~~Confidential~~

Helsinki, 17 September 2021

Addressees
Registrant(s) as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
30/10/2019

Registered substance subject to this decision ("the Substance")
Substance name: [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate
EC number: 255-288-2
CAS number: 41272-40-6

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed in A.1. and B.1. - B.4. below by ***3 Janury 2023*** and all other information listed below by ***25 March 2024***.

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

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

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

2. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202)

3. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method:EU C.3./OECD TG 201 // EU C.26./OECD TG 22)

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. Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.) to be combined with the Screening for reproductive/developmental toxicity below

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

5. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: OECD TG 203)

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

7. Simulation testing on ultimate degradation in surface water (triggered by Annex VIII, Section 9.2.; test method: EU C.25./OECD TG 309) at a temperature of 12 °C

8. Identification of degradation products (triggered by Annex VIII, Section 9.2; test method: using an appropriate test method or test method; simulation test method OECD TG 309)

9. Bioaccumulation in aquatic species (triggered by Annex I, sections 0.6.1. and 4.; Annex XIII, Section 2.1.; test method: OECD TG 305, aqueous exposure/dietary exposure) with, to the extent technically feasible, analytical monitoring of all transformation/degradation products identified in the study requested under B.8 above.

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

- Appendix entitled "Reasons common to several requests";
- Appendix/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".

The studies relating to biodegradation and bioaccumulation are necessary for the PBT assessment. However, to determine the testing needed to reach the conclusion on the persistency and bioaccumulation of the Substance you should consider the sequence in which

these tests are performed and other conditions described in Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes".

Appeal

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

Failure to comply

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

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

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

Appendix on Reasons common to several requests

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 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.)
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
- Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)
- Adsorption/desorption screening (Annex VIII, Section 9.3.1.)
- Bioaccumulation in aquatic species (Annex IX, Section 9.3.2.)

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

A. Predictions for (eco)toxicological properties and environmental fate

You have not provided a read-across justification document.

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

In your dossier *you used data from the following source substances:*

- Basic Violet 4 (EC 219-231-5)
- Basic Violet 1 (EC 616-846-4)
- [4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]diethylammonium acetate (EC 278-585-9)
- [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-

[2] 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

[3] 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)

[4] 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

ylidene]dimethylammonium chloride (EC 202-322-8)
- [4-[[4-(dimethylamino)phenyl][4-(methylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (EC 282-846-2)
- Fluorescein (EC 208-253-0)
- Patent Blue (EC 204-934-1)
- Basic Violet 14 (EC 211-189-6)
- Green S (EC 221-409-2)
- Disulphonato-1-naphthyl)benzylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylammonium, monosodium salt (EC 221-409-2)
- Fast green (EC 219-091-5)

Additionally, in your comments to the initial draft decision you used data from the following source substances:
- 4-[(4-dimethylaminophenyl)-[4-(methylamino)phenyl]methylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (EC: 616-846-4) [Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test]
- 4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC: 208-953-6) [developmental toxicity, short-term toxicity testing on fish]
- 4-[[4-(dimethylamino)phenyl]-phenylmethyl]-N,N-dimethylaniline (EC: 204-961-9)
- Acetic acid (EC: 200-580-7) [developmental toxicity]
- [4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]diethylammonium acetate (EC 278-585-9) [Repeated dose toxicity as per OECD 407, short-term toxicity testing on aquatic invertebrates]
- [4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC: 208-953-6) [Repeated dose toxicity, short-term toxicity testing on fish]
- Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate (EC 241-922-5)
- [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 209-322-8)
- [4-[[4-(dimethylamino)phenyl][4-[ethyl(3-sulphonatobenzyl)amino]phenyl]methylene]cyclohexa-2,5-dien-1-ylidene](ethyl)(3-sulphonatobenzyl)ammonium, sodium salt (EC 216-901-9) [bioaccumulation in aquatic species]
- N, N-dimethylaniline (EC 204-493-5) [adsorption / desorption screening]

You have provided in your comments the following reasoning for the prediction of (eco)toxicological properties and environmental fate: *" The target substance […] is commonly known as C. I. Solvent Green, acetate salt. This substance and most of the read-across analogues are used as dyes.The read-across substances have been identified using the OECD QSAR toolbox version 3.4, wherein the target substance profiling has been done in the initial activity, and the read-across substances have been identified based on various criteria of functional groups. […] the target and read-across substances covered in this justification have common properties and present comparable environmental fate, ecotoxicological and toxicological behaviour.".*

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 and environmental fate.

1. *Read-across documentation*

Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide a justification for the read-across including a hypothesis, explanation of the rationale for the prediction of properties and robust study summary(ies) of the source study(ies).[5]

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

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

In your comments on the initial draft decision you provided two attachments including a read-across justification document, addressed below (the numbering correspond to the list of attachment mentioned under Appendix E).

> Attachment 2: Consolidated comments to the draft decision on substance evaluation of [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (CAS no.41272-40-6; EC no. 255-288-2),
> Attachment 3: Read-across justification for the chemical CAS No. 41272-40-6 based on ECHA's Read-Across Assessment Framework (RAAF) document.

Furthermore you stated that the justification is attached in IUCLID section 13.2 : *"On Page 5 of ECHA Draft Decision is stated that the Registrants did not provided a Read Across Justification document. We checked the dossier submitted in February 2020 and the document was attached to section 13.2 (other assessment reports)".* However there is no attachment in section 13.2 in the dossier (submission number [redacted] and [redacted]), therefore ECHA is unable to assess the above mentioned information.

In your justification document (see 3, Read-across justification for the chemical CAS No. 41272-40-6) you have indicated that 'Scenario 2' of the RAAF was selected for the analogue approach.

You further state that *"according to to the OECD QSAR Toolbox v3.4, structural alerts for (eco)toxicological endpoints are consistent between the target substance and the read-across analogues. The target substance and the read-across analogues have several common alerts in general mechanistic and endpoint specific mechanisms. The mechanistic triggers of read-across analogues are comparable with the endpoint specific requirements, which further strengthen the target values. […] The structural alerts for (eco)toxicological endpoints and environmental fate are consistent between the target and read-across analogues and shown in Table 3.*

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

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

different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substances.

2. *No basis for prediction*

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 category members, including test materials.[6] Therefore, qualitative and quantitative information on the compositions of the test materials should be 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 substances in the dossier. Furthermore, for all the studies provided in the technical dossier that were conducted with these substances, as listed above, no information on the composition of the test material used to generate the source data is provided.

In your comments you acknowledge that there is no detailed description of the test material. The new source studies provided in attachment 2 in your comments for all properties do not include any description of the test material besides the CAS/EC number.

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.

3. *Missing supporting information to compare properties of the substances (only* toxicological properties*)*

Annex XI, Section 1.5 of the REACH Regulation states that "*physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)*". For this purpose "*it is important to provide supporting information to strengthen the rationale for the read-across*"[7]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

"Supporting information must include bridging studies to compare properties of the Substance and source substances.

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

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

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).

In your comments you have provided new studies with source substances but not with the target substance to fulfil the information requirement for repeated dose toxicity and screening for reproductive toxicity as listed under the relevant endpoints. Therefore, there is no endpoint-specific information (bridging studies) available to compare properties of the source substances with those of the target substance. The data set reported in the comments does not include relevant, reliable and adequate information for the Substance.

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

4. Adequacy and reliability of source study

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

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

Test material identity

As described above under "No basis for prediction", purity and impurity profiles of the substance and the structural analogue need to be assessed.

You do not provide any description of the source substances in your dossier or in your comments. 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. As explained above under "No basis for prediction", the new source studies provided in your comments do not contain the required information on the test material.

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.1-3.,B.2-3, and B.5. These deficiencies are discussed under the respective endpoints.

B. Conclusions on the read-across approach

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

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

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.)
4. Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
5. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
6. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
7. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
8. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)

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

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

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

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

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

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

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

Reliability of the read across approach

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

Assigned reliability of studies

The following studies have been given a reliability score of 4 (non-assignable) by you with limited reporting and no further justification:

1. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (study ii))
2. In vitro gene mutation study in mammalian cells (study ii))
3. Screening for reproductive/developmental toxicity (study ii))

Therefore the studies cannot be regarded as reliable.

Study conducted after 2008 and not GLP compliant

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

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

1. Short-term daphnia studies (study i., ii., and iii.);
2. Algae studies (study i. and ii.);
3. Ready biodegradation study (OECD TG 301 D, 2018);
4. Short-term fish studies (study i., ii., and iii);
5. Adsorption/desorption study (OECD TG 121, 2018).

Therefore the studies cannot be regarded as reliable.

3. Assessment of the identity of the test material

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

1. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
2. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
3. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)
4. Adsorption/ desorption screening (Annex VIII, Section 9.3.1.)
5. Simulation testing on ultimate degradation in surface water (Annex VIII, Section 9.2.)
6. Identification of degradation products (Annex VIII, Section 9.2.)
7. Bioaccumulation in aquatic species (Annex I, sections 0.6.1. and 4.; Annex XIII, Section 2.1.)

You have provided studies for 1 – 7 above under the endpoint in appendices A-B that you claim were conducted with the Substance.

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

To identify the test materials in all the studies for 1 – 7 above under the endpoint, you have provided the substance name, EC and/or CAS number, and/or the purity of the Substance in water. Information on the detailed composition, including quantitative and qualitative information on all constituents present in the test material, or production process of the test material has not been provided.

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

Therefore, the provided information is rejected.

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

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is a standard information requirement in Annex VII 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 to support your adaptations:

i) *In vitro* gene mutation study in bacteria (2006) with analogue substance Basic Violet 4 (EC 219-231-5).
ii) *In vitro* gene mutation study in bacteria (1981) with analogue substance Basic Violet 1 (EC 616-846-4).

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

A. Read-across

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

B. Weight of evidence

As explained in Section 2 of the Appendix common to several requests, the weight of evidence 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 471 must be provided. The key parameters investigated by this test are:

- Detection and quantification of gene mutations (base pairs, substitution or frame shift) in cultured bacteria including data on the number of revertant colonies; and
- Data provided on 5 bacterial strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

The provided studies detect and quantify mutations in bacteria. However, they do not include data on the required fifth strain, S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101).

Therefore, the provided studies only provide partly relevant information.

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

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

As a conclusion, sources of information as indicated above, provide information on mutations in bacteria which is only partly relevant, 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.

In your comments you have agreed to perform the requested test.

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

Possibility for data sharing:

The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[8].

2. Short-term toxicity testing on aquatic invertebrates

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

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. (weight of evidence) and Annex XI 1.5 (grouping of substances and read-across) of REACH.

You have provided the following sources of information to support your adaptations;

i. Weight of evidence: OECD TG 202 study (2018) not GLP compliant, with the Substance (2018)
ii. Weight of evidence: OECD TG 202 study (2018), not GLP compliant, with analogue substance [4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]diethylammonium acetate (EC 278-585-9)
iii. Weight of evidence: OECD TG 202 study (2019) GLP compliance not specified, with analogue substance [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 202-322-8)

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

A. Read-across

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

B. Weight of evidence

As explained in Section 2 of the Appendix common to several requests, the weight of evidence 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 202 must be provided. The key parameter investigated by this test is immobilisation of aquatic invertebrate.

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

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

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

In addition, the reliability of the sources of information is also affected by the following additional issues.

The conditions of exposure in OECD TG 202 specifies that:

Validity criteria

1. the dissolved oxygen concentration is ≥ 3 mg/L in all test vessels at the end of the test;

Technical specifications impacting the sensitivity/reliability of the test

2. *Daphnia magna* (or other suitable *Daphnia* species) is used as test species;
3. at least 20 animals are used at each test concentration and for the controls;

Characterisation of exposure

4. the concentrations of the test material are measured at least at the highest and lowest test concentration, at the beginning and end of the test;
5. the effect values can only be based on nominal or measured initial concentration if the concentration of the test material has been satisfactorily maintained within 20% of the nominal or measured initial concentration throughout the test (see also ECHA Guidance R.7b, Section R.7.8.4.1);

Reporting of the methodology and results

6. pH measured at least at the beginning and end of the test is reported and the pH variation is < 1.5 units;
7. adequate information on the analytical method (including performance parameters of the method) and on the results of the analytical determination of exposure concentrations are provided;

Regarding point 1 above, none of the source information provide information on the dissolved oxygen (a validity criterion of the TG). The conclusion of source information i) and ii) is that this criterion were fulfilled, but there is no supporting information for this claim.

Regarding point 2 above, source information iii) does not include the information on the test organism (e.g. no name and only stated as 'aquatic invertebrate', no source information).

Regarding point 3 above, in the source information i) and ii) only 10 organisms were used. The source information iii) does not include the information on the number of organism used.

Regarding points 4, 5, 6, 7 above, analytical monitoring was not performed in any of the source information although the effect concentrations are reported based on nominal concentrations. For source information iii), information on pH is not reported. This is important because, as stated below under "test design", the Substance exists as both malachite green cation and malachite green carbinol in solution and the relative portion depends on pH. The pH value reported in the source study i) with the Substance (i.e. pH 7.1) indicates that both malachite green cation and malachite green carbinol were present in the solution. However, analytical monitoring was not performed and it is thus not possible to determine the exposure concentrations of each chemical species.

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

As a conclusion, sources of information as indicated above, provide information on immobilisation of aquatic invertebrate but the information provided is not reliable.

Accordingly, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 202 study.

In your comments, you stated that information on short-term toxicity on aquatic invertebrates is available on the Substance and supporting information on analogue substances, and that you will provide this information in an update of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment, especially because raw data are missing and you did not provide information to address the issues listed above (3.-7.).

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)."

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

Study design

The Substance is difficult to test due to ionisable, hydrolysable and photodegradable properties of the Substance, and the substance is a colored dye.

The Substance is a soluble salt consisting of a cationic part (Malachite green) and an anionic part (acetate anion). In water, the coloured cation (Malachite green) is in equilibrium with its colourless carbinol base, usually called 'Malachite green carbinol' or 'Malachite green carbinol base' or 'Malachite green pseudo-base' (EC no. 208-109-7/ CAS no. 510-13-4). The equilibrium is pH dependent: according to available literature data, at pH 4 the main chemical species present is the coloured cation (i.e. Malachite green), at around pH 7 both chemical species are present (the time required to each equilibrium is ca. 2 hours), while at pH 9 the predominant chemical species is malachite green carbinol.

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.

In addition, 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."

3. Growth inhibition study aquatic plants

Growth inhibition study aquatic plants is a standard information requirement in Annex VII to REACH (Section 9.1.2).

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. (weight of evidence) and Annex XI 1.5 (grouping of substances and read-across) of REACH.

You have provided the following sources of information to support your adaptations;

i. Weight of evidence: OECD TG 201 study (2018) not GLP compliant, with the Substance (2018)
ii. Weight of evidence: OECD TG 201 study (2017), not GLP compliant, with analogue substance: [4-[[4-(dimethylamino)phenyl][4 (methylamino)phenyl]methylene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (EC 282-846-2)

We have assessed this information and identified the following issues:

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 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 201 must be provided. The key parameter investigated by this test is growth rate of algal cultures.

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

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

In addition, the reliability of the sources of information is also affected by the following additional issues.

The conditions of exposure in OECD TG 201 specify that:

1. the concentrations of the test material are measured at least at the beginning and end of the test;
2. the results can be based on nominal or measured initial concentration only if the concentration of the test material has been maintained within 20 % of the nominal or measured initial concentration throughout the test;

No analytical monitoring of exposure was conducted for both source information i) and ii), however, the effect concentration was reported based on nominal concentrations. The source information i) with the Substance was conducted at pH range 6.58-7.94. This is important because, as stated above in Appendix A-2 under "*test design*" section, the Substance exists as both malachite green and malachite green carbinol in solution within this pH range. However, analytical monitoring was not performed and it is thus not possible to determine the exposure concentrations of each chemical species.

As a conclusion, sources of information as indicated above, provide information on the growth rate of algal cultures but the information provided is not reliable.

Accordingly, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 201 study.

In your comments, you stated that information on a freshwater algal growth inhibition test is available on the Substance and supporting information on analogue substances, and that you will provide this information in an update of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment, because raw data are missing to verify the validity criteria and the characterisation of exposure of OECD TG 201. Furthermore, the results are based on nominal concentrations. However, you have not demonstrated the stability of exposure concentrations (i.e. measured concentration(s) within 80-120% of the nominal concentration(s)). Information on analytical monitoring and analytical method is missing. Without analytical monitoring, it is not possible to determine whether and to what extent the tested organisms were exposed to the test material.

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)."

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

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 Section A.2.

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

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

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

You have adapted 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 study of chromosomal aberrations (ABs) in cultured Chinese hamster ovary (CHO) cells (1990) with analogue substance Fluorescin (EC 208-253-0)

ii) *In vivo* chromosome aberration assay according to OECD TG 473 (1992) with analogue substance Basic Violet 4 (EC 219-231-5). Rel. 4.

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

A. Read-across

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

B. Weight of evidence

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

To fulfil the information requirement, normally a study according to OECD TG 473/487 must be provided. The key parameter investigated by this test is cytogenicity in mammalian cells. The provided sources of information investigate cytogenicity in mammalian cells. Therefore, they provide information that would contribute to the conclusion on this key parameter.

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

In addition, source study ii) has been given a reliability score of 4 by you (not assignable), with limited reporting and ECHA agrees that this source study is not reliable.

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

As a conclusion, 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 is rejected and the information requirement is not fulfilled.

In your comments you have agreed to perform the requested test.

Information on the study design

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

Possibility for data sharing:

The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[9].

2. In vitro gene mutation study in mammalian cells

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

Your dossier contains an adaptation for an *in vitro* gene mutation study in bacteria, and an adaptation 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 2 of Appendix A and section 1 of this Appendix B.

The result of the requests for information in section 2 of Appendix A and section 1 of this Appendix B will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

For Annex VIII, 8.4.3., you have not provided any study with the Substance in your dossier. However, you have adapted this information requirement by using Weight of Evidence under Annex XI, Section 1.2 of REACH and Annex XI 1.5 (grouping of substances and read-across).

You have provided the following sources of information:

i) *In vitro* mammalian cell gene mutation test (2006) with analogue substance Basic violet 4 (EC 219-231-5)

ii) *In vitro* mammalian cell gene mutation test (2013) with analogue substance Patent Blue (EC 204-934-1). Rel. 4.

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

A. Read-across

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

B. Weight of evidence

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

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

To fulfil the information requirement, normally a study according to OECD TG 476/490 must be provided. The key parameter investigated by this test is mammalian cell gene mutation.

The provided sources of information investigate mammalian cell gene mutation. Therefore, they provide information that would contribute to the conclusion on this key parameter.

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

In addition, source study ii) has been given a reliability score of 4 by you (not assignable), with limited reporting and ECHA agrees that this source study is not reliable.

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

As a conclusion, sources of information as indicated above, provide information on mammalian cells gene mutation 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.

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

In your comments you have agreed to provide the requested information.

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

Possibility for data sharing:

The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[10].

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

A Short-term repeated dose toxicity study (28 days) is a standard information requirement under Annex VIII to REACH (Section 8.6.1.).

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.5 (grouping of substances and read-across) of REACH and Weight of Evidence under Annex XI, Section 1.2 of REACH.

You have provided the following sources of information in your dossier:

i) Chronic toxicity study (1982) with analogue substance Basic Violet 14 (EC 211-189-6)
ii) Short-term repeated dose toxicity study (1987) with analogue substance Green S (EC no 221-409-2).

In your comments on the draft decision you indicate your intention to adapt this information request. You have provided new information in support of an adaptation.

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

iii) Subacute toxicity study (2018) with the analogue substance [4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]diethylammonium acetate [CAS: 76994-37-1; EC: 278-585-9],
iv) Chronic toxicity study (2 year study) with the analogue substance -[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride[CAS: 548-62-9; 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

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 407 must be provided. The key parameter investigated by this test is repeated dose toxicity.

The provided studies investigate repeated dose toxicity. Therefore, they provide information that would contribute to the conclusion on this key parameter.

However, the reliability of these studies, including those submitted in your comments on the draft decision, are significantly affected by the deficiencies identified in Section 2 of the Appendix on Reasons common to several requests.

In addition, the reliability of the source of information ii) for this endpoint is also affected by the following issue:

The conditions of this test guideline include
- dosing of the Substance daily for a period of 28 days until the scheduled termination of the study

The study ii) you have provided is a 2-week study and does not have the required exposure duration of 28 days.

Therefore, the condition is not fulfilled and the provided studies cannot be considered reliable sources of information.

As a conclusion, sources of information as indicated above, provide information on repeated dose toxicity 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.

Information on study design

Referring to the criteria provided in Annex VIII, Section 8.6.1, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the substance is a liquid with low vapour pressure.

REACH Annex VIII, Section 8.6.1. refers to short-term repeated dose toxicity (28 days), which can be tested by the oral route according to the test methods OECD TG 407 or 422. REACH Annex VIII, Section 8.7.1. refers to screening studies for reproductive/ developmental toxicity according to the test methods OECD TG 421 or 422. As pointed out below in section B.4 of this decision, the information provided under Annex VIII, Section 8.7.1. does not fulfil the information requirement for reproductive/developmental toxicity and therefore there is an information gap. To prevent unnecessary animal testing, an OECD TG 422 study is more appropriate to fulfil the information requirements of both Sections 8.6.1. and 8.7.1. of Annex VIII, as it provides initial information on reproductive/developmental toxicity and on short-term repeated dose toxicity.

Therefore the study must be performed according to the OECD TG 422, in rats and with oral administration of the Substance.

Possibility for data sharing:

The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[11].

4. Screening study 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 (Section 8.7.1), if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant. There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.5 (grouping of substances and read-across) of REACH and Weight of Evidence under Annex XI, Section 1.2 of REACH.

You have provided the following sources of information:

i) Non-guideline teratogenicity and embryotoxicity study (1987) with the analogue substance disulphonato-1-naphthyl)benzylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylammonium, monosodium salt (EC 221-409-2),
ii) Non-guideline 3-generation study (1987) with the analogue substance fast green (EC 219-091-5), RL 4.

In your comments you indicate your intention to adapt this information request. You have provided in the comments the following new studies:

iii) Screening for reproductive/developmental toxicity (no study year) with the source substance, 4-[(4-dimethylaminophenyl)-[4-(methylamino)phenyl]methylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium chloride (EC 616-846-4),
iv) Developmental toxicity study (no study year) with the source substance [4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 208-953-6),
v) Developmental toxicity study (2011) with the source substance 4-[[4-(dimethylamino)phenyl]-phenylmethyl]-N,N-dimethylaniline (EC 204-961-9),

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

vi) Developmental toxicity study (1974) with the source substance Acetic acid [CAS: 64-19-7; EC: 200-580-7].

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 422 must be provided. The key parameter investigated by this test is 1) sexual function and fertility, 2) toxicity to offspring, and 3) systemic toxicity.

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

However, the reliability of the sources of information is significantly affected by the deficiencies identified in Section 2 of the Appendix on Reasons common to several requests. In addition, source study ii) has been given a reliability score of 4 by you (not assignable), with limited reporting and ECHA agrees that this source study is not reliable.

The sources of information iii) and vi) may provide relevant information on these key parameters. However, the reliability of these sources of information is significantly affected by the deficiencies identified in the Appendix on Reasons common to several requests, section 2. (Reliability of the read across approach).

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

As a conclusion, sources of information as indicated above, provide information on sexual function and fertility, toxicity to offspring, and systemic toxicity 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.

In your comments you indicated your intention to adapt this information requirement according to Annex VIII, Section 8.7.1, Column 2, by submitting a pre-natal developmental toxicity study with the Substance. *"By weight of evidence, treatment with the registered substance in rats as per OECD 414 can be assumed to produce no adverse effects on development. By proposal, the reproduction/developmental toxicity study (OECD 421) can be omitted in accordance with REACH Annex VIII, Section 8.7.1, Column 2".* It is in your discretion to provide the necessary supporting information in the dossier in order to justify your adaptation. If doing so, you are responsible for demonstrating the fulfilment of the requirements of the relevant Annex(es) of REACH, taking into account the issues identified in this decision.

Information on study design

A study according to the test method EU B.64/OECD TG 422 must be performed in rats with oral[12] administration of the Substance, as already explained above.

Possibility for data sharing:

The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[13].

5. Short-term toxicity testing on fish

Short-term toxicity testing on fish is a standard information requirement in Annex VIII to REACH (Section 9.1.3).

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. (weight of evidence) and Annex XI 1.5 (grouping of substances and read-across) of REACH.

In support of your adaptation, you have provided the following sources of information:

i) Weight of evidence: OECD TG 203 study (2018) not GLP compliant, with the Substance.
ii) Weight of evidence: Data from J-Check database (2019) not specified to be GLP compliant, with [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride (EC 202-322-8)
iii) Weigt of evidence: OECD TG 203 study (2017) not GLP compliant, with analogue substance [4-[[4-(dimethylamino)phenyl][4-(methylamino) phenyl] methylene] cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (EC 282-846-2)

We have assessed this information and identified the following issues:

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 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 203 must be provided. The key parameter investigated by this test is mortality of fish.

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

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

[12] ECHA Guidance R.7a, Section R.7.6.2.3.2.
[13] https://echa.europa.eu/regulations/reach/registration/data-sharing

In addition, the reliability of the sources of information is also affected by the following additional issues.

The validity criteria of the OECD TG 203 include:

Validity criteria
1. the analytical measurement of test concentrations is conducted;
2. the dissolved oxygen concentration is ≥ 60% of the air saturation value in all test vessels throughout the exposure;
3. the test duration is 96 hours or longer;

Regarding point 1 above, you have indicated that no analytical monitoring of exposure was conducted for the source information i) and iii). Analytical monitoring was not specified for the source information ii). In addition, the studies were conducted at pH 7.1 and 7.12 for source study i) with the Substance. As stated Appendix A-2 under "*test design*" section, the Substance exists as both malachite green and malachite green carbinol in solution at these pH range. However, analytical monitoring was not performed and it is thus not possible to determine the exposure concentrations of each chemical species. For the source study ii) there is no information on test conditions provided, including pH.

Regarding point 2 above, no information on dissolved oxygen is available for the source information ii). For source information i) and iii), dissolved oxygen was provided as mg/L but not as % of the air saturation value.

Regarding point 3 above, you have indicated that the exposure duration of the source information iii) is 24 hours.

Therefore, source of information i), ii) and iii) are not reliable.

As a conclusion, sources of information as indicated above, provide information on mortality of fish but the information provided is not reliable.

Accordingly, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 203 study.

In your comments, you stated that information on short-term toxicity on fish is available on the Substance and supporting information on analogue substances, and that you will provide this information in an update of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment, because raw data are missing to verify the validity criteria. In particular it is unclear if there was any analytical monitoring of the test concentrations. Furthermore, the results are based on nominal concentrations. However, you have not demonstrated the stability of exposure concentrations (i.e. measured concentration(s) within 80-120% of the nominal concentration(s)). Information on analytical monitoring and analytical method is missing. Without analytical monitoring, it is not possible to determine whether and to what extent the tested organisms were exposed to the test material.

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)."

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

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 Section A.2.

6. Adsorption/ desorption screening

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

You have provided a key study (2018) corresponding to adsorption/desorption screening study according to OECD TG 121, not GLP, with the Substance.

We have assessed this information and identified the following issues:

A. Validity of the study

To fulfil the information requirement, a study must comply with the OECD TG 121 (Article 13(3) of REACH). Therefore, the following requirements must be met:

- The HPLC method has to be performed on analytical columns packed with a commercially available cyanopropyl solid phase containing lipophilic and polar moieties. Commercial cyanopropyl chemically bound resins on a silica base shall be used (e.g. Hypersil and Zorbax CN).
- Ionisable substances must be measured using a buffered mobile phase.

You have provided a study performed with an HPLC column '*ZORBAX Eclipse Plus C18*' where the stationary phase is C18. The mobile phase for the HPLC method was '*Acetonitrile : water (55:45)*'. The mobile phase used for the HPLC was not a buffered mobile phase.

The Substance is a salt consisting of a cationic dye and the acetate anion XX, and thus is ionisable.

The study you have provided does not fulfil the requirements of OECD TG 121. Specifically:
- The stationary phase of the HPLC column was not adequate for this kind of test.
- The mobile phase used for the HPLC was not a buffered mobile phase, although it is specified for ionisable substances.

These are critical methodological deficiencies resulting in the rejection of the study results. More specifically, the study is not reliable because the key parameter (adsorption coefficient Koc as determined by the partition of the Substance between the mobile solvent phase and the cyanopropyl stationary phase using revers phase HPLC) was not measured as indicated in the OECD TG 121. Furthermore the method is applicable to ionisable substances if an appropriate buffer (with a pH in the range of 5.5 to 7.5) is used for the mobile phase, which was not used in this study.

In your comments, you stated that information on adsorption/desorption is available on the Substance and supporting information on an analogue substance and that you will provide this information in an update of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment, because you did not provide the information on the stationary phase and the mobile phase of the HPLC column. Because of the issues identified under the Appendix on Reasons common to several requests, it is not possible to assess whether the requirements are met. Because of missing documentation (no

QMRF/QPRF provided), it is not possible to verify the validity of your QSAR prediction on the Substance using OPERA.

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)."

Therefore, this information is rejected.

B. Study conducted after 1 August 2008 and not GLP

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

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

Therefore, this information is rejected.

On this basis, the information requirement is not fulfilled.

7. Simulation testing on ultimate degradation in surface water

Further degradation testing must be considered if the chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the degradation of the substance (Annex VIII, Section 9.2., Column 2).

This information requirement is triggered in case the chemical safety assessment indicates the need for further degradation investigation, such as if the substance is a potential PBT or vPvB (Section 4, Annex I and Sections 2.1 and 3.2, Annex XIII to REACH and ECHA Guidance R.11.4). This is the case if the substance, a constituent, an impurity or a transformation / degradation product meets the PBT/vpvB criteria.

The information provided in your dossier indicates that the Substance may have PBT/vPvB properties following criteria:

1. the Substance is potentially persistent or very persistent (P/vP) if:
   - it is not readily biodegradable (i.e. <60/70% degradation in an OECD 301D), and
2. the Substance is potentially bioaccumulative or very bioaccumulative (B/vB) if:
   - other mechanisms than partitioning to lipids may drive bioaccumulation (e.g. binding to protein/cell membranes) and high potential for bioaccumulation cannot be excluded;
3. the Substance meets the T criteria set in Annex XIII: NOEC or EC10 < 0.01 mg/L or classification as carc. 1A or 1B, muta. 1A or 1B, repro. 1A, 1B or 2, or STOT RE 1 or 2.

The information provided in your dossier indicates that:

1. the Substance is potentially P/vP since it is not readily biodegradable (Key study (2018) showing 38% degradation after 35 days in OECD TG 301 D);
2. the Substance is ionisable and no reliable screening information is available to support that is not potentially B/vB;

3. the substance meets the criteria for T: currently self-classified in the technical dossier as Repro tox 2. You have concluded that the Substance is inherently biodegradable based on the key study (2018) on ready biodegradability. However this conclusion is not supported by the reported results of the key study (see above). Even though the reliability of the key study is affected by the fact that it is not performed according to GLP (without further explanation) after 1 June 2008, available literature[14] indicates that the Substance is not readily biodegradable and that they may persist in the environment. Thus the available screening information is not sufficient to conclude on the P/vP properties of the Substance.

Furthermore, the information in your dossier is currently incomplete and therefore:
- it is not possible to conclude on the bioaccumulation potential of the Substance (see Appendix B, Section 9 below of this decision), and
- it is not possible to conclude on the toxicity of the Substance see Appendix A, Sections 1-3 and Appendix B, Sections 1-5 of this decision).

Based on the above the Substance may have PBT or vPvB properties and therefore further information on biodegradation must be provided.

In your comments you have agreed to perform the requested test.

Study design

Under Annex XIII, the information must be based on data obtained under conditions relevant for the PBT/vPvB assessment. Therefore:

- You must perform the OECD TG 309 test, by following the pelagic test option with natural surface water containing approximately 15 mg dw/L of suspended solids (acceptable concentration between 10 and 20 mg dw/L) (ECHA Guidance R.11).
- You must perform the test at the temperature of 12 °C, the average environmental temperature for the EU (ECHA Guidance R.16, Table R.16-8). Performing the test at this temperature is in line with the applicable test conditions of the OECD TG 309.

Non-extractable residues (NER) must be quantified in all simulation studies. The reporting of results must include a scientific justification of the used extraction procedures and solvents. By default, total NER is regarded as non-degraded Substance. However, if reasonably justified and analytically demonstrated a certain part of NER may be differentiated and quantified as irreversibly bound or as degraded to biogenic NER. Such fractions could be regarded as removed when calculating the degradation half-life(s) (ECHA Guidance R.11).

8. Identification of degradation products

Further degradation testing must be considered if the chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the degradation of the substance (Annex VIII, Section 9.2., column 2). triggered by Annex XIII, Section 2.1.

This information requirement is triggered in case the chemical safety assessment indicates the need for further degradation investigation, such as if the substance is a potential PBT or vPvB (Section 4, Annex I and Sections 2.1 and 3.2, Annex XIII to REACH and ECHA Guidance R.11.4). This is the case if the substance, a constituent, an impurity or a transformation / degradation product meets the PBT/vPvB criteria.

[14] e.g. Annex 1 Background document of ECHA/RAC/CLH-O-0000001309-75-03/A1 (2010), Section 10.3 Annex III (page 50-); Schwarzbauer and Apel "Malachite green in suspended particulate matter and surface sediments in Germany" available from: http://www.umweltprobenbank.de/upb_static/fck/download/MG-UPB_20131011.pdf.

As already explained in Request B-4 above, the information provided in your dossier indicates that the Substance may have PBT/vPvB properties.

There is no adequate information in the dossier provided on the degradation products formed in the surface water/soil/sediment under environmentally relevant conditions and concentrations.

The available screening information is not sufficient to conclude on the P/vP properties of the Substance, therefore further testing is required. Furthermore, information on bioaccumulation and toxicity are currently incomplete and therefore it is not possible to evaluate the bioaccumulation (Request B-9 below of this decision) and toxicity (see Appendix A, Sections 1-3 and Appendix B, Sections 1-5 of this decision) of the Substance.

Based on the above the Substance may have PBT or vPvB properties and therefore further information on biodegradation must be provided.

In your comments you have agreed to perform the requested test.

Study selection and design

You must obtain this information while performing the simulation study requested in this decision (request B-7 above). You must provide a scientifically valid justification for any other method you have used for identification of the transformation/degradation products.

Identity, stability, behaviour, and molar quantity of the degradation/ transformation products relative to the Substance must be evaluated and reported, when analytically possible. In addition, degradation half-life, potential for bioaccumulation and toxicity of the transformation / degradation product must be investigated.

9. Bioaccumulation in aquatic species

This information requirement is triggered in case the results from screening tests or other information indicate that the substance is a potential PBT or vPvB (Annex I, sections 0.6.1. and 4; Annex XIII, Section 2.1; ECHA Guidance R.11.4).

This is the case if the Substance itself or any of its constituent, impurity or transformation/degradation product present in concentration ≥ 0.1% (w/w) meets the criteria, as already specified in the Appendix B-4 above. Specifically for bioaccumulation:

the Substance is potentially bioaccumulative or very bioaccumulative (B/vB) if:

- it has a high potential to partition to lipid storage (e.g. log Kow > 4.5); and/or
- other mechanisms than partitioning to lipids may drive bioaccumulation (e.g. binding to protein/cell membranes) and high potential for bioaccumulation cannot be excluded;

In the technical dossier, you have stated that "*the study [on bioaccumulation] does not need to be conducted because the Substance has a low potential for bioaccumulation based on logKow <=3".*

However, to use log Kow to support low potential for bioaccumulation, the partitioning to lipids must be the sole mechanism driving the bioaccumulation potential of a substance. However, the Substance is ionisable. Hence other partitioning mechanisms may drive bioaccumulation (e.g. binding to protein/cell membranes). For such substances log Kow is not considered a valid descriptor of the bioaccumulation potential (ECHA Guidance R.7c, Appendix R.7.10-3).

Furthermore, available literature[15] shows that the Substance is metabolically transformed by fish into leucomalachite green. It has been shown in recent studies[16] that both the Substance (i.e. malachite green) and leucomalachite green are detected in fish and aquaculture products within the EU.

Based on the available information the Substance may have PBT or vPvB properties and therefore further information on bioaccumulation must be provided.

QSAR predictions

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.3 (qualitative or quantitative structural-activity relationship (QSAR)) of REACH. In support of your adaptation, you have provided the following sources of information:

(i) a QSAR prediction BCFBAF (version 3.01) on the Substance;
(ii) a QSAR prediction OPERA (version 1.02) on the Substance;

We have assessed this information and identified the following issue:

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

1. adequate and reliable documentation of the applied method is provided;
2. the substance falls within the applicability domain of the QSAR model; and
3. 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 a QSAR prediction for this endpoint, concluding that the Substance is "*not expected to bioaccumulate in the food chain*".

Regarding point 1 above, you have not provided any documentation for the QSAR prediction. In particular, you have not included a QMRF and/or a QPRF in your technical dossier.

Regarding point 2 above:

- The Substance is an ionic compounds and a dye.
- For prediction ii), you did not indicate whether the prediction was inside or outside of the applicability of the model.

The information provided does not comply with point 1 above.

Regarding point 2 above, the Substance does not fall within the applicability domain of the model (see ECHA Guidance R.6, Section R.6.1.5, and ECHA's Practical guide "How to use and report (Q)SARs", section 3.2).

More specifically:

[15] e.g. Plakas et al., (1996) "Uptake, tissue distribution, and metabolism of malachite green". Aquaculture 44, 145-152; Schuetze et al., (2008) "Occurance of residues of the veterinary dung malachite green in eels caught downstream from municipal sewage treatment plants". Chemosphere 72, 1664-1670 (BfR study).
[16] e.g. EFSA report (2016) "Malachite green in food"; belpaire et al., (2015) "Toxic textile dyes accumulate in wild European eel *Anguilla anguilla"*. Chemosphere 138, 784-791.

- for the prediction i) BCFBAF (version 3.01) can be primarily used for non-ionic substance and is not suitable for ionic compounds and dyes. Therefore the Substance does not fall within the applicability domain of the QSAR model .
- For prediction ii), you did not indicate whether the prediction was inside or outside of the applicability of the model.

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

In your comments, you stated that supporting information bioaccumulation is available on analogue substances (a QSAR prediction and two experimental studies), and that you will provide this information in an update of your registration dossier. The information in your comments is not sufficient for ECHA to make an independent assessment. About the QSAR prediction there are the same issues listed above for point 1 and 2. It is not possible to make an independent assessment of the experimental studies provided because tabulated test material concentration data in individual fish and water (including mean values for test group and control, standard deviation and range, if appropriate) for all sampling times are not provided.

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)."

Study design

The test guideline OECD TG 305 (I-III) apply to the Substance.

Bioaccumulation in fish: aqueous and dietary exposure (test method EU C.13. / OECD TG 305) is the preferred test to investigate bioaccumulation (ECHA Guidance, Chapter R.7c, R.7.10.3.1). Whenever technically feasible, the aqueous route of exposure (OECD TG 305-I) must be used as the results obtained can be used directly for comparison with the B and vB criteria of Annex XIII of REACH. Therefore, the requested study must be conducted with aqueous exposure. If testing through aquatic exposure is technically not possible, you must provide scientifically valid justification for the infeasibility and testing must be conducted with dietary exposure.

For PBT purposes (Annex XIII of REACH), the information provided is to address the bioaccumulation of the Substance itself as well as any of its constituents, impurity or transformation/degradation products. In this case, there are indications of several chemical species present and that may be relevant for PBT assessment, including leucomalachite green[17].

Therefore, the study must monitor not only the Substance (i.e. malachite green), but also any other relevant transformation/degradation products identified under the request in Appendix B-5 above, to the extent technically feasible.

Otherwise, it is not possible to relate the observed effects to the Substance itself considering its properties described above. For the same reason, you must provide a description on the analytical method used, monitor the test concentration(s), indicate what has been monitored and on which chemical species the effect concentrations are based.

[17] Commission Decision of 22 December 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin (2440/25/EC).

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[18].

B. Test material

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have 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[19].

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

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

A. Strategy for the PBT/vPvB assessment

You are advised to consult ECHA Guidance R.7b (Section R.7.9.), R.7c (Section R.7.10) and R.11 on PBT assessment to determine the sequence of the tests needed to reach the conclusion on PBT/vPvB. The guidance provides advice on 1) integrated testing strategies (ITS) for the P, B and T assessments and 2) the interpretation of results in concluding whether the Substance fulfils the PBT/vPvB criteria of Annex XIII.

In particular, you are advised to first conclude whether the Substance fulfils the Annex XIII criteria for P and vP, and then continue with the assessment for bioaccumulation. When determining the sequence of simulation degradation testing you are advised to consider the intrinsic properties of the Substance, its identified uses and release patterns as these could significantly influence the environmental fate of the Substance. You must revise your PBT assessment when the new information is available.

B. Testing strategy for aquatic toxicity testing

You are advised to consult ECHA Guidance R.7b, (Section R.7.8.5) which describes the Integrated Testing Strategy, to determine the sequence of aquatic toxicity tests and testing needed.

Appendix E: Procedure

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

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

The compliance check was initiated on 17 March 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 requests.

In your comments on the initial draft decision you provide three attachments:
1. Comments to the ECHA draft decision Communication number CCH-D-2114510092-67-01/D of the 08 May2020 For Basic green 004 Acetate,
2. Consolidated comments to the draft decision on substance evaluation of [4-[α-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium acetate (CAS no.41272-40-6; EC no. 255-288-2),
3. Read-across justification for the chemical CAS No. 41272-40-6 based on ECHA's Read-Across Assessment Framework (RAAF) document.

Regarding the first attachment, no explanation was provided why or which part of such comments on a different draft decision addressing a different registration dossier would be relevant. Therefore, this information could not be taken into account.

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

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

Appendix F: List of references - ECHA Guidance[20] 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)[21]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[21]

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[22]
Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

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

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

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

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

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

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

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

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