Helsinki, 03 June 2020

Addressees
Registrant of JS_16090-02-1 listed in the last Appendix of this decision

Date of submission for the dossier subject of this decision
11 July 2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate
EC number: 240-245-2
CAS number: 16090-02-1

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

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed in A.2, C.2, C.3 and C.4 below by ***4 January 2021*** and all other information listed below by ***10 December 2021***.

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

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

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

B. Requirements applicable to 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) with the Substance;

2. Only if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. is obtained, In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method OECD TG 476 or TG 490) with the Substance;

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method OECD 422) in rats, oral route with the Substance;

C. Requirements applicable to all the Registrants subject to Annex IX of REACH

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

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method OECD TG 414) in a first species (rat or rabbit), oral route with the Substance;

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

4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method OECD TG 210) with the Substance

Conditions to comply with the requests

You are bound by the requests for information corresponding to the REACH Annexes applicable to your own registered tonnage of the Substance at the time of evaluation. Therefore you have to comply with the requirements of Annexes VII, VIII and IX of REACH, as you have registered a substance at 100-1000 tpa.

Appendix on general considerations addresses issues relevant for several requests while the other Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

Appendix on Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

Studies A.2, C.2, C.3 and C.4 listed above have already been requested from another registrant (decision CCH-D-2114450734-48-01/F) and the deadlines have been aligned.

You must also update the chemical safety report, where relevant, including any changes to classification and labelling based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

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 general considerations

Assessment of the weight-of-evidence adaptations, under the requirements of Annex XI, Section 1.2.

You have adapted the following standard information requirements by applying weight of evidence (WoE) approaches in accordance with Annex XI, Section 1.2:

- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.);
- *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);
- *In vitro* cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.);
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.);
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.);
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.).

In addition, in your comments to the draft decision you propose to adapt the following standard information requirement by applying weight of evidence (WoE) approach in accordance with Annex XI, Section 1.2:

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

Annex XI, Section 1.2 states that there may be sufficient weight of evidence (WoE) 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.

In accordance with the ECHA Guidance R.4.4, a WoE adaptation involves an assessment of the relative values/weights of different pieces of the available information which is defined by e.g. the reliability of the data, consistency of results/data, nature and severity of effects, and relevance of the information for the given regulatory endpoint. Subsequently, the lines of evidence should be integrated considering their relative values or weights in order to draw a conclusion. Adequate and reliable documentation shall be provided to describe your WoE approach, the assessment of relative weights of individual piece of information and the subsequent conclusions drawn.

You have based your WoE approach on the information provided on:

- the Substance itself
- predictions from analogue substances using the QSAR Toolbox
- experimental studies from analogue substances, further referred in this decision as "source substance(s)":
    - Tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate (CAS: 16470-24-9 / EC: 240-521-2);
    - Hexasodium 2,2'-[vinylenebis[(3-sulphonato-4,1-phenylene)imino(6-phenoxy-1,3,5-triazine-4,2-diyl)imino]]bis(benzene-1,4-disulphonate) (CAS: 41267-43-0 /EC: 255-284-0)

In your comments to the draft decsion you provided information also with the following analogue substances:

- Disodium 2,2'-([1,1'-biphenyl]-4,4'-diyldivinylene)bis(benzenesulphonate) (CAS: 27344-41-8; EC: 248-421-0)
- Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-[(4-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-, tetrasodium salt (CAS: 16470-

24-9; EC: 240-521-2)
- Hexasodium 2,2'-[vinylenebis[(3-sulphonato-4,1-phenylene)imino (6-phenoxy-1,3,5-triazine-4,2-diyl)imino]]bis(benzene-1,4-disulphonate) (CAS: 41267-48-0; EC: 255-284-0)

We have assessed this information according to the requirements of Annex XI, Section 1.2 of the REACH Regulation and identified the following general issues which are addressed directly below:

1. Reliability of the information

Information obtained from the QSAR Toolbox in your dossier

ECHA Guidance R.4., Section R.4.2 sets out the criteria for assessing the reliability of information provided as part of WoE adaptations. The availability of raw data from the studies and an adequate description of the studies are listed among the key elements to be assessed to determine if and how the information can be used in the adaptation. This ECHA Guidance indicates that "*where critical supporting information is not reported (e.g. species tested, substance identity and dose procedure) the test data should be considered to be unreliable for the purposes of REACH*".

Your WoE adaptations are partly based on:
- information on the Substance obtained from databases. This data do not include raw data from the studies or the adequate description of the studies (e.g. species tested or doses used)
- information on similar substances obtained through the use of data from the QSAR Toolbox.

The QSAR Toolbox prediction reports provided to support the use of this data do not include any information on the test procedures applied, and on the results of the studies on the similar substances. In the absence of this above indicated information, the information obtained from databases and the QSAR toolbox is considered unreliable.

Information on analogue substances referred to in your comments

Under Article 10(a)(vii) of the REACH Regulation, a technical dossier must include "*robust study summaries of the information derived from the application of Annexes VII to XI, if required under Annex I*". Annex I, Section 1.1.4/3.1.5 of REACH states that robust study summaries are "*required of all key data used in the hazard assessment*".

In the document attached to your comments to the draft decision you have identified studies conducted with analogue substances that you intend to use as sources of information in your weight of evidence approach and provided high-level narratives presenting these studies.
You have not provided robust study summaries for any of these source studies. In particular you have not provided detailed information on the methods, results and conclusions of these studies allowing for an independent assessment of the studies. In the absence of such information, we cannot assess the reliability of the information from these studies.

2. Relevance of information – requirement for a scientific justification for the use of information from similar substances

Based on ECHA Guidance R.4, Section R.4.3.2.2 a scientific justification needs to establish why the toxicological or ecotoxicological properties of the Substance can be determined from information on the similar substances. It must provide a reasoning why this information can

reliably be used to predict the toxicological/ecotoxicological properties of the Substance. It must also explain why the differences between these substances would not influence the properties of the Substance or should do so in a regular predictable pattern. The justification must further provide detailed characterisation of the identity and composition of the analogue substance(s).

Your WoE adaptations are partly or entirely based on information on similar substances obtained through the use of data from the QSAR Toolbox or directly from information on the source substances: CAS: 16470-24-9 and CAS: 41267-43-0. In addition, in your comments to the draft decision you refer to information on the analogue substances: CAS: 27344-41-8; CAS: 16470-24-9; CAS: 41267-48-0.

- Information from the QSAR Toolbox: You have provided QSAR Toolbox prediction reports detailing the structural and mechanistic criteria used for identifying the similar substances and providing high level information on the identity of these substances. No further endpoint-specific scientific explanation is provided. These descriptions of criteria do not constitute on their own scientific justifications establishing that the information on the similar substances is adequate and relevant for the purpose of identification of the hazard of the Substance by means of weight of evidence.
- Information from analogue substances (CAS: 16470-24-9 and CAS: 41267-43-0) in your dossier: You have provided information for reproductive and developmental toxicity, based on analogue substances. However, you did not provide:
  - detailed information on the identity of the source substances in particular the composition of the test materials;
  - any scientific evidence or justification establishing why the toxicological properties of the Substance can be determined from information on the source substances;
  - any reasoning establishing why information from analogue substances can reliably contribute to the WoE adaptation to conclude on the presence or absence of the particular dangerous property of the Substance.

  Consequently, this information cannot be considered as relevant for the purpose of identification of the hazard of the Substance by means of weight of evidence.

- Information on the similar substances CAS: 27344-41-8; CAS: 16470-24-9; CAS: 41267-48-0; CAS: 16470-24-9 and CAS: 41267-43-0, provided in your comments: You have attached a document to your comments to the draft decision intended to justify the use of information obtained on the aforementioned similar substances in your WoE adaptation. This document presents a set of physico-chemical properties and alerts obtained from the QSAR Toolbox v3.4 for the Substance and for each of these similar substances. On the basis of this information you derive conclusions on the structural similarities, similarities in physico-chemical properties and similarities in the alert profiles between the Substance and each of the above similar substance. You conclude that *"Based on structural similarity, physical-chemical properties, organic functional groups and several general and endpoint specific mechanistic approach using OECD QSAR toolbox v3.4 [...]"*, the aforementioned substances "[...] *were identified as read-across chemical with sufficient data for ecotoxicological and toxicological evaluations used for the target chemical Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate (CAS no. 16090-02-1)*".

  Provided that the applicability domain is appropriate, the results from using QSAR models may be used in a weight of evidence analysis where such data are considered

alongside other relevant data[2,3]. However, neither did you provide information on the applicability domain of the expert systems to generate the alert profiles nor did you provide any other scientific assessment of the information alongside these data. Considering the complexity and amount of information needed from various function and parameters to evaluate endpoints such as long-term toxicity on fish and reproductive and developmental toxicity, QSAR predictions alone do not establish that structurally similar substances have similar properties for these endpoints.

You have not provided robust scientific information, including relevant and reliable studies of comparable design and duration, establishing why the (eco)toxicological properties of the Substance can be determined from information on the similar substance.

3. Requirement for documentation of the WoE adaptations

ECHA Guidance R.4, Section R.4.4 specifies that a weight-of-evidence adaptation must involve an assessment of the relative values/weights of the several pieces of available information. This assessment must consider for instance the relevance and reliability of the information, the consistency of results/data, the nature and severity of effects. The lines of evidence should be integrated considering their relative values or weights in order to draw a conclusion. The assessment should be documented and included in your technical dossier.

You have provided WoE summaries in the endpoint summaries for growth inhibition study aquatic plants, repeated dose toxicity, genetic toxicity and toxicity to reproduction and development. In these summaries you briefly present each of the sources of information in a tabular format, specify the dose descriptors obtained from these studies and outline the effects observed/the absence of effects observed in these sets of data.
In each of the endpoint-specific sections of your comments to the draft decision you described the sources of information that you considered in your WoE adaptation and concluded that with this information you consider that you have been able to fulfil the different information requirements.
Whilst these reports can be regarded as integrated summaries of the data sets, you have not communicated and documented in a robust and transparent manner your considerations on the relevance, relialibity of the individual sources of information. You did not provide a critical assessment of their relative weight and of the overall adequacy of the data set in the context of these WoE. Therefore, your WoE adaptations are not supported by adequate documentation.

4. Conclusion of the WoE assessment

As your WoE adaptations are neither based on relevant and/or reliable data to allow reaching a conclusion on the relevant hazard properties of the Substance nor supported by adequate documentation for the reasons presented above, they do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2. Therefore, your adaptations are rejected.

[2] ECHA Guidance R.7a, Section R.7.6.4.1.2
[3] ECHA Guidance R.7a, Section R.7.5.4.1.1

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

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 1 to 10 tonnes or more per year must contain, as a minimum, the information specified in Annex VII to REACH.

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

An *In vitro* gene mutation study in bacteria is a standard information requirement in Annex VII to REACH.

In your dossier, you have provided

(i) one non-guideline and non-GLP study with the Substance (McGregor & Ainsworth, 1976) in bacteria with the following strains TA1535 and TA1538 which gave negative results with and without metabolic activation. You have named the study as "weight-of-evidence".

In your comments to the draft decision you have futher provided a brief summary of the following additional studies with the Substance:

(ii) *In vitro* gene mutation study in bacteria (OECD TG 471, published in the EPA HPVIS database)

(iii) *In vitro* gene mutation study in bacteria (OECD TG 471, publication in Chem Res Toxicol, 2006)

(iv) You refer to testing according to OECD TG 474 and 475, published in HPVIS database without reporting the studies

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

A. Annex XI, Section 1.2 specifies that a WoE must rely on several independent sources of information to support the assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property.

In your dossier, you have provided a single *in vitro* gene mutation study in bacteria with the Substance.

In your comments on the draft decision you refer to additonal sources of information, as already specified above. We understand that you intend to use this information alongside the information from study (i) included in your dossier as part of a weight of evidence adaptation according to Annex XI, Section 1.2.
In any case, the adaptation would still not be acceptable for the reasons set out under point C. below.

B. ECHA has further assessed the study in your dossier in accordance with Annex XI, Section 1.1.2. (although you do not explicitly claim an adaptation) but it is a non-guideline study conducted before REACH came into force. According to Annex XI, Section 1.1.2. a number of cumulative conditions for a non-guideline study to be considered equivalent to a guideline study have to be covered. In particular, the study must provide an adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3). To meet the requirements of OECD TG 471 (1997), the following key parameters, among others have to be met:

- The test must be performed with 5 strains: four strains of S. typhimurium (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101)

Study (i) was not conducted according to any of the recommended guidelines. The reported study is conducted using only two strains: TA1535 and TA1538.

Therefore, the study does not comply with the specific rules of adaptation as set out in Annex XI, Section 1.1.2. as it does not provide a reliable coverage of the key parameter foreseen to be investigated in OECD TG 471 study and it does not fulfil the information requirement.

C. Even if you would update your dossier with the additional information mentioned in your comments, this would not meet the requirements for an adaptation based on Weight of Evidence under Annex XI, Section 1.2.

The studies do not meet the information requirements of OECD TG 471 stated above, because they were not conducted with the appropriate 5 strains as they do not include results in the required fifth strain, *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101). Therefore, the studies are not providing adequate and reliable coverage of the key parameters.

Further, in your comments to the draft decision you refer to testing according to OECD TG 474 and 475, published in HPVIS database.
A study according to OECD TG 474/475 is a different endpoint (i.e. Section 8.4, Column 2 of Annex VII, VIII or IX) and therefore it cannot be used to fulfil the information requirements for this endpoint. Furthermore, the studies are not included in the dossier subject to the draft decision.

Therefore, the information requirement is not fulfilled.

Possibility for data sharing:

ECHA notes that the jointly submitted registration for the Substance contain data which is relevant for this endpoint. In accordance with Title III of the REACH Regulation, you may request it from the other registrant(s) and then make every effort to reach an agreement on the sharing of data and costs.

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

Growth inhibition study aquatic plants is a standard information requirement in Annex VII to REACH.

You have adapted this information requirement by using a WoE approach under Annex XI, Section 1.2. of REACH. In support of your adaptation of this information requirement, you have provided the following information for this endpoint:

(i) An OECD TG 201 study "Toxicity to aquatic algae and cyanobacteria" with the Substance (GLP not specified, assigned reliability score of 2; study report by Japan chemicals collaborative knowledge database (J-check), 2014). 72-h ErC50 reported to be >65 mg/L and NOEC 6.3 mg/L.
(ii) An OECD TG 201 study "Toxicity to algae" with the Substance (GLP not specified, assigned reliability score of 4; study report by IUCLID dataset CAS 16090-02-1 (2000). 96-h ErC50 reported to be 41.1 mg/L. NOEC not reported.

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

A. For the reasons explained in the Appendix on General considerations the information obtained from database is not considered reliable. Your WoE adaptation is also not supported by adequate documentation.

B. For a WoE information to be considered sufficient it must cover the particular dangerous (hazardous) properties of the Substance foreseen to be investigated in a growth inhibition study aquatic plants performed according to OECD TG 201. This information includes measurements of the algal biomass (e.g. cell counts) as a function of time.

Neither of the studies provide information on key parameters, specifically:
- the results of the analytical determination of exposure concentrations and (if necessary) the calculation of effect levels as measured concentrations
- raw data relative to cell density determination to allow a verification that the validity criteria of the method were fulfilled.

Therefore, neither of the sources of information alone or combined allows to conclude whether the Substance has or has not hazardous properties related to growth inhibition in aquatic algae.

For all the above reasons, your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2.

In your comments to the draft decision you say that you have *"adapted the weight of evidence approach by using the information of the target chemical Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate, CAS 16090-02-1 (EC 240-245-2) from several independent sources to suffice the data gap."* You continue that you *"have included the details on measurement of algal biomass, analytical monitoring, calculated effect levels and validity criteria."* In your comments you have referred to:

(i) a study according to OECD TG 201 on *Pseudokirchneriella subcapitata* cited from the *"Study report, 2019"*. 72 hour ErC50 value was reported to be > 100 mg/L. NOEC/EC10 was not provided;
(ii) a study according to OECD TG 201 on *Desmodesmus subspicatus* cited from the handbook (2008) and secondary sources. 96 hour EbC50 and NOEC values were determined to be 41.1 mg/L and 25 mg/L, respectively, and
(iii) a study according to OECD TG 201 on green algae (species not specified) cited from (J-CHECK, 2019). 72 hour ErC50 and NOEC values were determined to be >65 mg/L and 6.3 mg/L, respectively.

We note that the studies (ii) and (iii) (cited in your comments as handbook 2008 and J-CHECK 2019, respectively) seem to be the same studies that were already reported in the dossier (cited in the dossier as IUCLID dataset CAS 16090-02-1 and J-CHECK 2014, respectively) and assessed in the draft decision before your comments. Study (i) in your comments provides new information that was not reported in your dossier.

We understand that you intend to use all this information as part of a weight of evidence adaptation according to Annex XI, Section 1.2.

We have assessed this information and the reasons for rejecting your weight-of-evidence adaptation provided in the Appendix on General considerations also apply to your comments on the draft decision. In particular, you still have not provided a critical assessment of the

relative weight and of the overall adequacy of the data set in the context of WoE and your WoE adaptation is also not supported by adequate documentation. As another exemplary shortcoming it can be mentioned that no raw data (e.g. cell counts as a funtion of time in each replicate) or any growth curves were provided in the comments. Therefore the independent assessment of the validity of the studies and reliability of the results is not possible.

For all the above reasons, your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2.

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

Appendix B: Reasons for the requests to comply with Annex VIII of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 10 to 100 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII and VIII to REACH.

1. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

An *In vitro* cytogenicity study in mammalian cells or an *In vitro* micronucleus study is a standard information requirement in Annex VIII to REACH.

In your dossier, you have provided

(i) one non-guideline and non-GLP study with the Substance (Ishidate & Odashima, 1977), performed in Chinese hamster lung fibroblasts with negative results. You have named the study as "weight-of-evidence".

In your comments to the draft decision you referred to study (i) and have provided a brief summary of the following new study with the Substance:

(ii) *In vitro* cytogenicity study (according to OECD TG 473, published in the EPA HPVIS database) performed in V79 Chinese hamster cell line and giving negative results with and without metabolic activation.

In addition, you refer to testing according to OECD TG 474 and 475, published in HPVIS database without reporting the studies.

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

A. Annex XI, Section 1.2 specifies that a WoE must rely on several independent sources of information to support the assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property.

You have provided a single *in vitro* cytogenicity study in mammalian cells with the Substance.

In your comments on the draft decision you refer to additonal sources of information, as already specified above. We understand that you intend to use this information alongside the information from study (i) included in your dossier as part of a weight of evidence adaptation according to Annex XI, Section 1.2.

In any case, the adaptation would still not be acceptable for the reasons set out under point C. below

B. ECHA has further assessed the study in your dossier in accordance with Annex XI, Section 1.1.2., although you do not explicitly claim an adaptation. According to Annex XI, Section 1.1.2. a number of cumulative conditions for a non-guideline study to be considered equivalent to a guideline study have to be covered. In particular, the study must provide an adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3).

To fulfil the information requirement of OECD TG 473 or OECD TG 487, respectively[4], the following key parameters have to be met:

a) Two separate test conditions must be assessed: in absence of metabolic activation and in presence of metabolic activation.
b) At least 3 concentrations must be evaluated, in each test condition.
c) At least 300 well-spread metaphases must be scored per concentration
d) 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.
e) The response for the concurrent negative control must be inside the historical control range of the laboratory.
f) Data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures must be reported.

ECHA notes that the study was not conducted according to any of the recommended guidelines.
The reported data for the study you have provided did not include:

a) two separate test conditions, but the information about the metabolic activation is identified as "*not specified*".
b) the evaluation of at least 3 concentrations in each test condition. You state that "three different concentrations" are used, however they are not reported.
c) the scoring of at least 300 metaphases per concentration. Instead, you scored only 100 metaphases.
d) positive control
e) a negative control with a response inside the historical control range of the laboratory.
f) the reporting of data on cytotoxicity and/or the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures.

Therefore, study (i) does not comply with the specific rules of adaptation as set out in Annex XI, Section 1.1.2. as it does not provide a reliable coverage of the key parameter foreseen to be investigated in OECD TG 473 or OECD TG 487 studies.

C. For study (ii), you have provided a brief summary which does not cover all the key parameters mentioned above. Therefore the documentation of this study is insufficient and does not allow an independent assessment of its adequacy, its results and its use for hazard assessment as explain in the part of General considerations.

Further, in your comments to the draft decision you refer to testing according to OECD TG 474 and 475, published in HPVIS database.

A study according to OECD TG 474/475 is a different endpoint (i.e. Section 8.4, Column 2 of Annex VII, VIII or IX) and therefore it cannot be used to fulfil the information requirements for this endpoint. Furthermore, the studies are not included in the dossier subject to the draft decision, neither explained in the comments.

Therefore, the information requirement is not fulfilled.

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

Possibility for data sharing:

ECHA notes that the jointly submitted registration for the Substance contain data which is relevant for this endpoint. In accordance with Title III of the REACH Regulation, you may request it from the other registrant(s) and then make every effort to reach an agreement on the sharing of data and costs.

2. Only if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. is obtained, In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or OECD TG 490), with the Substance;

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

Your dossier contains data for an *in vitro* gene mutation study in bacteria, and data for an *in vitro* cytogenicity study in mammalian cells or in vitro micronucleus study.

The information for the *in vitro* gene mutation study in bacteria and for the *in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study provided in the dossier are rejected for the reasons provided in sections A.1. and B.1. above.

The result of the requests for information in sections A.1. and B.1. will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

Your dossier does not contain any study or adaptation in accordance with column 2 of Annex VIII, Section 8.4.3. or with the general rules of Annex XI for this standard information requirement.

In your comments to the draft decision you have provided a brief summary of the following study with the Substance:

(i) *In vitro* gene muattion study (OECD TG 476, in Chem Res Toxicol, 2006) in L5178Y TK+/- mouse lymphoma cells, which gives negative results with and without metabolic activation.

We have assessed the information you provided in your comments and identified the following issues:

To fulfil the information requirement, the in vitro gene mutation study on mammalian cells has to meet the requirements of OECD TG 476 or OECD TG 490. The key parameter(s) of these test guidelines include:

a) 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.
b) The response for the concurrent negative control must be inside the historical control range of the laboratory.
c) Data on the cytotoxicity and the mutation frequency for the treated and control cultures must be reported.

The brief summary of the study you have provided does not cover all the key parameters mentioned above. Therefore, the documentation of this study is insufficient and does not allow an independent assessment of its adequacy, its results and its use for hazard assessment.

Further, in your comments to the draft decision you refer to testing according to OECD TG 474 and 475, published in HPVIS database.

A study according to OECD TG 474/475 is a different endpoint (i.e. Section 8.4, Column 2 of Annex VII, VIII or IX) and therefore it cannot be used to fulfil the information requirements for this endpoint. Furthermore, the studies are not included in the dossier subject to the draft decision, neither explained in the comments.

Consequently, you are required to provide information for this endpoint, if the *in vitro* gene mutation study in bacteria and the *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study provides a negative result.

Possibility for data sharing:

ECHA notes that the jointly submitted registration for the Substance contain data which is relevant for this endpoint. In accordance with Title III of the REACH Regulation, you may request it from the other registrant(s) and then make every effort to reach an agreement on the sharing of data and costs.

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

A Screening for reproductive/developmental toxicity study (OECD TG 421 or 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 this information requirement by using a WoE approach under Annex XI, Section 1.2. of REACH.

You have provided the following information for this WoE adaptation:

(i) An automated report generated with the OECD QSAR Toolbox v 3.2 based on an information from analogue subtsnaces
(ii) Combined repeated dose and reproductive toxicity study in rats, peformed with the source substance CAS: 41267-43-0 (no guideline, no GLP compliant, assigned reliability score of 2; 2004). Doses: 0, 20, 60, 200 mg/kg bw/day. NOAEL reported to be 200 mg/kg bw/day
(iii) Two generation reproduction and fertility study via oral gavage in rats, performed with the source substance CAS: 16470-24-9 (no guideline, no GLP compliant, assigned reliability score of 4; [redacted] 2001). Doses: 0, 100, 300, 1000 mg/kg bw/day.

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

A. For the reasons explained in the Appendix on General considerations the information obtained from QSAR Toolbox and information on the source substance is not considered reliable and/or relevant for the purpose of identification of the hazard of

the Substance. Your WoE adaptation is also not supported by adequate documentation.

B. For a WoE information to be considered sufficient it must cover the particular dangerous (hazardous) properties of the Substance foreseen to be investigated in a screening for reproduction/developmental toxiicty study performed according to OECD TG 421/422. It must provide conclusive information on male and female reproductive performance which includes data on: gonadal function, mating behaviour, monitoring the oestrus cycle, conception, development of the conceptus and parturition, histopathology of reproductive organs and tissues. In addition, the highest dose level should be chosen with the aim of inducing toxic effects but not death nor obvious suffering.

The source studies you submitted do not provide infromation on male and female reproductive performance, specifically:

1. Investigations for sexual function and fertility, in particular duration of gestation, parturition, lactation and weight and histopathology of reproductive organs and tissues
2. Monitoring of the oestrus cycles
3. In study (ii), the highest dose level did not induce any toxicity. Therefore, the dose level selection is considered too low.

In your comments to the draft decision you did not provide any new information but refer to the studies (ii) and (iii) described above. Based on this, you request ECHA to remove this request from the decision.

We point out that the studies have already been assessed and rejected due to the reasons explained in this decision.

Further, as further explained under the Appendix on general considerations, your WoE adaptation is rejected, and therefore it cannot be used to fulfil the information requirement for this endpoint.

Therefore, none of the sources of information alone or combined allows to conclude whether the Substance has or has not hazardous properties related to pre-natal developmental toxicity.

For all the above reasons, your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2.

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[5] 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

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

material with diet constituents, testing should be done via oral. To minimise contact of the test material with the diet, the schedule described in Appendix E point 6 must be followed.

Appendix C: Reasons for the requests to comply with Annex IX of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 100 to 1000 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII to IX to REACH.

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.)

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

You have adapted the standard information requirement according to Annex XI, Section 1.2. Weight of evidence of REACH.

You have provided the following information for this WoE adaptation:

(i) An automated report generated with the OECD QSAR Toolbox v 3.2 based on an information from analogue substances.
(ii) 90-day oral dietary toxicity study in rats with the Substance (no guideline, no GLP compliant, [redacted] 1971). You assigned a reliability score of 4.
(iii) 90-day oral dietary toxicity study in dogs with the Substance (no guideline, no GLP compliant, [redacted] 1971). You assigned a reliability score of 4.

In your comments to the draft decision you have further provided a brief summary for the following studies with the Substance:
(i) Short-term (28-day) study in rats (OECD TG 407).
(ii) Combined chronic toxicity/carcinogenicity feeding study in rats.
(iii) 10-week study with no species, route of administration and test material identified.

We have assessed the information in your dossier and identified the following issue(s):

A. For the reasons explained in Appendix on General considerations the information obtained from QSAR Toolbox is not considered reliable and relevant for the purpose of identification of the hazard of the Substance. Your WoE adaptation is also not supported by adequate documentation.

B. For a WoE information to be considered sufficient it must moreover cover the particular dangerous (hazardous) properties of the Substance after prolonged administration via the most appropriate route. The study information must cover the following key parameters, among others: recording of body weight, hematology, clinical biochemistry, and pathology of sexual (male and female) organs; full detailed gross necropsy and subsequent histopathology.

You have disregarded the studies (ii) and (iii) by assigning a reliability score of 4. You did not provide any record of body weight, hematology and clinical biochemistry. You did not specify the organs and tissues examined under the gross and histopathological evaluation. Due to the known property of Substance, it may attach to constituents of the standard diet used in animal. Therefore, the dietary route is not the most appropriate one.

In your comments on the draft decision you refer to additonal sources of information, as specified above. We understand that you intend to use this information alongside the information from studies i-iii, included in your dossier as part of a weight of evidence adaptation according to Annex XI, Section 1.2.

We have assessed the information you provided in your comments and identified the following issues:

To be considered compliant and enable concluding whether the Substance has dangerous properties and supports the determination of the No-Observed Adverse Effect Level (NOAEL), a study has to meet the requirements of OECD TG 408. The following key parameter(s) of this test guideline include, among others:

- At least 10 female and 10 male animals should be used at each dose level (including control group)
- Dosing of the Substance daily for a period of 90 days until the scheduled termination of the study

The studies (iii) and (vi) you provided do not have the required exposure duration of 90 days as required in OECD TG 408, because you indicated an exposure duration of 28 days and 10 weeks, respectively. In addition study (iii) was conducted with less than 10 animals per sex per test dose group. The statistical power of the information provided is not sufficient because it does not fulfil the criterion of 20 animals (10 males + 10 females) for each test group set in OECD TG 408.

For study (iv) the test material was not identified and no documentation of this study is presented, therefore we cannot establish its relevance to the registered substance.

Regarding study (v) you have provided a brief summary but no documentation which allows an independent assessment of its adequacy, its results and its use for hazard assessment.

For all the above reasons, none of the sources of information alone or combined allows to conclude whether the Substance has or has not hazardous properties related to pre-natal developmental toxicity. Your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2.

Therefore, your 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 although the information indicate that human exposure to the Substance by the inhalation route is likely, the exposure concentrations reported in the chemical safety report for the inhalation route are low compared to the available data on toxicity of the substance.

Therefore the sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and 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 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. To minimise contact of the test material with the diet, the schedule described in Appendix E point 6 must be followed.

Possibility for data sharing:

ECHA notes that the other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider data sharing these information.

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first 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 according to Annex XI, Section 1.2. Weight of evidence of REACH.

You have provided the following information for this WoE adaptation:

(i) PNDT study in rat via feed, performed with the Substance (no guideline, no GLP compliant, assigned reliability score of 2; publication, 1976)
(ii) Pilot prenatal developmental toxicity study via oral gavage to rats, performed with the source substance CAS: 16470-24-9 (no guideline, no GLP compliant, assigned reliability 4; [redacted] report, 1999);
(iii) Prenatal developmental toxicity study via oral gavage to rats, performed with the source substance CAS: 16470-24-9 (no guideline, no GLP compliant, assigned reliability 4; [redacted] report, 1999)

In your comments to the draft decision you referred to studies (i) and (iii) and have provided a brief summary of the following new studies with analogue substances:

(iv) PNDT study in rabbit (equivalent to OECD TG 414, HPVIS database), performed with structural analogue CAS: 16470-24-9; EC: 240-521-2
(v) PNDT study in rats (no guideline specified), performed with structural analogue CAS: 60650-94-4; EC: 612-006-6

We have assessed the information in your dossier and identified the following issue(s):

A. For the reasons explained in the Appendix on General considerations the information on the source substance is not considered relevant for the purpose of identification of the hazard of the Substance. Your WoE adaptation is also not supported by adequate documentation.

B. For a WoE information to be considered sufficient it must cover the particular dangerous (hazardous) properties of the Substance foreseen to be investigated in a Pre-natal developmental toxicity (PNDT) study performed according to OECD TG 414, This information includes maternal effects and structural abnormalities or altered growth of the fetus observed after an exposure period ranging at least from implantation, throughout gestation until the day before normal delivery.

None of the studies provide information on key parameters, specifically:

- gravid uterus weight, uterine content, body weight of the dams/clinical signs of the dams.
- examination of the sex ratio, number of resorptions and or live foetuses, measurement of anogenital distance in live rodent foetuses
- structural malformations and variations

Therefore, none of the sources of information alone or combined allows to conclude whether the Substance has or has not hazardous properties related to pre-natal developmental toxicity.

In your comments to the draft decision you have provided additional sources of information. We understand that you intend to use this information alongside the information from studies i – iii, included in your dossier as part of a weight of evidence adaptation according to Annex XI, Section 1.2.

We have assessed this information but for the reasons explained in the Appendix on General considerations cannot accept the adaptation based on the information provided. The information obtained from QSAR Toolbox and information on the similar substances is not considered reliable and relevant for the purpose of identification of the hazard of the Substance. Your WoE adaptation is also not supported by adequate documentation. For all these reasons, your adaptation is rejected.

For all the above reasons, your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2.

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 should be performed in rat or rabbit as preferred species with oral[6] 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. To minimise contact of the test material with the diet, the schedule described in Appendix E point 6 must be followed.

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

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

You have provided a study "Long-term toxicity to aquatic invertebrate of disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'disulfonate" indicated as a key study (OECD TG 211, GLP not specified, assigned reliability score 2; Japan chemicals collaborative knowledge database (J-check), 2010).

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

A. According to Article 13(4) of REACH, ecotoxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP). According to Article 141(2), Article 13 applies from entry into application of the REACH Regulation on 1 June 2008.

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

You have not demonstrated that the study was conducted in accordance with GLP.

B. Tests on substances must be conducted in accordance with the OECD test guidelines or another recognised international test method (Article 13(3) of REACH).

The OECD TG 211 is preferred to cover this information requirement. The key parameter(s) of this test guideline include,

- validity criteria of the test guideline need to be met: The mortality of the parent animals (female Daphnia) does not exceed 20% at the end of the test and the mean number of living offspring produced per parent animal surviving at the end of the test is > 60.

Based on the information provided in your dossier, it was not demonstrated that the validity criteria were met because you did not report the mortality of the parent animals and the mean number of living offspring produced per parent animal but you only reported the effect values, NOEC 0.42 mg/L and EC50 1.3 mg/L. Furthermore, you do not specify if the effect values are based on nominal or measured concentrations.

In your comments to the draft decision you now say that you have *"adapted the weight of evidence approach by using the information of the target chemical Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate, CAS 16090-02-1 (EC 240-245-2) from several independent sources."* In your comments you have referred to:

(i) a study according to OECD TG 202 (part 2 "Daphnia sp., Reproduction Test" (1993) for 21 days) on *Daphnia magna* cited from the *"secondary source, 2019"*. 21-day NOEC value was reported to be 0.75 mg/L based on measured concentrations;
(ii) a study according to OECD TG 211 on *Daphnia magna* cited from the J-CHECK (2019). 21-day NOEC value was reported to be 0.42 mg/L based on nominal concentrations, and
(iii) a QSAR prediction based on EPI Suite ECOSAR version 1.11 (2018). NOEC value was estimated to be 0.327 mg/L.

You provide brief summaries from secondary sources on the *Daphnia magna* toxicity studies on the Substance (studies i-ii above). We note that the study ii (cited in your comments as J-CHECK 2019) seem to be the same study already reported in the dossier (cited in the dossier as J-CHECK 2010) and assessed in the draft decision sent to you. The study i (cited in your comments as *"Secondary source, 2019*) is new information that is not reported in your dossier.

We understand that you now intend to use all this information as part of a weight of evidence adaptation according to Annex XI, Section 1.2. We have assessed this additional information from your comments and identified the following issue(s):

The reasons explained in the Appendix on General considerations justifying the rejection of your weight-of-evidence adaptation provided in your dossier also apply to your comments on the draft decision. The documentation of the studies i-ii above is insufficient. More specifically, no raw data (e.g. potential mortality of the parent animals or the number of living offspring produced per parent animal surviving) or any indications whether or not the tests were conducted under the GLP criteria were provided in the comments and therefore the independent assessment of the validity of the studies and reliability of the results is not

possible.

In your comments on the draft decision, you also provide an estimate of the *Daphnia magna* NOEC value based on QSAR prediction (ECOSAR v. 1.11, 2018) (study iii above).

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.

No documentation was provided in your comments or in the dossier to support this prediction. Since no documentation was provided to support your prediction, the assessment of the reliability of the prediction is not possible.

In your comments to the draft decision, you also say that *"long-term toxicity testing on aquatic invertebrates only need to be proposed if the chemical safety assessment according to Annex I indicate the need to investigate further the effects on aquatic organisms. They can be waived based on risk assessment result according to column 2 of Annex IX of REACH regulation.* You continue that *"Also, classification of the substance is also finalized thus this testing request can be waived."*

Based on your comment above, we understand that you intend to adapt this standard information requirement according to Annex IX, Section 9.1.5, Column 2 of REACH.

ECHA has assessed this additional information in your comments and identified the following issue:

As specified in Annex IX, Section 9.1., Column 2, a long-term toxicity study on aquatic invertebrates must be performed unless the Chemical Safety Assessment demonstrates that risks towards the aquatic compartment arising from the use of the Substance are controlled (as per Annex I, section 0.1). The justification must be documented in the Chemical Safety Assessment.

In particular, the Chemical Safety Assessment must take into account the following elements to support that long-term toxicity testing is not required:

- all relevant hazard information from your registration dossier,
- the outcome of the exposure assessment in relation to the uses of the Substance,
- the outcome of the PBT/vPvB assessment including information on relevant degradation products and constituents present in concentration at or above 0.1% (w/w).

In your comments on the draft decision, you did not submit any specific justification as to why the risks of the substance are controlled.

As specified in the reasons for request A.2 and C.4 of this decision, the data on aquatic toxicity is not compliant. Hence your dossier currently does not include adequate information to characterize the hazard property of the Substance. Without this information your Chemical Safety Assessment does not demonstrate that the risks of the Substance are adequately controlled and the Substance is correctly classified for environmental hazards. As a consequence, your adaptation is rejected as it does not meet the specific rules for adaptation of Annex IX, Section 9.1., Column 2.

In conclusion, none of the sources of information alone or combined allows to conclude whether the Substance has or has not hazardous properties related to long-term toxicity to *Daphnia magna*.

For all the above reasons, your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2. or with specific rules for adaptation as set out in Annex IX, Section 9.1., Column 2.

Therefore the information provided does not fulfil information requirement.

4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method OECD TG 210) with the Substance

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

You have provided a study "Long-term toxicity to fish for the test material disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'disulfonate" indicated as a key study (no guideline, GLP not specified, assigned reliability score 2; Japan chemicals collaborative knowledge database (J-check), 2010).

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

A. According to Article 13(4) of REACH, ecotoxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP). According to Article 141(2), Article 13 applies from entry into application of the REACH Regulation on 1 June 2008.

You have not demonstrated that the study was conducted in accordance with GLP.

B. Tests on substances must be conducted in accordance with the OECD test guidelines or another recognised international test method (Article 13(3) of REACH).

The OECD TG 210 is preferred to cover this information requirement. The key parameter(s) of this test guideline include,

- mortality at each stage (embryo, larval, juvenile), hatching (days to hatch, hatching success) and length/weight of surviving fish;
- exposure duration required by OECD TG 210 (varying from 60 days post-hatch for rainbow trout to 30 days for warm water fish)

However, the provided study does not meet those conditions, for the following reasons:

- you only report the determined effect values (NOEC 14 mg/L, EC50 40 mg/L) based on mortality but provide no data or effect values for potential reproductive effects.
- The exposure duration of 14 days is less than the test duration required in OECD TG 210.

In your comments to the draft decision you agree that long-term toxicity testing on fish is a standard information requirement in Annex IX to the REACH Regulation and you continue that "*to fulfil this requirements, weight of evidence approach has been adapted by using the information of the target chemical Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-*

yl)amino]stilbene-2,2'-disulphonate, CAS 16090-02-1 (EC 240-245-2) and a read across analogue Disodium 2,2'-([1,1'-biphenyl]-4,4'-diyldivinylene)bis(benzenesulphonate), CAS 27344-41-8 (EC 248-421-0)." In your comments you have referred to:

(i) a study according to OECD TG 204 on the Substance cited from the "*J-CHECK, 2019*". 14-day NOEC value for the fish (species not specified) was reported to be 40 mg/L based on nominal concentrations;

(ii) a study according to OECD TG 204 on the Substance reported without any other reference to the source than the year (1993). 14-day NOEC value for *Brachydanio rerio* (Zebra fish) was reported to be 61.8 mg/L based on measured concentrations, and

(iii) a study according to OECD TG 210 on the analogue substance Disodium 2,2'-([1,1'-biphenyl]-4,4'-diyldivinylene) bis (benzenesulphonate), CAS 27344-41-8 (EC 248-421-0) without any reference to the source of the study. 28-day NOEC for the *Brachydanio rerio* (Zebra fish) was determined to be >1 mg/L based on nominal concentrations.

You provide brief summaries from secondary sources on the fish toxicity studies on the Substance (studies i-ii above) and on the analogue substance Disodium 2,2'- ([1,1'-biphenyl]-4,4'-diyldivinylene) bis (benzenesulphonate), CAS 27344-41-8 (EC 248-421-0) (study iii above). We note that the study i (cited in your comments as J-CHECK 2019) seem to be the same study already reported in the dossier (cited in the dossier as J-CHECK 2010) and assessed in the draft decision sent to you. The study ii (cited in your comments only by year 1993) and study iii (no reference given to the source of the study) are new information that are not reported in your dossier.

We understand that you now intend to use all the above information as part of a weight of evidence adaptation according to Annex XI, Section 1.2. We have assessed this additional information from your comments and identified the following issues:

The reasons explained in the Appendix on General considerations justifying the rejection of your weight-of-evidence adaptation provided in your dossier also apply to the your comments on the draft decision. The documentation of all these studies is insufficient. More specifically, the issues reported in the draft decision (*i.e.* study not conducted in accordance with GLP, exposure duration of 14 days is too short, and reproductive parameters were not recorded) apply also to the information that you provide in your comments for the studies i and ii.

Based on your comments, the study iii was conducted with the analogue substance but no documentation was provided to justify the use of the read across except the claim in the comments that the target chemical and the read across analogue are structurally similar. Therefore, independent assessments of the validity and the reliability of the results of the studies i and ii, and the assessment of the acceptability of the data on the analogue substance in the study iii are not possible.

In your comments to the draft decision, you also say that "*long-term toxicity testing on aquatic fish only need to be proposed if the chemical safety assessment according to Annex I indicate the need to investigate further the effects on aquatic organisms. They can be waived based on risk assessment result according to column 2 of Annex IX of REACH regulation.* You continue that "*Also, classification of the substance is also finalized thus this testing request can be waived.*"

Based on your comment above, we understand that you intend to adapt this standard information requirement according to Annex IX, Section 9.1.5, Column 2 of REACH.

ECHA has assessed this additional information in your comments and identified the following issue:

As specified in Annex IX, Section 9.1., Column 2, a long-term toxicity study on fish must be performed unless the Chemical Safety Assessment demonstrates that risks towards the aquatic compartment arising from the use of the Substance are controlled (as per Annex I, section 0.1). The justification must be documented in the Chemical Safety Assessment.

In particular, the Chemical Safety Assessment must take into account the following elements to support that long-term toxicity testing is not required:

- all relevant hazard information from your registration dossier,
- the outcome of the exposure assessment in relation to the uses of the Substance,
- the outcome of the PBT/vPvB assessment including information on relevant degradation products and constituents present in concentration at or above 0.1% (w/w).

In your comments on the draft decision, you did not submit any specific justification as to why the risks of the substance are controlled.

As specified in request A.2 and C.3 of this decision, the data on aquatic toxicity is not compliant. Hence your dossier currently does not include adequate information to characterize the hazard property of the Substance. Without this information your Chemical Safety Assessment does not demonstrate that the risks of the Substance are adequately controlled and the Substance is correctly classified for environmental hazards. As a consequence, your adaptation is rejected as it does not meet the specific rules for adaptation of Annex IX, Section 9.1., Column 2.

For all the above reasons, your adaptation do not comply with the general rules of adaptation as set out in Annex XI, Section 1.2. or with specific rules for adaptation as set out in Annex IX, Section 9.1., Column 2.

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

Appendix D: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The compliance check was initiated on 4 February 2019.

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

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

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

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

Appendix E: Observations and technical guidance

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

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

3. Test guidelines, GLP requirements and reporting

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

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

   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: 'How to report robust study summaries'[7].

4. Testing strategy for aquatic toxicity testing

   Before conducting the requested aquatic toxicity tests (requests A.2, C.3, C.4) you should consult the Integrated Testing Strategy described in ECHA Guidance R.7b, Section R.7.8.5 (including Figure R.7.8-4), on the sequence in which the aquatic long-term toxicity tests are to be conducted and the necessity to conduct all the requested studies, in particular the long-term fish toxicity study.

   If you decide to omit some of the studies requested in this decision, you must provide full documentation to justify the adaptation.

5. Test material

   Selection of the test material(s)

   The registrant of the Substance is responsible for the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected test material must contain that constituent/ impurity.

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

Technical reporting of the test material

The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance.

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[8].

6. 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[9]. For rabbits, the passage of food through the stomach is estimated to be 3 – 6 hours[10].

7. List of references of the ECHA Guidance and other guidance/ reference documents[11]

   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 in this decision.

   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 in this decision.

   ECHA Read-across assessment framework (RAAF, March 2017)[12]

   Physical-chemical properties

[8] https://echa.europa.eu/manuals
[9] R.A. Purdon and P. Bass (1973), Gastroenterology 64: 968-976
[10] R. R. Davies et al. (2003), Vet Clin Exot Anim 6: 139–153
[11] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[12] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

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.

OECD Guidance documents[13]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.
Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

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

Appendix F: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]