0). However, you have not provided any information on the other analogues (i.e EC 240-245-2, 240-521-2 and 241-164-5). Therefore, no comparison can be made between the Substance and the analogues to

support your claim of similarity in ecotoxicological properties.

Based on the above, the 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

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

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

II. Category approach:

a. Scope of the grouping

In your registration dossier you have performed categorisation based read-across through identification of 5 nearest neighbours compared by prediction descriptors using OECD QSAR Toolbox. You have provided automated reports generated from the OECD QSAR Toolbox software in IUCLID Section 13 (OECD Toolbox predictions for the Pre-natal developmental toxicity study and Toxicity to reproduction based on category members using read-across). Even though you have reported this information as '(Q)SAR' in the IUCLID, on the basis of the corresponding prediction report indicating prediction from category members using read-across, ECHA has evaluated this information as Annex XI, Section 1.5 Grouping of substances and read-across approach.

You define the applicability domains of the categories provided for PNDT study and toxicity to reproduction based on general structural and mechanistic criteria (referential boundaries), and logKow ranges (parametric boundaries).

ECHA notes the following shortcoming with regards to your grouping approach.

Applicability domain of the categories

According to the ECHA Guidance, a category (grouping) hypothesis should address "*the set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members for the given endpoint*"[5]. Particularly, "*the applicability domain of a (sub)category would identify the structural requirements and ranges of physico-chemical, environmental fate, toxicological or ecotoxicological properties within which reliable estimations can be made for the (sub)category members*".[6] Therefore, to reliably predict properties within a category the applicability domain should be described including the borders of the category, for which chemicals the category does not hold and a justification for the inclusion and/or exclusion rules.

You describe the applicability domains of the categories based on substance type classification by general mechanistic and structural criteria and logKow boundaries.

[5] ECHA Guidance R.6., Section R.6.2.4.1.
[6] ECHA Guidance R.6., Section R.6.2.1.2.

These descriptions of general structural and mechanistic criteria document the selection of the source substances but do not constitute on their own a description of the inclusion and exclusion criteria defining boundaries of the category.

Therefore, these applicability domains do not introduce unambiguous inclusion/exclusion criteria which would identify the structural requirements and ranges of physico-chemical, environmental fate, toxicological properties within which reliable estimations can be made for the (sub)category members.

b. Predictions for properties

You have provided predictions based on category members using read-across and calculating an average from 5 nearest neighbours derived by comparing prediction descriptors.

ECHA notes the following deficiencies with regards to predictions of toxicological properties based on category approach.

Read-across hypothesis

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. 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 (read-across approach).

A read-across hypothesis needs to be provided, establishing why a prediction for a toxicological or ecotoxicological property is reliable. This hypothesis should be based on recognition of the structural similarities and differences between the source substance(s) and your Substance[5]. It should explain why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern.

You have not provided a read-across hypothesis to establish a reliable prediction for the toxicological properties, based on recognition of the structural similarities and differences between the category members.

Reliability of information

According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results should:

- have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3)

In addition, the source studies should be in the form of the robust study summary(ies), in order to be assessed and to support the read-across justification.

In order to have a reliable prediction using multiple source substances, it is important to ensure that the read-across prediction is well founded and that the prediction accounts for the uncertainty in the approach. In cases, where there are multiple source substances, and consequently a range of possible NO(A)EL values available to read-across, the use of the most conservative (lowest)

value may be sufficient to account for the uncertainty in the read-across[7].

You have provided a list of NO(A)EL values for the source substances, and predictions for NO(A)ELs based on category members using read-across and calculating an average from 5 nearest neighbours.

You have not selected the most conservative NO(A)EL or provided justification why the target substance is expected be less potent than the calculated average from the 5 nearest neighbours. Therefore, the selection of NO(A)EL for the read-across from the source substances to the Substance is not justified, and the uncertainty in the approach for predictions has not been considered. Consequently, the results are not adequate for the purpose of the risk assessment.

In addition, you have not provided robust study summaries of the source studies for the NO(A)ELs. In their absence, you have not established that the results to be read across meet the criteria as defined above.

III. Conclusion for the analogue and category approach

Therefore, based on the information in the dossier and provided in the comments, the information from the analogue substances submitted (both in the analogue and category approaches) under your weight of evidence adaptation is not considered reliable.

Additional issues related to weight of evidence are addressed under the corresponding information requirement.

B. Assessment of the Qualitative or Quantitative Structure activity relationship (Q)SAR adaptations, under the requirements of Annex XI, Section 1.3

You have adapted the following standard information requirements by applying QSAR approach in accordance with Annex XI, Section 1.3:

a) Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)
b) Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)

In you dossier, you have provided:

i. a QSAR prediction for a 28-d NOEC for fish using the Neutral Organic SAR (Baseline Toxicity) from ECOSAR v1.11;
ii. a QSAR prediction for a 21-d NOEC for aquatic invertebrates using the Neutral Organic SAR (Baseline Toxicity) from ECOSAR v1.11.

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
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

[7] ECHA Guidance R.6., Section R.6.2.2.

establish the scientific validity of the model, to verify that the Substance falls within the applicability domain of the model, and to assess the adequacy of the prediction for the purposes of classification and labelling.

You have not provided 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 are rejected.

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

1. 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.

In the registration dossier, you have adapted the standard information requirement mentioned above according to Annex XI, Section 1.5. of REACH.

In the registration dossier you have provided:

i. QSAR toolbox category read-across prediction for Developmental Toxicity /Teratogenicity of the Substance in rabbits ([redacted] 2014), Key study;
ii. Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Test (National institute of technology and evaluation, 2004) conducted with the analogue substance hexasodium 2,2'-{ethene-1,2-diylbis[(3-sulfonato-4,1-phenylene)imino(6-phenoxy-1,3,5-triazine-4,2-diyl)imino]}dibenzene-1,4-disulfonate (CI Fluorescent Brightener 271; EC no 255-284-0; CAS no 41267-43-0) (National institute of technology and evaluation, 2004), Supporting study.

In your comments to the draft decision, you have clarified your intention to adapt according to Annex XI, Section 1.2. of REACH and Annex XI, Section 1.5. of REACH.

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

iii. Prenatal developmental toxicity study (guideline not specified) conducted with the analogue substance 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate (CAS no 16090-02-1; EC no 240-245-2) (Moriyama et al, 1976)
iv. Prenatal developmental toxicity study according to OPPTS 870.3700 (US EPA) conducted with the analogue substance tetrasodium2,2'-ethene-1,2-diylbis[5-({4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfonatophenyl)amino]-1,3,5-triazin-2-yl}amino) benzenesulfonate] (CAS no 16470-24-9 ; EC no 240-521-2) (US EPA HPVIS database)

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude on the "*treatment with the registered substance in rats as per OECD 414 can be assumed to produce no adverse effects on development*".

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

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 8.7.2 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.

1) Prenatal developmental toxicity

Prenatal developmental toxicity includes following information after prenatal exposure:

- information 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 (ii) and (iii) provide information on some of the elements of developmental toxicity, such as litter sizes, postnatal survival and growth of pups. However, the source of information (ii) does not inform on structural malformations and variations (external, visceral and skeletal) and the source of information (iii) does not inform on embryonic/foetal survival, skeletal malformations or variations (external, visceral and skeletal).

The source of information (iv) provides and the source of information (i) may provide relevant information on all aspects of the prenatal developmental toxicity.

However, the reliability of sources of information (i-iv) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on this key element.

2) Maternal toxicity

Maternal toxicity inlcudes information after gestational exposure on maternal survival, body weight and clinical signs and other potential aspects of maternal toxicity in the pregnant dam.

The source of information (iii) does not provide information on maternal toxicity.

The sources of information (ii) and (iv) provide and the source of information (i) may provide relevant information on all aspects of maternal toxicity.

However, the reliability of sources of information (i-iv) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on this key element.

3) Maintenance of pregnancy

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

The source of information (iii) does not provide information on maintenance of pregnancy.

The sources of information (ii) and (iv) provide and the source of information (i) may provide relevant information on all aspects of maternal toxicity and maintenance of pregnancy.

However, the reliability of sources of information (i-iv) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on this key element.

Conclusion

Taken together, the key elements: prenatal developmental toxicity, maternal toxicity, and maintenance of pregnancy are covered by sources of information. However, due to significant reliability issues, they cannot contribute to the conclusion on the potential of the Substance to cause pre-natal developmental toxicity.

Based on above, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous 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.

Information on study design

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral 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 cotton fibres. As a result of these properties, the Substance may also attach to constituents of the standard diet used in animal testing. Therefore, in order to minimise contact of the test material with diet constituents, testing should be done via oral gavage. The schedule described in Appendix C point A.4 must be followed.

2. Extended one-generation reproductive toxicity study

The basic test design of an extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is a standard information requirement under Annex IX to REACH, if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. Furthermore column 2 defines the conditions under which the study design needs to be expanded.

In the registration dossier, you have adapted the standard information requirement mentioned above according to Annex XI, Section 1.5. of REACH.

In the registration dossier you have provided:

i. QSAR toolbox category read-across prediction for Two-generation reproductive toxicity of the Substance in rats ([redacted], 2014), Key study;
ii. Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Test (National institute of technology and evaluation, 2004) conducted with the analogue substance CI Fluorescent Brightener 271 (EC no 255-284-0; CAS no 41267-43-0), Supporting study.

In your comments to the draft decision, you have clarified your intention to adapt according to Annex XI, Section 1.2. of REACH and Annex XI, Section 1.5. of REACH.

In your comments to the draft decision you have provided information on following study conducted with a source substance:

iii. A two-generation toxicity study (guideline not specified) conducted with tetrasodium2,2'-ethene-1,2-diylbis[5-({4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfonatophenyl)amino]-1,3,5-triazin-2-yl}amino) benzenesulfonate] (CAS no 16470-24-9 ; EC no 240-521-2) (US EPA HPVIS database)

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

Triggers for EOGRT study

A. An EOGRT study according to OECD TG 443 as specified in this decision is an information requirement for your registration, because Column 1 criteria at Annex IX, section 8.7.3 are met.

Adverse effects on reproductive organs or tissues or other concerns in relation with reproductive toxicity are observed in available studies, as explained in the following.

In the technical dossier you provided information from a 90-day dietary repeated dose toxicity study conducted with the Substance ([redacted] 1992). According to the information available from the robust study summary provided in your dossier a NOAEL of 0.2% was identified, corresponding to a dose of 100 mg/kg/d. Effects were observed in animals exposed to daily doses of 1.0% and 5.0%. Particularly, mortality of all animals in the high dose group (5.0%) occurred within the first five weeks of the study. Reduced testicular weight and testical atrophy were recorded in the animals dosed at 1.0%.

A more detailed report for this study is publicly available in the National Technical Reports Library - Microfiche number OTS 0571834. You have summarised this report in your comments. This report contains information on the test procedure applied and on the results obtained from this study. The test substance is identified as Tinopal CH 3669 (CAS 41098-56-0). The substance was mixed with the diet and administered to Wistar rats at 0, 0.2 %, 1 % and 5 %. Dose calculations were not provided in the report but food consumption data and body weights are reported. Using this information we calculated for week 12 of the study for the male 0.2 % group 115 mg/kg bw/day and for the female 0.2 % group 120 mg/kg bw/day[8]. For the 1 % group the dose was for males 723 mg/kg bw/day and for females 754 mg/kg bw/day. The 5 % group animals did not survive past week 9 of the study. The body weight in the 1 % groups was significantly reduced and at 0.2 % slightly lower. Relative liver and kidney weights were increased at 1 % in both sexes. Also, the relative brain weight was increased. Testicle weight was decreased in the 1 % group. Gross and microscopic examinations revealed severe toxic tubular nephrosis in both sexes and testicular atrophy in all males at 1 %. A number of seminiferous tubules contained sertoli cells only and occasional spermatogonia which contained irregular vacuolated cytoplasm. Partial inhibition of spermatogenesis occurred in other tubules. The authors of the 90-day study conducted with the Substance ([redacted] 1992) did not consider the effects in the testis to be secondary to systemic toxicity, but rather stated in their report that *"From gross and microscopical examination it appears that 1 % Tinopal in the diet induced distinct pathological changes in the kidney and the testis, whereas at a feeding level of 0.2 % both organs were indistinguishable from those of the controls."* No pathological changes occurred at 0.2 %. The NOAEL was identified at 0.2 % (about 120 mg/kg bw and day).

Taking into account all the information available, ECHA concludes that the results from this study are reliable for the purpose of identification of effects in reproductive organs/tissues and/or concern in relation with reproductive toxicity.

The observation of reduced testis weight, of histopathological findings such as testicular atrophy, alteration of the structure of seminiferous tubules and partial inhibition of spermatogenesis in other tubules indicate adverse effects on reproductive organs or tissues and reveal a concern in relation with reproductive toxicity.

In your comments to the draft decision you indicated that you consider that the testicular effects observed in this study are caused by decreased testosterone levels secondary to reduced body weight (by 30 %) and food consumption. You refer to the OECD guidance document for histological evaluation of endocrine and reproductive tests (OECD, 2008) and to a scientific publication (Vidal JD and Whitney KM, 2014) to support your conclusion. However, no information on testosterone

[8] The calculations used the w/w percentage of test material in food, the average body weights at week 12 and the average food consumption at week 12 as provided in the report. For example:
for male rats at week 12: 0.2 % of 17 g food/rat/day = 34 (mg/rat/day) /294 (g bw)*1000 = 115.65 mg/kg bw/day;
for female rats at week 12: 0.2 % of 10.9 g food/rat/day = 21.8 (mg/rat/day) /180.7 (g bw)*1000 = 120.64 mg/kg bw/day

levels is available from the 90-day repeated dose toxicity under consideration [redacted], 1992) to substantiate your assumption, and you have not provided any other data to support your conclusion.

ECHA points out that the OECD Guidance Document 151 presents data on the effect of decreased body weight caused by dietary modulation on absolute organ weights in rats (pp. 59-68). According to this data, a dietary restriction resulting in a 30 % decrease in body weight would not induce *"striking"* changes in testicular weight. This conclusion was based on a 90-days study (Seki et al. 1997) which was conducted under the same conditions as the study under consideration [redacted] 1992): 13 weeks of feed restriction, 6-week old rats at onset of the treatment. The observed decrease in testis weight in the study by Seki et al. was only 3.6%. In the same conditions, the reduction in weight of androgen-dependent prostate was 18.6%. Therefore, if the testosterone levels were reduced in the provided 90-day study due to the reduced body weight/food consumption, a signifcant reduction in the weight of androgen-dependent prostate would be expected. No such effect was detected in the study conducted with the Substance.

Having regard to the above, ECHA considers that there is no evidence to support the conclusion that the testicular effects were secondary to body weight/food consumption and mediated by reduced testosterone levels. Therefore, the effects identified in the 90-day dietary repeated dose toxicity study conducted with the Substance ([redacted] 1992) do reveal a concern in relation with reproductive toxicity.

Therefore, an EOGRT study according to OECD TG 443 as specified in this decision is an information requirement for your registration, because Column 1 criteria at Annex IX, section 8.7.3 are met.

Evaluation of Annex XI weight of evidence approach

As explained under Appendix on 'Reasons common to several requests', section A, 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 IX includes similar information that is produced by the OECD TG 443 with the test design as requested in this decision, i.e. with extension to mate the Cohort 1B animals to produce the F2 generation because the criteria at Annex IX section 8.7.3 column 2 are met (explained below in the 'Specifications for the study design'). At general level, it includes information on following key elements: 1) sexual function and fertility, 2) toxicity to offspring, 3) systemic toxicity, and 4) information on sexual function and fertility of the offspring, and toxicity to F2 offspring.

1) 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, oestrous cyclicity, sperm count, sperm analysis, hormone levels, litter sizes, nursing performance and other potential aspects of sexual function and fertility.

The sources of information (ii) and (iii) provide and the source of information (i) may provide relevant information on maternal toxicity and maintenance of pregnancy.

However, the reliability of sources of information (i-iii) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on these key element.

2) Toxicity to offspring

Toxicity to offspring must cover information on deaths before, during or after birth, growth, external malformations, clinical signs, sexual maturity, oestrous cyclicity, organ weights and histopathology of reproductive organs and tissues in adulthood and other potential aspects of toxicity to offspring.

The source of information (ii) provides some information on toxicity to the offpsring up to post-natal day 4, but does not inform on toxicity to the offspring up to adulthood.

The source of information (iii) provide and the source of information (i) may provide relevant information on toxicity to the offspring in the adulthood.

However, the reliability of sources of information (i-iii) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on these key element.

3) Systemic toxicity

Systemic toxicity must include information on clinical signs, survival, body weights, food consumption, haematology, clinical chemistry, organ weights and histopathology of non-reproductive organs and tissues and other potential aspects of systemic toxicity in the parental P and F1 generation up to adulthood.

The source of information (ii) informs on systemic toxicity, but the information provided is limited and does not cover all relevant and essential aspects as defined above. In particular, there is no information on systemic toxicity from F1 generation, such as clinical signs, body weights, haematology, clinical chemistry, organs weights and histopathology of non-reproductive organs in adulthood.

The source of information (iii) provide and the source of information (i) may provide relevant information on toxicity to the offspring up to adulthood.

However, the reliability of sources of information (i-iii) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on these key element.

4) Information on sexual function and fertility of the offspring, and toxicity to F2 offspring

Sexual function and fertility of the offspring includes the same key investigations than in P0 animals (above section "sexual function and fertility") and developmental toxicity in F2 generation includes investigations up to weaning similar to F1 generation.

The source of information (ii) does not investigate the sexual function and fertility in the offspring (F1 generation producing the F2 generation) or toxicity to F2 offspring.

The source of information (iii) provide and the source of information (i) may provide relevant information on all aspects of the prenatal developmental toxicity.

However, the reliability of sources of information (i-iii) is significantly affected by the deficiencies identified and explained under Appendix on Reasons common to several requests (analogue approach and category approach), and cannot contribute to the conclusion on this key element.

Conclusion

Taken together, the key elements: sexual function and fertility, toxicity to offspring, systemic toxicity, and information on sexual function and fertility of the offspring, and toxicity to F2 offspring are covered by sources of information. However, due to significant reliability issues, they cannot contribute to the conclusion on the potential of the Substance to cause pre-natal developmental toxicity.

Based on above, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in OECD TG 443, extended one generation reproductive toxicity study.

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

The specifications for the study design

Premating exposure duration and dose-level setting

The length of premating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Because the F1 animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be 10 weeks for these Cohort 1B animals, at least a 2-week premating exposure duration for P0 animals is sufficient for your Substance.

The highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no relevant data to be used for dose level setting, it is recommended that range-finding results are reported with the main study. You have to provide a justification with your study results that demonstrates that the dose level selection meets the conditions described above.

Cohorts 1A and 1B

Cohorts 1A and 1B belong to the basic study design and shall be included.

Extension of Cohort 1B

If the Column 2 conditions of 8.7.3., Annex IX are met, Cohort 1B must be extended.

The extension is inter alia required, if the use of the registered substance is leading to significant exposure of consumers and professionals (column 2, first paragraph, lit. (a) of Section 8.7.3., Annex IX) and there are indications of one or more relevant modes of action related to endocrine disruption from available *in vivo* studies or non-animal approaches (column 2, first paragraph, lit. (b), third indent of Section 8.7.3., Annex IX).

The use of the Substance is leading to significant exposure of consumers and professionals

because the Substance is used by professionals as cleaning and maintenance products (PROCs 1, 2, 8a, 8b, 9, 11, 13, 15, 19) and consumers as cleaning and maintenance products such as detergents.

Furthermore, there are indications of one or more modes of action related to endocrine disruption because changes in organs sensitive to endocrine activity are observed. Specifically, reduced testis weight, histopathological findings such as testicular atrophy, alteration of the structure of seminiferous tubules and partial inhibition of spermatogenesis in other tubules were observed in males exposed to a dose of 1% of the Substance in a dietary sub-chronic toxicity study ([redacted] 1992).

Therefore, Cohort 1B must be extended.

The F2 generation shall be followed to weaning allowing assessment of nursing and lactation of the F1 parents and postnatal development of F2 offspring.

Species and route selection

The study must be performed in rats with oral[9] administration. The technical applications of the Substance as fluorescent whitening agent in paper, textile and household detergents is based on its property to bind to organic matter such as cellulose or cotton fibres. As a result of these properties, the Substance may also attach to constituents of the standard diet used in animal testing. Therefore, in order to minimise contact of the test material with diet constituents, testing should be done via oral gavage. The schedule described in Appendix B point A.4 must be followed.

Further expansion of the study design
No triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during the conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex IX. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance[10].

3. Long-term toxicity testing on aquatic invertebrates

Long-term toxicity testing on aquatic invertebrates is a standard information requirement under Annex IX to the REACH Regulation.

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 to the draft decision 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

[9] ECHA Guidance R.7a, Section R.7.6.2.3.2.
[10] ECHA Guidance R.7a, Section R.7.6.

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 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. For the reasons explained in the Appendix on Reasons common to several requests, section B, the QSAR based adaptation is rejected.

B. As explained under Appendix on Reasons common to several requests, section A, 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) and (iii) 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) and (iii) is significantly affected by the deficiency identified and explained under Appendix on Reasons common to several requests, section A.

In addition, the reliability of sources of information (ii) and (iii) 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:

- For study (ii), you have specified that the mortality of the parent animals did not exceed 20% at the end of the test. However for none of the studies you provided the number of deaths among the parent animals and the day on which they occurred.

- For study (ii) you have mentioned that mean number of live offspring produced per animal surviving was ≥60%, however you have not provided for any of the study detailed information on the full record of the daily production of living offspring during the test.

- You have not provided details on the analytical methods used, such as LOQ and LOD, for any of the studies;

- For study (ii) you have specified that analytical monitoring was performed and the results are reported based on measured concentrations. For study (iii) you have specified that analytical monitoring was performed and the results are reported based on nominal concentrations, however no data has been provided on the measured concentration of the substance during the test.

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 study (ii) 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. Furthermore, for study (iii) you have reported results based on nominal concentrations but you have not provided data on measured concentrations to prove that the test material was maintained within 20 % during the test. Lacking all this information, sources (ii) and (iii) cannot be considered as reliable/or have low reliability.

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

The sources of information (ii) and (iii) 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) and (iii) cannot contribute to the conclusion on this key investigation.

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

Sources of information (ii) and (iii) 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 forseen to be investigated in an OECD TG 211 study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

4. Long-term toxicity testing on fish

Long-term toxicity testing on fish is a standard information requirement under Annex IX to the REACH Regulation.

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 fish by ECOSAR Version 1.11".

In addition, in your comments to the draft decision 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 204 study (2013) 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).
iii. A second OECD TG 204 study (1993) 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. 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)
v. 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)

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

A. For the reasons explained in the Appendix on Reasons common to several requests, section B the QSAR based adaptation is rejected.

B. As explained under Appendix on Reasons common to several requests, section A, 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 (ii), (iii), (iv) and (v) 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 (ii, iii, iv and v) provide partial information on this key investigation as only survival of juvenile fish is reported. Information on hatching of fertilized eggs and survival of embryos, larvae is not provided.

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

a) The reliability of sources of information (ii), (iii) (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. [11]

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 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,

[11] ECHA Guidance R.6: Section R.6.2.5.5

according to Annex 2 of OECD TG 210).

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

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

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 (iii), (iv) and (v) 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 (iii), (iv) and (v) are significantly affected. Therefore, source of information (iii), (iv) and (v) cannot contribute to the conclusion on this key investigation.

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

Conclusion

Accordingly, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an OECD TG 210 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled.

Appendix B: equirements 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[12].

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

   It is therefore necessary to minimize the contact of the test material with diet constituents. In the requested 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.[13] For rabbits, the passage of food through the stomach is estimated to be 3 – 6 hours.[14]

B. Test material

1. Selection of the Test material(s)

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

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

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.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[15].

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

Appendix C: Procedure

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

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 7 October 2019.

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

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

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

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

Appendix D: List of references - ECHA Guidance[16] 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)[17]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[18]

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.

[16] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[17] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[18] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

OECD Guidance documents[19]

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.

[19] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

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

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.