Confidential

Helsinki, 12 May 2021

Addressees
Registrant(s) of JS_6786-83-0_[redacted] as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
30/04/2015

Registered substance subject to this decision ("the Substance")
Substance name: a,a-bis[4-(dimethylamino)phenyl]-4-(phenylamino)naphthalene-1-methanol
EC number: 229-851-8
CAS number: 6786-83-0

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***19 May 2022***.

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

Many of this type of organic substances 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. Water solubility (Annex VII, Section 7.7.; test method: EU A.6./OECD TG 105)

2. Partition coefficient n-octanol/water (Annex VII, Section 7.8.; using an appropriate test method)

3. Skin sensitisation (Annex VII, Section 8.3.) with the Substance

   i. *in vitro/in chemico* skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD

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

TG 442D) and activation of dendritic cells (EU B.71/OECD TG 442E)(Annex VII, Section 8.3.1.); and

ii. Only if the *in vitro/in chemico* test methods specified under point (2.i.) are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment, *in vivo* skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429);

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

5. Only if study under section A.1 shows the substance is poorly soluble, Long-term toxicity testing on aquatic invertebrates (triggered by Annex VII, Section 9.1.1., column 2; test method EU C.20/OECD TG 211)

6. Only if study under section A.1 shows the substance is not poorly soluble, Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.20./OECD TG 202)

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

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

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

2. 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. and 4. Combined repeated dose toxicity study with the reproduction/developmental toxicity screening study (Annex VIII, Sections 8.6.1. and 8.7.1.; test method: OECD TG 422) in rats, oral route (gavage))

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

6. Only if study under section A1 shows the Substance is poorly water soluble, Long-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: OECD TG 210)

7. Only if study under section A1 shows the Substance is not poorly water soluble, Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: OECD TG 203)

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

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

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and

in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 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:
- Skin sensitisation (Annex VII, Section 8.3.)
- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

In your comments on the initial draft decision:
- Long-term toxicity testing on aquatic invertebrates (triggered by Annex VII, Section 9.1.1., column 2)
- Long-term toxicity testing on fish (Annex VIII, Section 9.1.3.)

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].

You have provided the following read-across and grouping approach and supporting documentation:

i) An analogue approach for the endpoints addressed above, except reproductive toxicity, using a read-across justification document in IUCLID Section 13.

In addition you have provided the following supporting documents:

ii) QSAR Toolbox prediction based on read-across for skin sensitisation,

iii) QSAR Toolbox prediction based on read-across for *in vitro* gene mutation study in bacteria,

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[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

iv) QSAR Toolbox prediction based on read-across for reproductive toxicity.

In QSAR toolbox predictions ii) to iv) you carried out predictions "*from category members using read-across based on nearest neighbours*" using the OECD QSAR Toolbox.

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

In your comments on the initial draft decision you further provided information on the following source substances supporting analogue approaches:

- [4-[(4-dimethylaminophenyl)-[4-(methylamino)phenyl]methylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (EC 616-846-4)
- [4-[[4-anilino-1-naphthyl][4-(dimethylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 219-943-6)
- [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (EC 255-288-2)
- 6-dimethylamino-3,3-bis(4-dimethylaminophenyl)phthalide (EC 216-293-5)
- [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:
Ferrate(4-), hexakis(cyano-C)-, methylated 4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]benzenamine copper(2+) salts (235-468-7)

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

These category approaches are addressed under sections A and B below, and the analogue approaches under section B only.

A. Scope of the grouping

i. Description of the grouping

For the endpoints listed above, you provided experimental data from the following source substance identified in read-across justification document i):

- [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 209-322-8)

The selection of category members listed in OECD Toolbox QSAR reports ii) to iv) was done without a read-across hypothesis on basis of log Kow and a large number of profilers.

ii. Assessment of the grouping

ECHA notes the following shortcomings with regards to your grouping approach in QSAR Toolbox predictions ii) to iv).

Applicability domain of the category

According to the ECHA Guidance, a category (grouping) hypothesis should address "*the set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members for the given endpoint*".[6] Particularly, "*the applicability domain of a (sub)category would identify the structural requirements and ranges of physico-chemical, environmental fate, toxicological or ecotoxicological properties within which reliable estimations can be made for the (sub)category members*".[7] Therefore, to reliably predict properties within a category the applicability domain should be described including the borders of the category, for which chemicals the category does not hold and a justification for the inclusion and/or exclusion rules.

In the dossier you describe the applicability domains of the substances covered by the groupings are based on logKow boundaries. In your comments on the initial draft decision you suggest analogue approaches with the above related source substances. Analogue approaches are further discussed under section B. Predictions for (eco)toxicological properties.

The applicability domains of QSAR Toolbox predictions ii) to iv) do not introduce unambiguous inclusion/exclusion criteria for chemical structures allowed in the category substances. Furthermore, the provided applicability domain criteria based physico-chemical property (logKow) cannot predict type of toxicity or the mode/ mechanism of actions of the substances for repeat dose toxicity or reproductive/developmental toxicity.

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

In addition, you state that the skin sensitisation and reproductive toxicity QSAR Toolbox predictions ii) and iv) for the target substance do not fall within the applicability domains, respectively.

ECHA notes that no reliable estimations can be made outside of these applicability domains.

B. Predictions for (eco)toxicological properties

In the dossier, you have not provided a read-across hypothesis to establish a reliable prediction for the toxicological properties, based on recognition of the structural similarities and differences between the category members for the QSAR Toolbox predictions ii) to iv).

In your read-across justification document i) you have provided the following reasoning for the prediction of toxicological properties:

Read-across source substances were screened "*using mechanistic approaches such as protein binding mechanism, DNA binding mechanism, estrogen receptor binding mechanism (as presented in table below) which were related to the chemicals behavior when exposed to*

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

human or environment and its possible systemic impact on human body related to such as repeated dose toxicity, carcinogenicity, mutagenicity, toxicity to reproduction and developmental toxicity."

You also conclude that "*Based on the human health data it is observed that all chemical are not toxic in nature at the mentioned dose levels. Therefore the chemical category is likely justified with similar category members.*"

In your read-across justification document v) 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".*

Furthermore, 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. 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 in your read-across justification document i).

Missing supporting information (skin sensitisation and genotoxicity endpoints)

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 substance 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).

While there is no other information available for comparing the target and source substances for skin sensitisation and genotoxic properties, your reasoning is that the QSAR profiling and the related mechanistic approaches outlines in read-across justification document i) is the basis for comparing properties of the target and source substances for these endpoints.

Information such as the mechanistic approaches or the QSAR supporting information provided in the read-across justification document i) does not include relevant, reliable and adequate information for the Substance and of the source substance(s) to support your read-across hypothesis specifically with regard to skin sensitisation and genotoxicity endpoints. Moreover, the information does not meet the criteria outlined in this section under Adequacy and

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

Read-across hypothesis contradicted by existing data (all toxicological endpoints)

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances. The ECHA Guidance[8] indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance. The observation of differences in the toxicological properties between the source substance(s) and the Substance would contradict the hypothesis that the properties of the Substance can be predicted from the data on the source substance. An explanation why such differences do not affect the read-across hypothesis needs to be provided and supported by scientific evidence.

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

The results of the information on repeated dose toxicity obtained with the target and source substance vary. Specifically, mortality is observed in the sub-acute repeated dose toxicity study conducted with the Substance while no mortality is observed in equivalent sub-acute repeated dose toxicity studies conducted for the source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 209-322-8) using higher administered doses.

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

Characterisation of the source substance(s) (all (eco)toxicological endpoints)

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.[9] Therefore, qualitative and quantitative information on the compositions of the test materials should be

[8] Guidance on information requirements and chemical safety assessment (version 6.0, July 2017), Chapter R.6, Section R.6.2.2.1.f
[9] 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 other category members.

You do not provide any description of the source substance identified in read-across justification document i). 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 v) provide short summaries of additional source studies on additional source substances. You did not provide any characterisation on purity profile and composition of the substances tested in these studies.

Regarding your consolidated comments to the draft decision for the source substances for the environment, you have indicated these substances can be considered as potential read-across source substances 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 source substances, EC 235-468-7 / 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.

There are deficiencies with the studies provided identified in the corresponding Appendices.

ECHA notes the following shortcomings with regards to prediction of toxicological properties in your QSAR Toolbox predictions ii) to iv).

Absence of read-across documentation (all (eco)toxicological endpoints)

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).[10]

You have provided QSAR Toolbox predictions ii) to iv) without specifying the exact studies

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

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

In the absence of such documentation, ECHA cannot verify that the properties of your Substance can be predicted from the QSAR Toolbox predictions ii) to iv).

In addition, a robust study summary must cover sufficient information to make an independent assessment of the study.[11]

The OECD QSAR Toolbox predictions ii) to iv) do not provide robust study summaries of any source studies.

Furthermore, the documentation of the studies provided in your comments to the draft decision do not cover sufficient information to make an independent assessment of the study as indicated under the endpoint sections in the below Appendices.

In the absence of such documentation, ECHA cannot verify that the results to be read across meet the criteria above.

Predictions for no observed (adverse) effect level (NO(A)EL) or mode values (positive/negative or unknown/known) (all toxicological endpoints)

Annex XI, Section 1.5. requires that the relevant properties (i.e. key parameters foreseen to be investigated in corresponding test methods) of a substance within the group may be predicted from data for reference substance(s) within the group.

When conducting a hazard and risk assessment based on read-across, all results of a study conducted with the source substance(s) are read across to the target substance. These results thereafter form the basis for establishing the no observed (adverse) effect level (NO(A)EL) or the mode value (positive/negative or unknown/known) for the target substance.

In order to have a reliable prediction using multiple source substances, the NO(A)ELs or mode values need to be based on the same key parameters. Furthermore, it is important to ensure that the read-across prediction is well founded and that the prediction accounts for the uncertainty in the approach. In cases where there are multiple source substances, and consequently a range of possible NO(A)EL values available to read-across, the use of the most conservative (lowest) value may be sufficient to account for the uncertainty in the read-across.[12]

No information on what type of toxicity forms the basis to establishing the NO(A)ELs or mode values have been provided for any of the source substances identified in OECD QSAR Toolbox predictions ii) to iv). Therefore, ECHA cannot verify that the predicted NO(A)EL or mode values are based on the same key parameter(s).

In addition, for reproductive toxicity, you have provided a list of NO(A)EL values for the source substances, and predictions for reproductive toxicity NO(A)ELs based on category members using read-across and calculating an average from 4 nearest neighbours. You have not selected the most conservative (i.e. the lowest) reproductive toxicity NO(A)EL or provided a justification why the target substance is expected be less potent than the calculated average from the nearest 4 selected read-across source substances. Therefore, the selection of

[11] How to report robust study summaries Practical Guide 3, Version 2.0 – November 2012
[12] ECHA Guidance R.6., Section R.6.2.2.

NO(A)EL for the read-across from the source substances to the Substance is not justified, and the uncertainty in the approach for predictions has not been considered.

Test material identity (all toxicological endpoints)

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 substance identified in read-across supporting documentation ii) to iv). Furthermore, for all the studies provided in the technical dossier that were conducted with this substance, as listed above, no information on the composition of the test material used to generate the source data is provided.

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.

There are additional deficiencies with the studies you have provided for the endpoints A.3-4.,B.1, and B.3-4. These deficiencies are discussed under the respective endpoints.

C. 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.

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. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
2. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
3. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

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 findings apply equally to the sources of information relating to analogue substances submitted under your weight of evidence adaptations.

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

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

1. Water solubility

Water solubility is an information requirement under Annex VII to REACH (Section 7.7).
You have provided the following information:

- Water solubility study according to the shake flask method with the Substance (2015).

We have assessed this information and identified the following issue:

To fulfil the information requirement, a study must comply with the OECD TG 105 or the EU Method A.6 (Article 13(3) of REACH). Therefore, the following specifications must be met:

- the shake-flask method is applicable to test material with a water solubility ≥ 10 mg/L;
- solids are pulverized before testing;
- the test is conducted with a loading of about five times the quantity required to saturate a given volume of water;
- three flasks are included which are shaken/stirred for 24, 48 and 72 hours, respectively;
- after shaking/stirring, each flask is equilibrated for 24 hours at 20°C;
- the results are considered acceptable, if the results of the flasks shaken for 48 and 72 hours differ by ≤ 15%. If the results shows a tendency of higher solubility with longer shaking/stirring period, the test is repeated with longer equilibration times;
- a reliable analytical method is available.

You have provided a study performed with the flask method and you report a water solubility 0.05 mg/L. The study report does not report anything on the other study specifications mentioned above.

The reported result falls outside of the applicability domain of the method. Furthermore, in the absence of any information on the other study specifications, the reporting of the study is not sufficient to conduct an independent assessment of its reliability.

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

Based on the above assessment, the information requirement is not fulfilled.

Study design

Considering the properties of the Substance (solubility < 10 mg/L), the column elution described in EU A.6/OECD TG 105 is the most appropriate method to fulfil the information requirement for the Substance.

2. Partition coefficient n-octanol/water

Partition coefficient in n-octanol/water is an information requirement under Annex VII to REACH (Section 7.8).

You have provided the following information:

- Partition-coefficient by Shake-flask method with the Substance (2012).

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

To fulfil the information requirement, a study must comply with the OECD TG 107 or OECD TG 117 or OECD TG 123 or the EU Method A.8 (Article 13(3) of REACH). These test guidelines establish the requirements for the data to be reported for a partition coefficient study. For the shake-flask method, especially the following is required:

- chemical identity and impurities;
- the results of the preliminary estimation (when the shake flask method is not applicable, e.g. surface active material, a calculated value or an estimate based on the individual n-octanol and water solubilities should be provided);
- all information relevant for the interpretation of the results, especially with regard to impurities and physical state of the substance;
- the test conditions: temperature, the amount of test substance introduced in the test vessels,
- the volume of each phase in each vessel and the calculated total amount of test substance based on the analytical data;
- pH of the water used and of the aqueous phase during the experiment.

You have reported the Log Pow using the shake-flask method was found to be 0.8055 at temperature 23 deg C and pH of 5.7.

ECHA has assessed the information and identified the following issues:

You have not reported the parameters listed above except for temperature and pH of the water used.

In the absence of this information, the reporting of the study is not sufficient to conduct an independent assessment of its reliability.

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

On the basis of the above, the information requirement is not fulfilled.

Study design

Considering the properties of the Substance (sparingly soluble particles), the Partition Coefficient (n-octanol/water), HPLC Method (test method: OECD TG 117) or alternatively the Partition Coefficient (1-Octanol/Water): Slow-Stirring Method (test method: OECD TG 123) are the most appropriate method to fulfil the information requirement for the Substance.

3. Skin sensitisation

Skin sensitisation is an information requirement under Annex VII (Section 8.3.). Column 1 of Section 8.3. requires the registrants to submit information allowing a conclusion whether the substance is a skin sensitiser and whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and risk assessment, where required.

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

You have provided the following sources of information in support for your adaptations:

i) QSAR toolbox (version 3.1) prediction for the Substance
ii) Non guideline patch test in human (1969) on source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethyl ammonium chloride (EC 209-322-8)

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

iii) Skin sensitisation (2007), modified Buehler and Klecak method, on source substance [4-[(4-dimethylaminophenyl)-[4-(methylamino)-phenyl]-methylidene]-cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (EC 616-846-4)

iv) Skin sensitisation (1988), guinea pig maximisation test, on source substance [4-[[4-anilino-1-naphthyl][4-(dimethylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 219-943-6)

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

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 information provided has the following deficiencies affecting its reliability.

Human data
As provided under Appendix on Reasons common to several requests, Section 1.B, the results to be read across should be adequate for the purpose of classification and labelling and/or risk assessment.

For that purpose, the study ii) has to meet the general requirements for human studies. Among others, the key elements of these studies (i.e. human maximization study) include [13,14]:

a) information on number of induction and challenge exposure
b) information on duration of induction and challenge exposures including potential rest period
c) information on pre-treatment with sodium lauryl sulfate (SLS), if applied
d) information on test volume and patch size or a direct statement of the dose per square area
e) information on justification for dose level selection for induction and challenge

The provided study ii) does not cover the above key elements needed in a human maximization test, and cannot be used to fulfil the information requirement.

Animal data
In your comments on the initial draft decision, you stated that studies iii) and iv) are available and that you will provide this information in an updated of your registration dossier. As discussed further below, the information in your comments is not sufficient for ECHA to make a independent assessment. Please note that this decision does not take into account updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation).

In particular:

A study has to meet the requirements of the EU Method B.6/OECD TG 406 to have adequate and reliable coverage of its key parameters (Annex XI, Section 1.5).

The key parameter(s) of this test guideline (Buehler method) include:
- dose selection rationale, the induction concentration must be the highest causing mild

[13] ECHA Guidance R.7a, Section R.7.3.5.2
[14] Kligman AM, The identification of contact allergens: III: The Maximization test: A procecure for screening and rating contact sensitisers (1966). The journal of investigative dermatology, Vol. 47, No. 5.

irritation to the skin and the challenge dose must be the highest non-irritation concentration (OECD TG 406, paragraph 27), and
- 20 animals in test group and 10 in control group.

The key parameter(s) of this test guideline (guinea pig maximisation test) include:
- dose selection rationale, the induction concentration must be the highest causing mild-to-moderate irritation to the skin and the challenge dose must be the highest non-irritation concentration (OECD TG 406, paragraph 14), and
- 10 animals in test group and 5 in control group (in case of negative results, 20 in test and 10 in control group highly recommended).

In the short summaries provided for the study iii) and iv), no dose level selection rationale or number of the animals were provided. Reportedly the concentrations used for induction or the challenge did not cause irritation in test groups, nor did the challenge concentration cause irritation in the control group. The selected doses seem therefore too low, and require a justification.

You have not demonstrated that studies iii) and iv) have adequate and reliable coverage of its key parameters.

Based on the above assessment, ECHA concludes that the information requirement is not fulfilled.

To fulfil the information requirement for the Substance for skin sensitisation, *in vitro/in chemico* studies (OECD TG 442C, 442D and 442E) are considered suitable. In case *in vitro/in chemico* methods are not suitable for the Substance or the results cannot be used for classification and risk assessment an *in vivo* skin sensitisation study (OECD TG 429) must be performed.

4. 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 adapted this information requirement by using Grouping of substances and read-across approaches under Annex XI, Section 1.5. and Weight of Evidence under Annex XI, Section 1.2. of REACH.

You have provided the following sources of information in support for your adaptations:
i) QSAR toolbox (version 3.1) prediction for the Substance
ii) In vitro gene mutation study in bacteria (2004) on source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethyl ammonium chloride (EC 209-322-8)

In your comments on the initial draft decision you provide short summaries of three additional studies:
iii) In vitro gene mutation study in bacteria (2020) on source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (EC 255-288-2)
iv) In vitro gene mutation study in bacteria (1997) on source substance [4-[[4-anilino-1-naphthyl][4-(dimethylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 219-943-6))
v) In vitro gene mutation study in bacteria (1999) on source substance N,N,N',N'-tetramethyl-4,4'-benzylidenedianiline (EC 204-961-9)

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 3 of the Appendix 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.

The sources of information you provided investigate the key element of gene mutation foreseen to be investigated by OECD TG 471.

However, the information provided has the following deficiencies affecting its reliability.

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

To fulfil the information requirement, normally a study according to OECD TG 471 must be provided. The key parameters investigated by this test are:

a) Triplicate plating must be used at each dose level.
b) One positive control must be included in the study. The positive control substance must produce a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control.
c) The number of revertant colonies per plate for the concurrent negative control must be inside the historical control range of the laboratory.
d) The mean number of revertant colonies per plate must be reported for the treated doses and the controls.

The studies ii), iii), iv and v) detect and quantify mutations in bacteria.

However, the reported data for study ii) you have provided did not include:

a) triplicate plating at each dose level.
b) a positive control.
c) a negative control with a number of revertant colonies per plate inside the historical control range of the laboratory.
d) data on the number of revertant colonies per plate for the treated doses and the controls

Furthermore, the reliability of these sources of information i) and ii) is significantly affected by the deficiencies identified in Section 2 of the Appendix on Reasons common to several requests.

In your comments on the initial draft decision, you stated that studies iii) to v) are available and that you will provide this information in an updated of your registration dossier. As discussed further below, the information in your comments is not sufficient for ECHA to make an independent assessment. Please note that this decision does not take into account updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation).

In particular, the short summaries provided for the studies iii) to v) did not include data for the negative controls (and whether the number of revertant colonies per plate were inside

the historical control range of the laboratory), and data on the number of revertant colonies per plate for the treated doses. In addition, experiments in studies iv) and v) were reported to be conducted in duplicates instead of triplicates. This is despite the corresponding specifications of the OECD T 471.

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

As a conclusion, the sources of information as indicated above provide information on mutations in bacteria, but the information provided is not reliable.

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.

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

5. Only if study under section A.1 shows the substance is poorly soluble, Long-term toxicity testing on aquatic invertebrates (triggered by Annex VII, Section 9.1.1., column 2; test method EU C.20/OECD TG 211)

Short-term toxicity testing on aquatic invertebrates is a standard information requirement in Annex VII of REACH. However, pursuant to Annex VII, section 9.1.1, column 2, for poorly water soluble substances (e.g. water solubility below 1 mg/L or below the detection limit of the analytical method of the test substance) long-term toxicity study on aquatic invertebrates (Annex IX, Section 9.1.5) must be considered instead of an acute test.

You have not provided any data on long-term toxicity to aquatic invertebrates. You have adapted this information requirement based on Column 2 arguing that: *"this requirement shall also not apply given the water solubility value 0.05 mg/L of the chemical."*

As explained under request A.1, your dossier currently does not include reliable data on the water solubility of the substance. More specifically, the studies discussed under request A.1 are not compliant and do not allow the determination of a precise value for water solubility.

Poorly water soluble substances require longer time to reach steady-state conditions. Hence, the short-term tests may not give a true measure of toxicity for this type of substances. Therefore, if the information requested on water solubility (request A.1) confirms that the substance is poorly water soluble (<1 mg/L), a long-term toxicity test on aquatic invertebrates must be conducted.

In your comments on the initial draft decision you agree to perform the requested study on the Substance and on the analogue substance, EC 235-468-7 (CAS no. 12237-62-6). However, only one valid study is required to fulfil the standard information requirement.

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.

Study design

The Substance is difficult to test due to (1) its low water solubility (below < 1 mg/L) depending on the results of requests A.1, (2) its potential to ionise and (3) the use of the Substance as a dye indicating adsorptive properties. 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 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 solutions.

6. Only if study under section A.1 shows the substance is not poorly soluble, Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.20./OECD TG 202)

Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.).

You have provided the following information:

i. an adaptation under Annex VII, Section 9.1.1, Column 2 with the following justification: *"this end point was considered for waiver in accordance with the adaptation mentioned in Column 2 of Annex VII of the REACH regulation which mentions that the study need not be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur since the substance is highly insoluble in water (solubility value 0.05 mg/L)."*

We have assessed this information and identified the following issues:

Under Section 9.1.1., Column 2, first indent, Annex VII to REACH, the study may be omitted if aquatic toxicity is unlikely, for instance if the Substance is highly insoluble in water. ECHA Guidance R.7.8.5 explains that there is no scientific basis to define a cut off limit for solubility below which toxicity is unlikely. Therefore, the justification must demonstrate very low water solubility and low likelihood to cross biological membranes. For the latter, the indicators used for low likelihood of a high bioaccumulation potential (ECHA Guidance R.11, Figure R.11-4) must be considered, including:

- physico-chemical indicators of hindered uptake due to large molecular size (*e.g.* D_{max} > 17.4 Å and MW > 1100 or MML > 4.3 nm) or high octanol-water partition coefficient (log K_{ow} > 10) or low potential for mass storage (octanol solubility (mg/L) < 0.002 x MW), and
- supporting experimental evidence of hindered uptake (no chronic toxicity for mammals and birds, no chronic ecotoxicity, no uptake in mammalian toxicokinetic studies, very low uptake after chronic exposure).

Unless it can reliably be demonstrated that aquatic toxicity is unlikely to occur, the Substance must be considered as poorly water soluble.

Your registration dossier provides:

- information on the solubility of the Substance in water (0.05 mg/L) based on a non-guideline study (2015).

As explained under request A.1, your dossier currently does not include reliable data on the water solubility of the substance. More specifically, the studies discussed under request A.1 are not compliant and do not allow the determination of a precise value for water solubility.

Furthermore, you have not demonstrated the low likelihood of the substance to cross biological membranes.

Therefore, you have not demonstrated that toxicity is unlikely to occur and your adaptation is rejected.

Annex VII, section 9.1.1, column 2, requires to perform a long-term toxicity study on aquatic invertebrates instead of an acute test when the substance concerned is poorly water soluble. In that respect, as explained under request A.1, your dossier currently does not include reliable value on the water solubility of the substance. Therefore, a short-term toxicity testing on aquatic invertebrates must only be conducted if the data generated under request A.1 do not confirm that the substance is poorly water soluble (i.e. water solubility below 1 mg/L).

In your comments on the initial draft decision, you indicated that you will be adapting this information requirement based on the results of the long term toxicity testing data on aquatic invertebrates. Under this decision, it is, however, either the short invertebrates or the long term invertebrates, depending exclusively on whether the Substance is poorly soluble.

Study design

The Substance is difficult to test due to its potential to ionise and its function as a coloured dye with expected adsorptive properties. OECD TG 202 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. 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 202. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

7. 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 adaptation under Annex VII, Section 9.1.1, Column 2 with the following justification: "This end point was considered for waiver in accordance with the adaptation mentioned in Column 2 of Annex VII of the REACH regulation which mentions that the study need not be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur since the substance is highly insoluble in water (solubility value 0.05 mg/L)"

ECHA has assessed this information and identified the following issue:

Your adaptation is rejected for the same reasons as those explained under request A.6.

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

Therefore, you have not demonstrated that toxicity is unlikely to occur and your adaptation is rejected.

Study design

OECD TG 201 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 Appendix A.6.

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.).

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

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

You have provided the following sources of information:

i) Non-guideline in vitro chromosomal aberrations (1990) on source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethyl ammonium chloride (EC 209-322-8)

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

ii) In vitro chromosomal aberration study (1979) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6)
iii) In vitro chromosomal aberration study (2002) on source substance 6-dimethylamino-3,3-bis(4-dimethylaminophenyl)phthalide (EC 216-293-5)
iv) In vivo micronucleus study (1997) on source substance [4-[[4-anilino-1-naphthyl][4-(dimethylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 219-943-6)
v) Sister chromatid exchange assay (1979) on source substance [4-[[4-anilino-1-naphthyl][4-(dimethylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 219-943-6)
vi) In vivo chromosomal aberration study (1979) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6)

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

Annex XI, Section 1.2 states that there may be sufficient weight of evidence "*from several independent sources of information*".

You have only provided one source of information in your registration dossier.

Irrespective of this deficiency, which in itself could lead to the rejection of the adaptation, ECHA has assessed the provided sources of information and found the following deficiency.

To fulfil the information requirement, normally a study according to OECD TG 473/487 must be provided. The key element investigated by this test is cytogenicity in mammalian cells (i.e. detection and quantification of structural or numerical chromosomal aberrations in cultured mammalian cells including data on the cytotoxicity and the frequency of cells with chromosomal aberrations or micronuclei).

The studies ii), iii), iv) and vi) investigate cytogenicity in mammalian cells. Therefore, they provide relevant information that would contribute to the conclusion on this key element.

The study ii) outcome is considered positive by you as it "*was clastogenic in the absence of S9 metabolic activation*". *In vivo* follow-up study vi) (OECD TG 475) on the same source substance is discussed further below.

The reliability of the source of information i) to vi) is significantly affected by the deficiencies identified in Section 2 of the Appendix on Reasons common to several requests as well as the deficiencies identified below.

In your comments, you stated that studies iii) to iv) and vi) are available and that you will provide this information in an updated of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment as discussed below. Please note that this decision does not take into account updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation).

Specifications of the OECD test guidelines 473/487 include:

a) At least 300 well-spread metaphases must be scored per concentration
b) Data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures must be reported

The provided short summary of study iii) does not provide data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures. The study was also reported to have conducted analysing only "*200 metaphase cells per group for structural abnormalities and 800 metaphase cells per group for ploidy cells.*" ECHA notes that significant increase in the number of polyploid cells observed in high dose group was judged as "*biologically insignificant*" by you. Independent evaluation of the negative interpretation of the results by ECHA is not possible based on the provided information.

Study iii) do not therefore cover key parameter(s) required by OECD TG 474 or 475.

Specifications of OECD TGs 474 and 475 include:

a) The study must include a minimum of three doses/groups of treated animals, as well as a negative control group and a positive control group.
b) Each group must have a minimum of 5 analysable animals (the test can be performed in either sex).
c) The proportion of immature among total (immature + mature) erythrocytes must be determined for each animal (by counting a total of at least 500 erythrocytes for bone marrow and 2000 erythrocytes for peripheral blood) (OECD TG 474 only).
d) At least 4000 immature erythrocytes per animal must be scored for the incidence of micronucleated immature erythrocytes (OECD TG 474 only).
e) At least 200 metaphases must be analysed for each animal for structural chromosomal aberrations including and excluding gaps (OECD TG 475 only).
f) The mitotic index and the mean number of cells with aberrations per group must be reported for each group of animals (OECD TG 475 only).

Study iv) was conducted analysing only 1000 immature erythrocytes. In addition, the short summary provided does not specify the number of animals analysed or provide data on the proportion of immature among total erythrocytes in each dose group.

Study vi) was conducted using 2 dose groups of treated animals (2 animals analysed). In addition, the number of analysed metaphases for each animal for structural chromosomal aberrations, or the mitotic index was not reported. The study did not include a positive control.

Studies iv) and vi) do not therefore cover key parameter(s) required by OECD TG 474 or 475.

As a conclusion, the sources of information as indicated above provide information on cytogenicity in mammalian cells, but the information provided is not reliable.

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 according to Annex XI, Section 1.2 is rejected and the information requirement is not fulfilled.

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

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.

i. Triggering of the study

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 4 of Appendix A.

The result of the requests for information in section 1 of this Appendix and section 4 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.

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.

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 on the initial draft decision you indicated your intention to adapt this information requirement by using Grouping of substances and read-across approaches under Annex XI, Section 1.5., and Weight of Evidence under Annex XI, Section 1.2 of REACH.

You provide short summaries of three additional studies in your comments:

i) In vitro mammalian cell gene mutation test (1999) on source substance N,N,N',N'-tetramethyl-4,4'-benzylidenedianiline (EC 204-961-9)
ii) In vitro mammalian cell gene mutation test (1994) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6)
iii) Sex-linked recessive lethal mutations assay (1985) on source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6)

ECHA assessed the data provided in your comments 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 476/490 must be provided. The key element investigated by this test is gene mutation in mammalian cells.

The study iii) does not investigate gene mutation in mammalian cells. Therefore, it does not provide relevant information that would contribute to the conclusion on this key element.

The provided sources of information i) and ii) investigate gene mutation in mammalian cells. Therefore, they provide relevant information that would contribute to the conclusion on this key element.

ECHA has made the following observations on studies i) and ii):

In your comments, you stated that studies i) and ii) are available and that you will provide this information in an updated of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment. Please note that this decision does not take into account updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation").

The key parameters of OECD TG 476 or OECD TG 490[15] 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

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

cultures must be reported.

The provided short summary on study i) does not include data on the cytotoxicity and the mutation frequency for the treated and control cultures and if the required level of cytotoxicity was achieved in the conducted experiments. ECHA notes that you report "*mutant frequencies were repetitively above the control values only for one dose*" in the study. Independent evaluation of the negative interpretation of the results by ECHA is not possible based on the provided information.

The provided short summary on study ii) does not include data on the mutation frequency for the treated and control cultures. ECHA notes that you report a "*weak positive response*" in the study. Independent evaluation of the negative interpretation of the results by ECHA is not possible based on the provided information.

Studies i) and ii) do not therefore cover key parameter(s) required by OECD TG 476 or 490.

As a conclusion, the sources of information as indicated above provide information on gene mutation in mammalian cells, but the information provided is not reliable.

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.

Study design

To fulfil the information requirement for the Substance, either the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) or the thymidine kinase gene (OECD TG 490) are considered suitable.

3. Short-term repeated dose toxicity (28 days)

A Short-term repeated dose toxicity study (28 days) is a standard information requirement in Annex VIII to REACH.

While you have provided a study with the Substance, you have also adapted this information requirement by using Grouping of substances and read-across approaches under Annex XI, Section 1.5. of REACH.

You have provided a key study and supporting studies for this endpoint in your dossier:

i) Sub-acute toxicity study (2015) in rat on the Substance
ii) Sub-acute toxicity study (2004) in rat on source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethyl ammonium chloride (EC 209-322-8)
iii) Sub-acute toxicity study (2004) in mouse on source substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethyl ammonium chloride (EC 209-322-8)

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 to "*waive any potential testing requirement for OECD 407 to minimise the use of animals and the suffering of animals.*"

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

As explained in the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.5. is rejected and therefore the studies ii) and iii) cannot be used for fulfilling the information requirement.

In addition, ECHA has made the following observations:

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 407 or, in the case of read-across, to have adequate and reliable coverage of its key parameters (Annex XI, Section 1.5). The following key parameter(s) of this test guideline include

- 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 i) you have provided was conducted with too high dose levels for establishing a NOAEL in consequence of "*total mortality observed in mid and high-dose groups in both the sexes.*" In addition, the study does not cover organ weight and histopathology of thyroid and thyroid hormone measurements.

The studies ii) and iii) you have provided were conducted without pathology of sexual (male and female) organs, and full detailed gross necropsy and subsequent histopathology of both types of tissues.

Therefore, the studies you have provided were not performed according to the criteria of the OECD TG 407, and studies ii) and iii) do not have adequate and reliable coverage of its key parameters.

Column 2 of Annex VIII, section 8.6.1 outlines the applicable criteria for waiving the information requirement. ECHA observes that the information requirement at Annex **VIII** cannot be waived on the basis of Annex **IX**, Section 8.6.2, Column 2, meeting criteria for classification as STOT RE. Furthermore, the available 28d studies (i-iii) are unreliable. Neither did you provide a legal basis for waiving on the basis of minimising the use of animals/reducing animal suffering.

Based on the above, the information you provided do not fulfil the information requirement.

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

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407), nor for the screening study for reproductive/ developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the

same time fulfil the information requirement of REACH Annex VIII, 8.6.1 and that of REACH Annex VIII, 8.7.1.[16]

In your comments to the draft decision you indicate concerns in "*differences in sensitivity in pregnant vs. non-pregnant animals*", and intend to "*waive the testing requirement for OECD 422 to avoid potential issues of determining both general and reproductive/developmental toxicity in a combined test.*" Furthermore, following the intentions to classify the Substance as STOT RE 2, you state that "*it is the proposal of the registrant to conduct an OECD TG 421 study with the registered substance.*"

ECHA notes the OECD TG 422 paragraph 11 addressing the technical complexities related to performance of the combined screening test. You have not provided specific reasons why this would not be sufficient.

It is recommended that the registrant should consider a dose-range finding study using pregnant rats to obtain the best possible information as basis for selecting the dose levels in the study. A classification as STOR RE 2 does not, in itself, demonstrate that the study is not technically possible under Annex XI, Section 2 or is not appropriate.

In addition, ECHA notes that an OECD TG 421 study is not sufficient for fulfilling the information requirement. As explained above, the criteria for waiving the information requirement is not met and an OECD TG 422 study is therefore required.

Referring to the criteria in Annex VIII, Section 8.6.1, Column 2, ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. More specifically, even though 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), the available oral study indicates treatment related adverse effects (including mortality) and there is concern for systemic toxicity. The available study was not successful in establishing NOAEL. An oral repeated dose toxicity study is therefore required to evaluate systemic toxicity and to establish a NOAEL. Hence, the test shall be performed by the oral route using the test method OECD TG 422.

4. 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.

While you have provided a study with the Substance, you have also adapted this information requirement by using Grouping of substances and read-across approaches under Annex XI, Section 1.5. of REACH.

You have provided the following sources of information:

i) QSAR Toolbox (version 3.3) prediction for the Substance
ii) Sub-acute toxicity study (2015) with the Substance

[16] ECHA Guidance, Section R.7.6.2.3.2., pages 484 to 485 of version 6.0 – July 2017. (https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf)

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

As explained in the Appendix on Reasons common to several requests your adaptation under Annex XI, Section 1.5. is rejected and the study i) cannot be used for fulfilling the information requirement.

In addition to the observations made under section 3 of this Appendix, ECHA has made the below observations regarding the study ii):

To be considered compliant and to generate information concerning the effects of the Substance on male and female reproductive performance, the study has to meet the requirements of EU B.63/OECD TG 421 or EU B.64/OECD TG 422. The criteria of this test guideline include for example:

- At least 10 male and 12-13 female animals for each test and control group
- Dosing of the 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 parameters for sexual function and fertility such as those for mating and fertility, duration of gestation, parturition, lactation and weight and histopathology of reproductive organs and tissues
- Examination of offspring parameters such as number and sex of pups, stillbirths and live births, gross abnormalities, pup body weight, litter weight, anogenital distance, number of nipples/areolae in male pups

The animals were not mated in the study for a number of toxicological investigations listed above. The exposure duration does not cover the relevant life stages and the statistical power/number of animals is lower than in EU B.63/OECD TG 421 or EU B.64/OECD TG 422. Toxicological examinations of the key parameters listed above have not been conducted.

The study you have provided was not performed according to an adequate test method in the meaning of Article 13(3).

Based on the above, the information you provided do not fulfil the information requirement.

Information on study design

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407), nor for the screening study for reproductive/ developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the same time fulfil the information requirement of REACH Annex VIII, 8.6.1 and that of REACH Annex VIII, 8.7.1.[17]

However, as explained under section 3 of this Appendix, a study according to the test method OECD TG 422 must be performed in rats with oral[18] administration of the Substance.

[17] ECHA Guidance, Section R.7.6.2.3.2., pages 484 to 485 of version 6.0 – July 2017. (https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf)
[18] ECHA Guidance R.7a, Section R.7.6.2.3.2.

5. Adsorption/ desorption screening

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

ECHA understands that you have provided QSAR adaptations based on Annex XI, Section 1.3 of REACH, using the following information:

i) Adsorption / Desorption by EPI (Estimation Programs Interface) Suite (2011)

We have assessed this information and identified the following issues:

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:

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 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.

You have provided an estimated Log Koc (7.334) using the KOCWIN™ v2.00 module of EPI Suite™ v.4.11., KOCWIN™ (MCI method) for the substance and QPRF-like information.
Your Substance is a dye.

Your adaptation does not meet the general rule for adaptation of Annex XI, Section 1.3. because:

- QSAR results must be adequate for classification and labelling/risk assessment and thus be reliable.
- You have used a QSAR model lacking data in its training set for dyes, or substances mostly dissociated and highly ionisable at pH 5-8.
- The Substance is a dye that in environmental pH (5-8) will be mostly dissociated and highly ionisable.

By not taking into account the specific properties of the Substance provided above, you have not demonstrated that the prediction is adequate for the purpose of classification and labelling and risk assessment.

Therefore, your adaptation of the information requirement cannot be accepted.

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

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.

6. Only if study under section A1 shows the Substance is poorly water soluble, Long-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: OECD TG 210)

Short-term toxicity testing on fish is an information requirement under Annex VIII to REACH (Section 9.1.3). Long-term toxicity testing on fish must be considered (Section 9.1.3., Column 2) if the substance is poorly water soluble.

You have provided the following information on short-term toxicity testing on Fish :

- an adaptation under Annex VIII, Section 9.1.3., Column 2 with the following justification: the study need not be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur since the substance is highly insoluble in water (solubility value 0.05 mg/L).

You have not provided information on long-term toxicity which could be used to cover the information requirement on Section 9.1.3., Column 2:

ECHA has assessed this information and identified the following issue:

Your adaptation is rejected for the same reasons as those explained under request A.6.

Therefore, you have not demonstrated that toxicity is unlikely to occur and your adaptation is rejected.

On this basis, the information requirement for long-term toxicity testing on fish is triggered. Poorly water soluble substances require longer time to reach steady-state conditions. Hence, the short-term tests may not give a true measure of toxicity for this type of substances. Therefore, if the information requested on water solubility (request A.1) confirms that the substance is poorly water soluble (<1 mg/L), a long-term toxicity test on aquatic invertebrates must be conducted.

Your comments to the initial draft decision are the same as in the request for Long term testing on aquatic invertebrates and so have been addressed above under Appendix A section 5.

Study design

OECD TG 210 specifies that for difficult to test substances OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design' under Appendix A.5.

7. Only if study under section A1 shows the Substance is not poorly water soluble, Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: OECD TG 203)

Short-term toxicity testing on fish is an information requirement under Annex VIII to REACH (Section 9.1.3.).

You have provided the following information:

- an adaptation under Annex VIII, Section 9.1.3., Column 2 with the following justification: the study need not be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur since the substance is highly insoluble in water (solubility value 0.05 mg/L).

We have assessed this information and identified the following issue:

Your adaptation is rejected for the same reasons as those explained under request A.6.

Therefore, you have not demonstrated that toxicity is unlikely to occur and your adaptation is rejected.

In your comments on the initial draft decision, you indicated that you will be adapting this information requirement based on the results on the long term toxicity testing data on fish. Under this decision, it is, however, either the short term fish or the long term fish, depending exclusively on whether the Substance is poorly soluble.

Study design

OECD TG 203 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 Appendix A.6.

Appendix C: 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[19].

B. Test material

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the 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.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[20].

[19] https://echa.europa.eu/practical-guides
[20] https://echa.europa.eu/manuals

Appendix D: 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 12 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).

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: List of references - ECHA Guidance[21] 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)[22]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[23]

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

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

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

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

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

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

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

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

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

OECD Guidance documents[24]

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

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix F: 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]

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.