~~Confidential~~

Helsinki, 12 May 2021

Addressees
Registrant(s) of JS_12237-62-6_[redacted] as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
10/01/2020

Registered substance subject to this decision ("the Substance")
Substance name: Ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts
EC number: 235-468-7
CAS number: 12237-62-6

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***17 August 2023***.

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

Many of this type of organic pigments are listed in various national inventories of nanomaterials, such as the French nano-particulate substances reporting system.[1] In the case where the Substance is manufactured and/or imported in the European Union in nanoforms by any addressee of the present decision, the REACH Regulation (as amended by Regulation Commission Regulation (EU) 2018/1881) sets out explicit information requirements for nanoforms of substances. Manufacturers and/or importers of nanoforms must have fulfilled these specific information requirements by 1st January 2020. As far as the registration dossiers currently submitted on the Substance by any addressee of the present decision they do not cover any nanoform. Any incompliances identified in the present decision on the Substance relate only to information required on non-nanoforms.

Based on the above, the requested information in this present decision must be generated using exclusively non-nanoforms of the Substance.

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

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

2. Long-term toxicity testing on aquatic invertebrates requested below (triggered by Annex VII, Section 9.1.1., column 2; test method EC 20/OECD TG 211)

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

[1] "*Dispositif de déclaration des substances à l'état nanoparticulaire* », Decree 2012-232 of French *Conseil d'Etat* of 17 February 2012.

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

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

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

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) by oral route, in rats

4. Adsorption/ desorption screening (Annex VIII, Section 9.3.1.; test method: OECD TG 106 or OECD TG 121)

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

1. Dissociation constant (Annex IX, Section 7.16.; test method OECD TG 112)

2. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 413) by inhalation route, in rats

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

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

5. 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/appendices:

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

Information required depends on your tonnage band

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

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

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

How to comply with your information requirements

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

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

Appeal

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

Failure to comply

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

Authorised[2] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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

Appendix on Reasons common to several requests

1. Assessment of your read-across approach under Annex XI, Section 1.5.

You seek to adapt the following standard information requirements by applying (a) read-across approach(es) in accordance with Annex XI, Section 1.5:

In your dossier:
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
- Adsorption/desorption screening (Annex VIII, Section 9.3.1)

In your comments on the initial draft decision:
- Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2 and Annex IX, Section 9.1.5.)
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6.)

ECHA has considered the scientific and regulatory validity of your read-across approach(es) in general before assessing the specific standard information requirements in the following appendices.

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[3] and related documents[4, 5].

In your comments to the initial draft decision you provided a read-across justification document and indicated your intention to update the registration dossier accordingly.

As the analogues are used as source substances to predict the property of the Substance, we understand that you have adapted the standard information requirements under Annex XI, Section 1.5 to REACH (grouping and read-across). You clarify in your comments that you used the QSAR Toolbox for the identification of analogues and use information on these analogues to predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are

[3] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals. 2008 (May) ECHA, Helsinki. 134. pp. Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

[4] Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)

[5] Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017 (March) ECHA, Helsinki. 40 pp. Available online: https://doi.org/10.2823/794394

predicted to be quantitatively equal to those of the source substance(s).

A. Predictions for (eco)toxicological properties

You have not provided a read-across justification document in your technical dossier, but you have provided it with your comments on the initial draft decision.

For the endpoints listed above, you used data from the following source substances:

- Green S / Hydrogen[4-[4-(dimethylamino)-α-(2-hydroxy-3,6-disulphonato-1-naphthyl) benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium, monosodium salt (EC 221-409-2)
- Magenta / Benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)-methyl]-2-methyl (EC 211-189-6)
- C.I. Basic Violet 2 / Benzenamine,4,4'-((4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methylene)bis(2-methyl-,monohydrochloride (EC 221-831-7)
- C.I. basic violet 4 / 4-{bis[4-(diethylamino)phenyl]methylidene}-N,N-diethylcyclohexa-2,5-dien-1-iminium chloride (EC 219-231-5)
- Acid Green 50 / Hydrogen[4-[4-(dimethylamino)-α-(2-hydroxy-3,6-disulphonato-1-naphthyl)benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium, monosodium salt (EC 221-409-2)
- Fast green FCF / Dihydrogen(ethyl)[4-[4-[ethyl(3-sulphonatobenzyl)amino](4-hydroxy-2-sulphonatobenzhydrylidene]cyclohexa-2,5-dien-1-ylidene] (3-sulphonatobenzyl)ammonium, disodium salt (EC 219-091-5)
- 1,4-Bis(p-tolylamino)anthraquinone (EC 204-909-5)
- 4,4'-methylenebis(N-methylaniline) (EC 217-309-3)
- [4-[[4-(dimethylamino)phenyl][4-(methylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (EC 282-846-2).

In your comments on the initial draft decision you provided further information on the following source substances in your read-across justification document

- [4-[(4-dimethylaminophenyl)-[4-(methylamino)phenyl]methylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (EC 616-846-4)
- [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6)
- [4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]diethylammonium acetate (EC 278-585-9)
- 4-[[4-(dimethylamino)phenyl]-phenylmethyl]-N,N-dimethylaniline (EC 204-961-9)

For the environmental endpoints, you have mentioned the following additional source substance in the comments on the initial draft decision, however the read-across justification document doesn't include it:

- α,α-bis[4-(dimethylamino)phenyl]-4-(phenylamino)naphthalene-1-methanol (EC 229-851-8)

You have provided the following reasoning for the prediction of toxicological properties: *"the target and read-across substances covered in this justification have common properties and present comparable toxicological behavior".*

You provided a read-across justification with your comments on the initial draft decision and indicated your intention to update the registration dossier accordingly. In your justification document you have indicated that RAAF 'Scenario 2' was selected for the analogue approach. You provided the following reasoning for the prediction of (eco)toxicological properties: *"read-across of toxicological data from an analogue may be justified based on:*

- *Identifying the read across substances based on common functional groups and further*

filled with relate mechanistic approaches and finally fine-tuned with structural similarity using the QSAR Toolbox Version 3.4
- *Common structural alerts or reactivity*
- *Common physico-chemical properties*
- *Likelihood of common breakdown products via biological/degradation processes"*

You conclude that "*the descriptors, various alerts and scenario (for analogue approach) which were taken into consideration for toxicological assessment as reported in this RA justification document obtained by using OECD QSAR toolbox v.3.4 of the target substance and source substances (i.e., read across analogues) were evaluated to be similar and therefore justified and appropriate*".

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects, and that the properties of your Substance are predicted to be quantitatively equal to those of the source substance.

ECHA notes the following shortcomings with regards to prediction of (eco)toxicological properties.

Read-across documentation

Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide a justification for the read-across including a hypothesis, explanation of the rationale for the prediction of properties and robust study summary(ies) of the source study(ies).[6]

You have provided studies conducted with other substances than your Substance in order to comply with the REACH information requirements. In your dossier, you have not provided documentation as to why this information is relevant for your Substance.

The documentation of the studies provided in your comments on the initial draft decision do not cover sufficient information to make an independent assessment of the study as indicated under the endpoints.

In the absence of such documentation, ECHA cannot verify that the properties of your Substance can be predicted from the data on the source substance(s).

Characterisation of the source substance(s)

Annex XI, Section 1.5 states that "*physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)*".

According to the ECHA Guidance, "*the purity and impurity profiles of the substance and the structural analogue need to be assessed*", and "*the extent to which differences in the purity and impurities are likely to influence the overall toxicity needs to be addressed, and where technically possible, excluded*". The purity profile and composition can influence the overall toxicity/properties of the potential source substances, including test materials.[7] Therefore, qualitative and quantitative information on the compositions of the test materials should be

[6] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.6.1
[7] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.4.1

provided to allow assessment whether the attempted predictions are compromised by the composition and/or impurities.

The provided 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 source substances.

You do not provide any description of the source substances introduced in your dossier. Furthermore, for all the studies provided in the technical dossier that were conducted with these substances, as listed above, no information on the composition of the test material used to generate the source data is provided.

The read-across justification document attached to your comments on the initial draft decision specifies the type of the additional source substances (mono-constituent or UVCB) without further characterisation on purity profile and composition.

Regarding your consolidated comments on the initial draft decision for the sources substances for the environment, you have indicated these substances can be considered as potential read-across due to the presence of a common organic moiety "triphenyl methane, despite the % of similarity being low. You state further that this similarity is based on the presence of inorganic moiety "copper ferrocyanide" in one of the sources substances, CAS 12237-62-6. However, you have not provided any further characterisation on purity profile and compositional information that could support your comments.

Without such information, no qualitative or quantitative comparative assessment of the compositions of the different test materials can be completed. Therefore, is not possible to assess whether the attempted predictions are compromised by the composition of the test materials and their relation to source and target substances.

Adequacy and reliability of source study

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

- be adequate for the purpose of classification and labelling and/or risk assessment;
- have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3);
- cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter.

Test material identity

As described above under "Characterisation of the source substance(s)", purity and impurity profiles of the substance and the structural analogue need to be assessed.

You do not provide any description of the source substances introduced in your dossier. Furthermore, for all the studies provided in the technical dossier that were conducted with these substances, as listed above, no information on the composition of the test material used to generate the source data is provided.

The read-across justification document attached to your comments on the initial draft decision provide short summaries of additional source studies on additional source substances. You did not provide characterisation on purity profile and composition of the substances tested in these studies.

Due to the above deficiency, it is not possible to assess whether the test material is representative for the source substance and thus relevant to the Substance. Therefore, the studies listed above are not adequate for the purpose of classification and labelling and/or risk assessment.

Missing supporting information to compare properties of the substances

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*"[8]. 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 bridging studies to compare properties of the Substance and source 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 types of 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).

You have provided studies in the dossier and in the comments on the draft decision which have been conducted with source substances. You have not provided studies that were conducted with the Substance on the endpoints for which you have submitted a read-across adaptation. ECHA notes the *in vitro* mammalian cell gene mutation study on the Substance is considered as not fulfilling the adequacy and reliability criteria, as explained in Appendix B.2 of this decision.

Therefore, there is no endpoint-specific information (bridging studies) available to compare properties of the source substances with those of the target substance. The data set reported in the technical dossier and with the comments on the draft decision does not include relevant, reliable and adequate information for the Substance and of the source substance(s) to support your read-across hypothesis.

In the absence of such information, you have not established that the Substance and of the source substance(s) are likely to have similar properties. Therefore, you have not provided sufficient supporting information to strengthen the rationale for the read-across.

There are additional deficiencies with the studies you have provided for the endpoints A.1.,B.2-3, and C.2-3. These deficiencies are discussed under the respective endpoints.

B. Conclusions on the read-across approach

As explained above, you have not established that relevant properties of the Substance can be predicted from data on the analogue substances. Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. and your grouping and read-across approach is rejected.

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

[8] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

ECHA understands that you have adapted the following standard information requirements by applying weight of evidence (WoE) adaptation in accordance with Annex XI, section 1.2:

1. Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
2. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)
4. Adsorption/ desorption screening (Annex VIII, Section 9.3.1.)

Your weight of evidence adaptation raises the same deficiencies irrespective of the information requirement for which it is invoked. Accordingly, ECHA addressed these deficiencies in the present Appendix, before assessing the specific standard information requirements in the following appendices.

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

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

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

However, for each relevant information requirement, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has or has not a particular dangerous property.

In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation.

The issue identified below is essential for all the information requirements in which you invoked a weight of evidence.

Reliability of the read across approach

Section 1. of the present Appendix identifies deficiencies of the grouping and read across approach used in your dossier. These finding apply equally to the sources of information relating to analogue substances submitted under your weight of evidence adaptations.

Assigned reliability of studies

The following study has been given a reliability score of 4 (non-assignable) by you with no further justification:

1. Non-guideline 3-generation study (1987)

Therefore the studies cannot be regarded as reliable.

Study conducted after 2008 and not GLP compliant

Since 1 June 2008, toxicological and eco-toxicological tests and analyses on substances must be carried out in compliance with the principles of good laboratory practice (GLP) (Article 13(4) and Article 141(2) of REACH).

The following studies listed below have been performed after 1 August 2008 and not GLP or with GLP compliance not specified

1. Adsorption/desorption study (OECD TG 121, 2018).

Therefore the study cannot be regarded as reliable.

3. Assessment of your Qualitative and Quantitative structure-activity relationship ((Q)SAR) under Annex XI section 1.3

You have adapted the following standard information requirements by applying Qualitative and Quantitative structure-activity relationship ((Q)SAR) adaptation in accordance with Annex XI, section 1.3:

- Adsorption/desorption (Annex VII, Section 9.1.3.)
- 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.)

While an adaptation was not specifically indicated by you, ECHA has evaluated the provided information under the rules set in Annex XI, Section 1.3. Qualitative or quantitative structure-activity relationship (QSAR).

Annex XI, Section 1.3. states that 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 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.

Selection of the representative structure

Under ECHA Guidance R.6.1.7.3., that a prediction is adequate for the purpose of classification and labelling and/or risk assessment if the following cumulative conditions are met:

- the composition of the substance is clearly defined, and
- representative structure(s) for the assessment are selected.

Your registration dossier provides the following information:

- In Section 1.1 of your technical dossier, you define the Substance as organic UVCB
- In Section 1.2, you indicate the following components in the composition of your Substance:
    - Pigment Violet 27 / ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts ([redacted]
    - Pigment Violet 27 (Hexa isomer) ([redacted]

  - Pigment Violet 27 (Hexa isomer) ([redacted]
  - Sodium acetate ([redacted]
- For the assessment, you provided predictions for the following structures:
  - ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts ([redacted]

You have considered Pigment Violet 27 as representative structure(s) but failed to explain how you made this selection.

Therefore, you have not demonstrated that the prediction is adequate for the purpose of classification and labelling and/or risk assessment.

The substance is outside the applicability domain of the model.

Under ECHA Guidance R.6.1.5.3. a prediction is within the applicability domain of the model, when, among others, the substance and the structures selected for the prediction fall within descriptor, structural, mechanistic and metabolic domain.

Your registration dossier provides the following information

- The substance is an UVCB. There are 4 constituents indicated in section 1.2 of the dossier.:
  1. Pigment Violet 27 / ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts ([redacted]
  2. Pigment Violet 27 (Hexa isomer) ([redacted]
  3. Pigment Violet 27 (Hexa isomer) ([redacted]
  4. Sodium acetate [redacted]

Three out of four of the constituents of the UVCB substance (constituents 1, 2, and 3) fall outside the applicability domain of the model because they contain the following fragments not covered by AD of EPISUITE:

[redacted]

Therefore, you have not demonstrated that the Substance falls within the applicability domain of the model.

Lack of documentation of the model (QMRF)

The Appendix C of the OECD Guidance document on the validation of (Q)SAR models (ENV/JM/MONO(2007)2) and ECHA Guidance R.6.1.6.3 state that the information specified in or equivalent to the (Q)SAR Model Reporting Format (QMRF) template must be provided to have adequate and reliable documentation of the applied method. For a QMRF this includes:

- The predicted endpoint, including information on experimental protocol and data quality for the data used to develop the model,
- An explicit definition of the algorithm and the descriptor used,
- The definition of applicability domain,
- The goodness-of-fit and robustness of the model, including information on training set and validation statistics.

You have not provided any QMRF document in your technical dossier.

In absence of such information, you have not provided adequate and reliable documentation and therefore ECHA is not in a position to conclude on the validity of the model for prediction of the toxicological properties.

Lack of documentation of the prediction (QPRF)

ECHA Guidance R.6.1.6.3 states that the information specified in or equivalent to the (Q)SAR prediction reporting format (QPRF) template must be provided to have adequate and reliable documentation of the applied method. For a QPRF this includes, among others:

- the model prediction(s), including the endpoint,
- a precise identification of the substance modelled,
- the relationship between the modelled substance and the defined applicability domain,
- the identities of close analogues, including considerations on how predicted and experimental data for analogues support the prediction.

You have not provided information on any of the elements mentioned above

In absence of such information, you have not provided adequate and reliable documentation and therefore ECHA is not in a position to conclude on the validity of the prediction of the toxicological properties.

Further, specific considerations are addressed under the individual information requirements.

Therefore, your adaptations do not comply with the general rules of adaptation as set out in Annex XI, Section 1.3. and your (Q)SAR adaptations are rejected.

4. Assessment of the identity of the test material

The following issue concerns all the studies conducted for the following standard information requirements:

1. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
2. Adsorption/ desorption screening (Annex VIII, Section 9.3.1.)

You have provided studies for 1 and 2 above under the endpoint in Appendices A-B that you claim were conducted with the Substance.

To comply with these information requirements, the test material in a study must be representative for the Substance (Art. 10 and Recital 19 of REACH; ECHA Guidance R.4.1).

To identify the test materials in all the studies for 1 and 2 above under the endpoint, you have provided the substance name, EC and/or CAS number, and/or the purity of the Substance in water. Information on the detailed composition, including quantitative and qualitative information on all constituents present in the test material, or production process of the test material has not been provided.

Without comprehensive reporting of all constituents present in the test material (including their identity and concentrations) ECHA is unable to confirm that the test materials are representative of the Substance.

Therefore, the provided information is rejected.

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

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is an information requirement under Annex VII to REACH (Section 8.4.1.).

You have provided a key study in your dossier:

i. In vitro gene mutation study in bacteria (2018) with the Substance with the following strains, TA 98, TA 100, TA 1535, TA 1537, and TA 102 which all gave negative results.

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

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471[9] (1997). The key parameters of this test guideline include:

a) The maximum dose tested must induce a reduction in the number of revertant colonies per plate compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test dose must correspond to 5 mg/plate or 5 ml/plate.
b) The number of revertant colonies per plate for the concurrent negative control must be inside the historical control range of the laboratory.

The reported data for the study you have provided did not include:

a) a maximum dose of 5 mg/plate or 5 ml/plate or that induced a reduction in the number of revertant colonies per plate compared to the negative control, or the precipitation of the tested substance.
b) a confirmed negative control with a number of revertant colonies per plate inside the historical control range of the laboratory, because the historical control range of the laboratory is not reported.

The information provided does not cover the key parameters required by OECD TG 471.

In your comments to the draft decision you indicate your agreement and intention to re-test the Substance according to OECD TG 471.

Therefore, the information requirement is not fulfilled.

Study design

To fulfil the information requirement for the Substance, the *in vitro* gene mutation study in bacteria (OECD TG 471) is considered suitable.

2. Long-term toxicity testing on aquatic invertebrates

Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.). Long-term toxicity testing on aquatic invertebrates must be considered (Section 9.1.1., Column 2) if the substance is poorly water soluble.

You have provided a short term toxicity study on aquatic invertebrates according to EEC Guideline - L251 Vol. 27, 84/449/EEC: C. (static procedure) (1989) but no information on long-term toxicity on aquatic invertebrates for the Substance.

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

We have assessed this information and identified the following issue:

Poorly water soluble substances require longer time to reach steady-state conditions. As a result, the short-term tests does not give a true measure of toxicity for this type of substances and the long-term test is required. A substance is regarded as poorly water soluble if, for instance, it has a water solubility below 1 mg/L or below the detection limit of the analytical method of the test material (ECHA Guidance R.7b, Section 7.8.5).

In the provided OECD TG 105 (2018), the substance is poorly water soluble (<1mg/L) as the solubility was determined to be 0.2843 mg/L at 25°C

Your comments ono the initial draft decision on this request are addressed under section C.4.

Therefore, the information on long-term toxicity on aquatic invertebrates must be provided.

The examination of the information provided, as well as the selection of the requested test and the test design are addressed under section C.4.

3. Growth inhibition study aquatic plants

Growth inhibition study aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

You have provided the following information:

i) An OECD TG 201 key study (2018) with the registered Substance
ii) An adaptation under Annex XI, Section 1.5. In support to your adaptation, you have provided the following study:
   - An OECD TG 201 supporting study (2017) with the analogue substance EC 282-846-2

We have assessed this information and identified the following issues

A. To fulfil the information requirement, a study must comply with OECD TG 201 and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test (Article 13(3) of REACH). The following specifications must be met:

Characterisation of exposure

- 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. Alternatively, a justification why the analytical monitoring of exposure concentrations is not technically feasible must be provided;
- the concentrations of the test material are measured at least at the beginning and end of the test:
  1) at the highest, and
  2) at the lowest test concentration, and
  3) at a concentration around the expected EC_{50}.

Additional requirements applicable to difficult to test substances

- if the test material is poorly water soluble, the maximum dissolved concentration that can be achieved in the specific test solution under the test conditions is determined;
- if losses of the test material are expected within the timeframe of the test, a preliminary stability study is conducted.

In your registration dossier, you provided the following on study i):
Characterisation of exposure

- no analytical monitoring of exposure was conducted with no further justification;

Additional requirements applicable to difficult to test substances

- the maximum dissolved concentration that can be achieved in the specific test solution is not reported;
- the substance has a low solubility and high adsorption potential and therefore losses of the test material may be expected. The result of a preliminary stability study is not reported in the study;

In addition, tests and analyses on the intrinsic properties of substances must be carried out in compliance with the principles of good laboratory practice (GLP) provided for in Directive 2004/10/EC or other international standards recognised as being equivalent by the Commission or ECHA and with the provisions of Directive 86/609/EEC, if applicable (Article 13(4) of REACH). According to Article 141(2), Article 13 applies from 1 June 2008.

The provided study i) was indicated as not being performed according to GLP without further explanation.

Therefore, the study i) must be rejected.

As explained in the Appendix on Reasons common to several requests your read-across adaptation (Annex XI, Section 1.5) is rejected for study ii).

In your comments to the draft decision you agree to perform the requested study.

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

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

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is an information requirement under Annex VIII to REACH (Section 8.4.2.).

You have provided a key study in your dossier:

i. In Vitro Mammalian Chromosome Aberration Test (2019) with the Substance

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

To fulfil the information requirement, the study has to be an *in vitro* chromosomal aberration test or an *in vitro* micronucleus test, conducted in mammalian cells in accordance with OECD TG 473 or OECD TG 487, respectively[10]. The key parameter(s) of these test guidelines include:

a) The maximum concentration tested must induce 55+5% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration must correspond to 10 mM, 2 mg/mL or 2 µl/mL, whichever is the lowest.

The reported data for the study you have provided did not include:

a) a maximum tested concentration of 10 mM, 2 mg/mL or 2 µl/mL, or that induced 55+5% of cytotoxicity compared to the negative control, or the precipitation of the tested substance.

The information provided does not cover key parameter required by OECD TG 473.

In your comments to the draft decision you indicate your agreement and intention to re-test the Substance according to OECD TG 473.

Therefore, the information requirement is not fulfilled.

2. In vitro gene mutation study in mammalian cells

An *in vitro* gene mutation study in mammalian cells is an information requirement under Annex VIII to REACH (Section 8.4.3.) 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 section 1 of this Appendix and section 1 of Appendix A.

The result of the requests for information in section 1 of this Appendix and section 1 of Appendix A 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.

For Annex VIII, 8.4.3., you have provided a study with the Substance in your dossier.

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

However, you have adapted this information requirement also by using grouping of substances and read-across approach under Annex XI 1.5.

You have provided the following sources of information:

i. In vitro mammalian cell gene mutation test (2015) on the Substance
ii. In vitro mammalian cell gene mutation test (2006) on source substance 4-{bis[4-(diethylamino)phenyl]methylidene}-N,N-diethylcyclohexa-2,5-dien-1-iminium chloride (EC 219-231-5)

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

Study on the Substance

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[11]. The key parameter(s) of these test guidelines include:

a) The maximum concentration tested must induce 80-90% of cytotoxicity compared to the negative control (according to relative survival percentage calculations), or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration must correspond to 10 mM, 2 mg/mL or 2 µl/mL, whichever is the lowest.
b) 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.
c) The response for the concurrent negative control must be inside the historical control range of the laboratory.
d) Data on the cytotoxicity for the treated and control cultures must be reported (including relative survival percentages calculations).
e) The minimum number of cells used for each test (control and treated) culture at each stage in the test should be based on the spontaneous mutant frequency, in order to ensure a sufficient number of spontaneous mutants in every culture in all phases of the test.

The reported data for the study i) you have provided do not include:

a) a maximum tested concentration of 10 mM, 2 mg/mL or 2 µl/mL, or that induced 80-90% of cytotoxicity compared to the negative control (presentation of data with relative survival percentage calculations not provided), or the precipitation of the tested substance.
b) positive control that produced a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control in cultures with the selection agent in absence of metabolic activation.
c) a negative control with a response inside the historical control range of the laboratory.
d) data on the cytotoxicity for the treated and control cultures (presentation of data with relative survival percentage calculations).
e) a sufficient number of spontaneous mutants in every culture in all phases of the test, as zero spontaneous mutants were observed in several test conditions.

The information provided does not cover key parameter(s) required by OECD TG 476.

In addition, tests and analyses on the intrinsic properties of substances must be carried out in compliance with the principles of good laboratory practice (GLP) provided for in Directive 2004/10/EC or other international standards recognised as being equivalent by the

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

Commission or ECHA and with the provisions of Directive 86/609/EEC, if applicable (Article 13(4) of REACH). According to Article 141(2), Article 13 applies from 1 June 2008.

The provided study i) was indicated as not being performed according to GLP without further explanation.

Therefore, this information is rejected.

On this basis, the information requirement is not fulfilled.

Annex XI adaptation

As explained in Section 1 of the Appendix common to several requests, your adaptation under Annex XI, Section 1.5. is rejected. In addition, the grouping of substances and read-across approach must fulfil the information requirement based on reliable sources of information. The reliability of the study ii) of information is affected by the following issues.

According to Annex XI, Section 1.5., the *in vitro* gene mutation study on mammalian cells must have adequate and reliable coverage of the key parameters of OECD TG OECD TG 490[12]. The key parameter(s) of these test guidelines include:

a) The maximum concentration tested must induce 80-90% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration must correspond to 10 mM, 2 mg/mL or 2 µl/mL, whichever is the lowest.
b) Data on the cytotoxicity and the mutation frequency for the treated and control cultures must be reported.

The reported data for the study ii) you have provided do not include:

a) a maximum tested concentration of 10 mM, 2 mg/mL or 2 µl/mL, or that induced 80-90% of cytotoxicity compared to the negative control, or the precipitation of the tested substance.
b) data on the cytotoxicity and the mutation frequency for the treated and control cultures (including number of colonies).

The information provided does not cover key parameter(s) required by OECD TG 490.

Therefore, the information requirement is not fulfilled.

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 provide a negative result.

In your comments to the draft decision you indicate your agreement to re-consider the information requirement after the results of the studies requested under section 1 of Appendix A and section 1 of this Appendix B become available.

3. Screening for reproductive/developmental toxicity

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

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

You have adapted this information requirement by using Weight of Evidence under Annex XI, Section 1.2 of REACH and Annex XI, Section 1.5 (grouping of substances and read-across).

You have provided the following sources of information:

i) Non-guideline teratogenicity and embryotoxicity study (1987) on source substance Hydrogen[4-[4-(dimethylamino)-α-(2-hydroxy-3,6-disulphonato-1-naphthyl)benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium, monosodium salt (EC 221-409-2)

ii) Non-guideline 3-generation study (1987) on source substance Dihydrogen(ethyl)[4-[4-[ethyl(3-sulphonatobenzyl)amino](4-hydroxy-2-sulphonatobenzhydrylidene]cyclohexa-2,5-dien-1-ylidene] (3-sulphonatobenzyl)ammonium, disodium salt (EC 219-091-5). RL 4.

In your comments to the draft decision you provide short summaries of two additional studies:

iii) Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (2009) on source substance [4-[(4-dimethylaminophenyl)-[4-(methylamino)phenyl]methylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (CAS 8004-87-3; EC 616-846-4)

iv) Generation reproductive toxicity study (1988) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (CAS 548-62-9; EC 208-953-6). RL 4.

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

A. Read-across

As explained in the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.5. is rejected.

B. Weight of evidence

As explained in Section 2 of the Appendix common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

To fulfil the information requirement, normally a study according to OECD TG 421/422 must be provided. The key elements investigated by this test is 1) sexual function and fertility, 2) toxicity to offspring, and 3) systemic toxicity.

The provided sources of information investigate all three key elements. Therefore, they provide information that would contribute to the conclusion on them.

However, the reliability of the sources of information is significantly affected by the deficiencies identified in Section 2 of the Appendix on Reasons common to several requests.

In addition, ECHA has made the following observations on the data provided in the comments:

The OECD TG 421/422 provides the following specifications:

- highest dose level should aim to induce some systemic toxicity, but not death or severe suffering
- a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL)
- dosing of the test substance for a minimum of four weeks for males and approx. 63

days for females to cover premating, conception, pregnancy and at least 13 days of lactation
- examination of key parameters for toxicity such as thyroid hormone assessment (P0 and F1)
- examination of the animals for histopathology (including thyroid gland)
- pathology of sexual (male and female) organs, full detailed gross necropsy and subsequent histopathology of both types of tissues

The study iii) does not provide any consideration on the setting of the highest dose level. Mortality was observed in the high dose group (4/12 and 5/12 males and females). Therefore, you have not demonstrated that the highest dose level was aiming to induce some systemic toxicity but not death or severe suffering. In addition, you report "*female rats were treated 14 days before mating and up to day 4 of lactation (for 41 to 48 days in total)*". It is not clear whether the conducted investigations cover organ weight and histopathology of thyroid and thyroid hormone measurements or sexual organs based on the short summary provided for the study in your comments.

In addition, source study ii) and iv) have been given a reliability score of 4 by you (not assignable), and ECHA agrees that these source studies are not assignable.

Therefore, the provided studies cannot be considered a reliable source of information.

As a conclusion, the sources of information as indicated above, provide information on sexual function and fertility, toxicity to offspring, and systemic toxicity; but their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated by the required study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

In your comments to the draft decision you indicate your intention to apply self-classification to the Substance for Reproductive Toxicity Category 2 (H361d: Suspected of damaging the unborn child), and waiving the information requirement as not justified in light of the classification.

ECHA notes that the information requirement cannot be waived on basis of classification for Reproductive toxicity Category 2. Column 2 of Annex VIII, section 8.7.1 outlines the applicable criteria for waiving the information requirement.

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[13] administration of the Substance, as already explained above.

4. Adsorption/ desorption screening

Adsorption/desorption screening is an information requirement under Annex VIII to REACH (Section 9.3.1.).

You have adapted this information requirement by using Grouping of substances and read-across approaches under Annex XI, Section 1.5., Weight of Evidence under Annex XI, Section

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

1.2. and Qualitative or Quantitative structure-activity relationship ((Q)SAR) under Annex XI, Section 1.3. of REACH.

You have provided the following sources of information:

i) EPI suite KOCWIN Program (v2.00) as by means of MCI method

ii) Experimental study (2017) with an analogue substance (EC 204-909-5)

iii) Experimental study (2017) with an analogue substance 4,4'-methylenebis(N-methylaniline) (EC 217-309-3)

We have assessed this information and identified the following issues:

A. Read across

As explained in Section 1 of the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.5. is rejected.

B. Weight of evidence

As explained in Section 2 of the Appendix on Reasons common to several requests, the weight of evidence must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

To fulfil the information requirement, normally a study according to OECD TG 106 or 121 must be provided. The key element investigated by this test is the adsorption/desorption behaviour of the substance on soil.

All the sources of information you provided investigate this key element. Therefore, they provide information that would contribute to the conclusion on this key element.

However, the reliability of these sources of information is significantly affected by the deficiencies identified in Section 2 of the Appendix common to several requests.

Taken together, even if these sources of information provide information on the key element, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated by in an OECD TG 106 or OECD TG 121 study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

C. QSAR calculations

As explained in Section 3 of the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.3. is rejected.

In your comments on the initial draft decision you provide additional information on an experimental study according to OECD TG 121 performed using the Reverse Phase High Performance Liquid Chromatographic (HPLC) method, and you have reported a Log Koc value of 2.787 at 25 °C.

OECD TG 121 establishes the requirements for the data to be reported for the estimation of the adsorption coefficient on soil and sewage. For the HPLC method, the following parameters are required to be reported (among others):

- identity of test and reference substances and their purity, and pKa values if relevant;
- description of equipment and operating conditions, e.g. type and dimension of analytical (and guard) column, means of detection, mobile phase (ratio of components and pH), temperature range during measurements;
- dead time and the method used for its determination;
- retention times of reference compounds used for calibration;
- details of fitted regression line (log k'vs log Koc) and a graph of the regression line;
- average retention data and estimated d log Koc value for the test compound;- chromatograms.

As you have not provided information on the parameters listed above, an independent assessment of the study reliability is not possible. On the basis of the above, the information requirement is not fulfilled.

Study design

Considering the properties of the Substance (sparingly soluble particles), the Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (test method: OECD TG 121) or alternatively the Adsorption/Desorption Using a Batch Equilibrium Method (test method: OECD TG 106) are the most appropriate method to fulfil the information requirement for the Substance.

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

1. Dissociation constant

Dissociation constant is an information requirement under Annex IX to REACH (section 7.16).

You have provided the following information on dissociation:

- A key study (2012) following a conductometric method

We have assessed the information and identified the following issues:

A. To comply with this information requirement, the test material in a study must be representative for the Substance (Article 10 and Recital 19 of REACH; ECHA Guidance R.4.1).

You have reported dissociation constant for only one constituent of your UVCB Substance.

You have not reported a dissociation constant value for the other identified constituents of your Substance, or justified why the result of single constituent represents the whole Substance.

In the absence of this information, you have not demonstrated that the test material is representative for the Substance.

B. To fulfil with this information requirement, a study must comply with the OECD TG 112. The specifications of OECD TG 112 for the conductometric method include the following:

- Reporting of the equivalent conductance Λ for each acid concentration and for each mixture of one equivalent of acid plus 0.98 equivalent of carbonate-free sodium hydroxide
- Reporting of $1/\Lambda$ plotted against $\sqrt{C}$ and Λ_0 of the salt extrapolated to zero concentration

For the key study, you have not reported the parameters listed above.

In the absence of this information, it is not possible to make an independent assessment of the reliability of the key study.

In your comments on the initial draft decision you agree to perform the requested study.

Therefore, the provided study does not fulfil the information requirement.

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

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

You have adapted this information requirement by using Weight of Evidence under Annex XI, Section 1.2 of REACH and Annex XI 1.5 (grouping of substances and read-across).

You have provided the following sources of information:

i) Subacute toxicity study (1987) in rat on source substance hydrogen [4-[4-(dimethylamino)-α-(2-hydroxy-3,6-disulphonato-1-

naphthyl)benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium, monosodium salt (EC 221-409-2)

ii) Chronic toxicity study (1982) in rat on source substance benzenamine, 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-2-methyl (EC 211-189-6)

In your comments on the initial draft decision you provide short summaries of two additional studies:

iii) Sub-acute toxicity study (2018) on source substance [4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]diethylammonium acetate (EC 278-585-9)

iv) Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (2009) on source substance [4-[(4-dimethylaminophenyl)-[4-(methylamino)phenyl]methylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (EC 616-846-4)

v) Chronic toxicity study (1989) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6)

vi) Chronic toxicity study (2005) on source substance 4-[[4-(dimethylamino)phenyl]-phenylmethyl]-N,N-dimethylaniline (EC 204-961-9)

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

A. Read-across

As explained in the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.5. is rejected.

B. Weight of evidence

As explained in Section 2 of the Appendix common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

For this endpoint your study needs to have adequate and reliable coverage of the key elements foreseen to be investigated in an OECD TG 408 test. The key elements investigated by this test is systemic toxicity in intact, non-pregnant and young adult males and females from: 1) in-life observations, 2) blood chemistry, 3) organ and tissue toxicity.

The provided studies investigate the above mentioned key elements. Therefore, they provide information that would contribute to the conclusion on them.

However, the reliability of this study is significantly affected by the deficiencies identified in Section 3 of the Appendix on Reasons common to several requests.

In addition to the reliability issues raised under B.3, the reliability of the sources of information for this endpoint are also affected by the following issue:

The conditions of OECD TG 408 include:

- testing of at least three dose levels and a concurrent control
- 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 study ii) you have provided was conducted with less than three dose levels.

The study i) you have provided was conducted with less than 10 animals per sex per test dose group.

The studies i), ii), iii) and iv) you have provided do not have the required exposure duration of 90 days. In study ii) dosing was discontinued after 21 and 83 days for one week before continuing the chronic exposure.

In addition, ECHA has made the following observations on the data provided in your comments on the initial draft decision:

OECD TG 408 includes the following specifications:

- highest dose level should aim to induce some systemic toxicity, but not death or severe suffering
- a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL)
- examination of the animals for histopathology (including thyroid gland/ thyroid hormone measurements)
- pathology of sexual (male and female) organs, full detailed gross necropsy and subsequent histopathology of both types of tissues

The study iv) does not provide any consideration on the setting of the highest dose level. Mortality was observed in the high dose group (4/12 and 5/12 males and females). Therefore, you have not demonstrated that the highest dose level was aiming to induce some systemic toxicity but not death or severe suffering. In addition, it is not clear whether the conducted investigations of studies iii), iv), v) and vi) cover organ weight and histopathology of thyroid and thyroid hormone measurements or sexual organs based on the short summary provided for the study in your comments on the initial draft decision.

Taken together, even if these sources of information provide information on the key parameters, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated by the required study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

In your comments on the initial draft decision you indicated your intention to classify the substance as STOT RE 2 (H373: may cause damage to organs through prolonged or repeated exposure), and seek to waive the testing requirement for OECD TG 413.

ECHA notes that, although you did not specifically claim an adaptation, your comments to the draft decision could be interpreted as intention to adapt the information requirement according to Annex IX, Section 8.6.2, Column 2. As provided in Annex IX, Section 8.6.2, Column 2, you may adapt the information requirement, provided you fulfil the following criterion:

- a reliable short-term toxicity study (28-day) is available and shows severe toxicity effects leading to the classification of the Substance, and where the NOAEL-90 days can be extrapolated for the same route of exposure

Your adaptation under Annex XI, Section 1.5. is rejected for the same reasons as those explained in the Appendix on Reasons common to several requests. The repeated dose toxicity information you provided is therefore not considered compliant. Therefore, the derived NOAEL-90-days cannot be considered reliable and also the classification intention is not reliable. Consequently your intended adaptation is not meeting the above criteria.

Information on the design of the study to be performed (route/ species/ strain)

Following the criteria provided in Annex IX, Section 8.6.2, Column 2, the inhalation route is the most appropriate route of administration to investigate repeated dose toxicity. As the substance to be tested is a fine particles, the sub-chronic toxicity study must be performed according to the OECD TG 413, in rats and with administration of the Substance by inhalation. The information provided in the technical dossier and the chemical safety report on properties of the Substance and its uses (including for example exposure to workers in other hot work operations with metals) indicate that human exposure to the Substance by the inhalation route is likely. More specifically, the Substance is reported to occur as a dust with a significant proportion (>1% on weight basis) of particles of inhalable size (MMAD < 50 µm). Furthermore, the Substance is of low water solubility and consequently there is a potential for accumulation of the Substance in the lungs.

Therefore the sub-chronic toxicity study must be performed according to the OECD TG 413, in rats and with administration of the Substance by inhalation.

3. Pre-natal developmental toxicity study in one species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

You have adapted this information requirement by using Weight of Evidence under Annex XI, Section 1.2 of REACH and Annex XI 1.5 (grouping of substances and read-across).

You have provided the following sources of information:

i) Non-guideline teratogenicity and embryotoxicity study (1987) on source substance Hydrogen[4-[4-(dimethylamino)-α-(2-hydroxy-3,6-disulphonato-1-naphthyl)benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium, monosodium salt (EC 221-409-2)

ii) Non-guideline 3-generation study (1987) on source substance Dihydrogen(ethyl)-[4-[4-[ethyl(3-sulphonatobenzyl)amino](4-hydroxy-2-sulphonatobenzhydrylidene]-cyclohexa-2,5-dien-1-ylidene] -(3-sulphonatobenzyl)ammonium, disodium salt (EC 219-091-5). RL 4.

In your comments on the initial draft decision you provide short summaries of two additional studies:

iii) Prenatal developmental toxicity study (2011) on source substance 4-[[4-(dimethylamino)phenyl]-phenylmethyl]-N,N-dimethylaniline (EC 204-961-9)

iv) Prenatal developmental toxicity study (no study year or exact reference provided) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6). RL 4

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

A. Read-across

As explained in the Appendix on Reasons common to several requests your adaptation under

Annex XI, Section 1.5. is rejected.

B. Weight of evidence

As explained in Section 2 of the Appendix common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

For this endpoint your study needs to have adequate and reliable coverage of the key elements foreseen to be investigated in a OECD TG 414 study in a first species. The key elements of this study cover the following aspects: 1) prenatal developmental toxicity, 2) maternal toxicity, and 3) maintenance of pregnancy.

The provided source of information i) investigate all three key elements. Therefore, they provide information that would contribute to the conclusion on them.

However, the reliability of the sources of information is significantly affected by the deficiencies identified in Section 2 of the Appendix on Reasons common to several requests.

In addition, ECHA has made the following observations:

OECD TG 414 includes the following specifications:

- dosing of the Substance from implantation until the day prior to scheduled caesarean section
- examination of the dams for weight and histopathology of the thyroid gland and thyroid hormone measurements

In the study iii) you have provided, the animals were exposed during GD 6 to 15. The study does not have a required exposure duration because the exposure duration is not from implantation until the day prior to scheduled caesarean section as required in OECD TG 414. In addition, it is not clear whether the conducted investigations of the study cover organ weight and histopathology of thyroid and thyroid hormone measurements as required by OECD TG 414 based on the short summary provided for the study in your comments.

In addition, source study ii) and iv) have been given a reliability score of 4 by you (not assignable), and ECHA agrees that this source study is not assignable.

Therefore, the provided studies cannot be considered a reliable source of information.

As a conclusion, the sources of information as indicated above provide information on sexual function and fertility, toxicity to offspring, and systemic toxicity; but their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated by the required study. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

In your comments on the initial draft decision you indicate your intention to apply self-classification to the Substance for Reproductive Toxicity Category 2 (H361d: Suspected of damaging the unborn child), and waive the information requirement as not justified in light of the classification.

ECHA notes that the information requirement cannot be waived on basis of classification for Reproductive toxicity Category 2. Column 2 of Annex IX, section 8.7 outlines the applicable criteria for waiving the information requirement.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[14] administration of the Substance.

4. Long term toxicity testing on aquatic invertebrates

Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

You have adapted this information requirement by using a Qualitative or Quantitative structure-activity relationship ((Q)SAR under Annex XI, Section 1.3. of REACH
In support of your adaptation, you provided the following study:

i) A key study (2018) Long-term toxicity to aquatic invertebrates by ECOSAR Version 1.11

We have assessed this information and identified the following issues:

As explained in the Appendix on Reasons common to several requests, Section 3, your adaptation under Annex XI, Section 1.3. is rejected.

On this basis, the information requirement is not fulfilled.

In your comments on the initial draft decision you agree to perform the requested study on the Substance and on the analogue substance, EC 229-851-8 (CAS no. 6786-83-0). However, a single valid study should fulfil the standard information requirements, no requirement to perform two studies.

ECHA notes that the read-across justification document attached to your comments on the initial draft decision does not include information on this analogue substance. However, not withstanding that as explained in the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.5. is rejected.

Regarding your comment on the initial draft decision on animal welfare, either on its own or with considerations of "overall available data", it does not constitute as such a valid justification to omit the information requirement or a valid adaptation to this information requirement.

Study design

The Substance is difficult to test due to the low water solubility (below 1 mg/L). OECD TG 211 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations.

Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 211. In case a dose-response relationship cannot be established (no

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

observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

5. Long-term toxicity testing on fish

Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

You have adapted this information requirement under Annex XI, 1.3. In support of your adaptation, you provided the following study:

i) A key study (2018) Long-term toxicity to fish by ECOSAR Version 1.11

We have assessed this information and identified the following issue:

As explained in the Appendix on Reasons common to several requests, Section 3, your adaptation under Annex XI, Section 1.3. is rejected.

On this basis, the information requirement is not fulfilled.

Your comments on the initial draft decision are the same as the above request and so have been addressed above under section 4.

Study design

To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (ECHA Guidance R.7.8.2.).

OECD TG 210 specifies that for difficult to test substances OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design' under Section C4.

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

A. Test methods, GLP requirements and reporting

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

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

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[15].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[16].

[15] https://echa.europa.eu/practical-guides
[16] https://echa.europa.eu/manuals

Appendix E: General recommendations when conducting and reporting new tests for REACH purposes

A. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in ECHA Guidance R.11 (Section R.11.4.2.2), you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the "known constituents approach" (by assessing specific constituents), or
- the "fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the "whole substance approach", or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

Appendix F: Procedure

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

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

The compliance check was initiated on 13 February 2020.

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

ECHA took into account your comments and did not amend the request(s). However, for the screening for reproductive/developmental toxicity request for EU B.64/OECD TG 422, ECHA included the option of EU B.63/OECD TG 421 OR EU B.64/OECD TG 422. Based on this the draft decision was amended.

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

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

Appendix G: List of references - ECHA Guidance[17] 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)[18]

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

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

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

OECD Guidance documents[20]
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.

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

Appendix H: Addressees of this decision and their corresponding information requirements

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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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.