~~Confidential~~

Helsinki, 03 November 2023

Addressees
Registrant(s) of JS_41272-40-6 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
25/11/2020

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/List number: 255-288-2

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***25 March 2024.***

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

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: OECD TG 471, 2020)

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 or EU C.26./OECD TG 221)

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

4. 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)

5. Only if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. is obtained, In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: EU B.17./OECD TG 476 or EU B.67./OECD TG 490)

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

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

8. 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.

9. Identification of degradation products (triggered by Annex VIII, Section 9.2; test method: simulation test method OECD TG 309)

10. Bioaccumulation in aquatic species (triggered by Annex VIII, Section 9.3; test method: EU C.13./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 9 above.

The information in the current draft decision has already been requested from one or several other registrants of the Substance. Where the information was requested from several other registrants, they have informed ECHA of their agreement as to who is to carry out the tests under Article 53(1). Once the current draft decision becomes adopted following procedure of Art. 50 and Art. 51, obligations and rights expressed in Article 53 will apply to you. Under Article 53(2 and 3) of the REACH Regulation if a registrant performs a test on behalf of other registrants, they shall all share the cost of that study equally and the registrant performing the test shall provide each of the others concerned with a copy/copies of the full study report(s). As a consequence, the same deadline shall apply to this decision, with a minimum of six months to allow for contacting the other registrants and organising the application of Article 53(2 and 3).

The reasons for the decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

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 requirements for testing and reporting new tests under REACH, see Appendix 4. In addition, 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 this Appendix.

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 Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[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 1: Reasons for the decision

Contents

0. Reasons common to several requests

0.1. Assessment of weight of evidence adaptations

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

(i) In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
(ii) In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
(iii) In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
(iv) Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
(v) Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
(vi) Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
(vii) Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
(viii) Bioaccumulation in aquatic species (Annex IX, Section 9.3.2.)

2 Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

3 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

4 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 on the corresponding information requirement.

5 Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach. This documentation must include robust study summaries of the studies used as sources of information and a justification explaining why the sources of information together provide a conclusion on the information requirement.

6 You have not included a justification for your weight of evidence adaptation for each of the relevant information requirement, which would include an adequate and reliable (concise) documentation as to why the sources of information provide sufficient weight to conclude on the information requirements under consideration.

7 In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation. Your weight of evidence approach has deficiencies that are common to all information requirements under consideration and also deficiencies that are specific for these information requirements individually.

8 The common deficiencies are set out here, while the specific ones are set out under the information requirement concerned in the Sections below.

0.1.1. Reliability of the read across approach

9 Section 0.2. 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.

0.1.2. Assigned reliability of studies

10 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);
4. Short-term daphnia studies (study i., ii., and iii.);
5. Short-term daphnia studies (study iiii.);
6. Bioaccumulation (studies i and ii)

11 Therefore the studies cannot be regarded as reliable.

0.1.3. Study conducted after 2008 and not GLP compliant

12 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).

13 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);

14 Therefore the studies cannot be regarded as reliable.

0.2. Read-across approach

15 You have adapted the following standard information requirements by using grouping and read-across approach under 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.)
- Bioaccumulation in aquatic species (Annex IX, Section 9.3.2.)

16 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 sections.

17 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.

18 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

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

19 For the endpoints listed above, 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-[[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)
- N, N-dimethylaniline (EC 204-493-5)
- [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)

20 In your comments on the initial draft decision, you provided a read-across justification document.

21 You 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. […T]he target and read-across substances covered in this justification have common properties and present comparable environmental fate, ecotoxicological and toxicological behaviour".*

22 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects.

23 We have identified the following issue(s) with the prediction(s) of (eco)toxicological properties and environmental fate:

0.2.1.1. Read-across documentation

24 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 include a an explanation why the properties of the Substance may be predicted from information on the source substance(s).

25 You have provided robust study summaries for studies conducted with other substances than the Substance in order to comply with the REACH information requirements.

26 In your comments on the initial draft decision, you provided a read-across justification document.

27 In your justification document you indicate that 'Scenario 2' of the RAAF was selected for the analogue approach.

28 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."

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

30 The properties of your Substance are predicted to be quantitatively equal to those of the source substances.

0.2.1.2. Characterisation of the source substances

31 Annex XI, Section 1.5 of the REACH Regulation provides that "substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group."

32 Therefore, qualitative and quantitative information on the compositions of the Substance and of the source substance(s) must be provided to allow assessing whether the attempted predictions are compromised by the composition and/or impurities.

33 You do not provide any description of the source substances. 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.

34 Without qualitative or quantitative information on the compositions of the Substance and of the source substance(s), it is not possible to assess whether the attempted predictions are compromised by the composition of the source substance(s).

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

0.2.2. Conclusion on the read-across approach

36 For the reasons above, you have not established that relevant properties of the Substance can be predicted from data on the source substance(s). Your read-across approach under Annex XI, Section 1.5. is rejected.

Reasons related to the information under Annex VII of REACH

1. In vitro gene mutation study in bacteria

37 An in vitro gene mutation study in bacteria is an information requirement under Annex VII, Section 8.4.1.

1.1. Information provided

38 You have adapted this information requirement by using weight of evidence and a Grouping of substances and read-across approach based on the following experimental data:

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).

1.2. Assessment of the information provided

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

1.2.1. Missing documentation of the weight of evidence

40 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

41 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).

42 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).

43 Therefore, the provided studies only provide partly relevant information.

44 Furthermore, the reliability of these sources of information is significantly affected by the deficiencies identified in the Section 0.1 Reasons common to several requests.

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

46 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.

47 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.

1.2.2. Read-across adaptation rejected

48 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

49 On this basis, the information requirement is not fulfilled.

50 In the comments to the draft decision, you agree with the request.

1.3. Specification of the study design

51 To fulfil the information requirement for the Substance, the in vitro gene mutation study in bacteria (OECD TG 471, 2020) is considered suitable.

1.4. Possibility for data sharing

52 The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[2].

2. Short-term toxicity testing on aquatic invertebrates

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

2.1. Information provided

54 You have adapted this information requirement by using weight of evidence and a Grouping of substances and read-across approach based on the following experimental data:

i. Weight of evidence: OECD TG 202 study (2018) not GLP compliant, with the Substance
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: EU Method C.2 study (2001) GLP compliance not specified, with analogue substance N, N-dimethylaniline (EC 204-493-5)

2.2. Assessment of the information provided

55 We have assessed this information and identified the following issue[s]:

2.2.1. Missing documentation of the weight of evidence

56 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

57 The key parameter investigated by this test is immobilisation of aquatic invertebrate.

58 All the sources of information you provided investigate immobilisation of aquatic invertebrate.

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

59 Therefore, they provide information that would contribute to the conclusion on this key parameter.

2.2.2. The provided studies do not meet the information requirement

60 However, the reliability of these sources of information is significantly affected by the deficiencies identified in Section 0.1.

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

62 The conditions in OECD TG 202 specifies that:

63 Validity criteria

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

64 Technical specifications impacting the sensitivity/reliability of the test

2. at least 20 animals are used at each test concentration and for the controls;

65 Characterisation of exposure

3. 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;
4. 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);

66 Reporting of the methodology and results

5. pH measured at least at the beginning and end of the test is reported and the pH variation is < 1.5 units;
6. 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;

67 Regarding point 1 above, source information iii) does not provide information on the dissolved oxygen (a validity criterion of the TG).

68 Regarding point 2 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.

69 Regarding points 3-6 above, analytical monitoring was not performed in any of the source information although the effect concentrations are reported based on nominal concentrations. For all sources information (i-iii), information on the pH variation is not reported. This is important because, as stated below under "Study design and test specifications", 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.

70 In your comments on the initial draft decision, you provide new information (regarding points 1., 2. and 5. above) on short-term toxicity on aquatic invertebrates on the Substance and supporting information on analogue substances. 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 specific issues listed above (points 3., 4. and 6. above) on the characterisation of exposure. 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)) (points 3. and 4. above). Information on analytical monitoring and analytical method is missing (points 3 and 6. above). Without analytical monitoring, it is not possible to determine whether and to what extent the tested organisms were exposed to the test material.

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

72 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.

2.2.3. Read-across adaptation rejected

73 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

2.3. Study design and test specifications

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

76 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.

77 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.

78 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

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

3.1. Information provided

80 You have adapted this information requirement by using weight of evidence and a Grouping of substances and read-across approach based on the following experimental data:

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)

3.2. Assessment of the information provided

81 We have assessed this information and identified the following issue[s]:

3.2.1. Missing documentation of the weight of evidence

82 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

3.2.2. The provided studies do not meet the information requirement

83 To fulfil the information requirement, a study must comply with OECD TG 201 and the requirements of OECD GD 23 if the substance is difficult to test (Article 13(3) of REACH).

84 The key parameter investigated by this test is growth rate of algal cultures.

85 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.

86 However, the reliability of these sources of information is significantly affected by the deficiencies identified in the Section 0.1 Reasons common to several requests.

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

88 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;

89 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 Section 2.3, 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.

90 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.

91 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.

92 In your comments, you provide more information on a freshwater algal growth inhibition test on the Substance and supporting information on analogue substances. 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)) (point 2 above). Information on analytical monitoring and analytical method is missing (point 1 above). Without analytical monitoring, it is not possible to determine whether and to what extent the tested organisms were exposed to the test material.

3.2.1. Read-across adaptation rejected

93 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

3.3. Study design and test specifications

95 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 and test specifications' under Request 2.

Reasons related to the information under Annex VIII of REACH

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

4.1. Information provided

96 You have adapted this information requirement by using weight of evidence and a Grouping of substances and read-across approach based on the following experimental data:

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 (1999, NICNAS secondary source) with analogue substance Basic Violet 4 (EC 219-231-5). Rel. 4.

4.2. Assessment of the information provided

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

4.2.1. Missing documentation of the weight of evidence

98 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

99 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.

100 The provided sources of information investigate cytogenicity in mammalian cells. Therefore, they provide information that would contribute to the conclusion on this key parameter.

101 However, the reliability of the sources of information is significantly affected by the deficiencies identified in theSection 0.1 Reasons common to several requests.

102 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.

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

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

105 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.

4.2.2. Read-across adaptation rejected

106 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

108 In the comments to the draft decision you agree with the request.

4.3. Specification of the study design

109 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.

4.4. Possibility for data sharing

110 The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[3].

5. In vitro gene mutation study in mammalian cells

111 An in vitro gene mutation study in mammalian cells is an information requirement under Annex VIII, Section 8.4.3., in case of a negative result in the in vitro gene mutation test in bacteria and the in vitro cytogenicity test.

5.1. Triggering of the information requirement

112 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 requests 1 and 4.

113 The result of the request for an in vitro gene mutation study in bacteria and for an in vitro cytogenicity study in mammalian cells 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.

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

5.2. Information provided

115 You have adapted this information requirement by using weight of evidence based on the following experimental data:

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.

5.3. Assessment of the information provided

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

5.3.1. Missing documentation of the weight of evidence

117 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In

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

addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

118 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.

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

120 However, the reliability of the sources of information is significantly affected by the deficiencies identified in the Section 0.1 Reasons common to several requests.

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

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

123 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.

124 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.

5.3.2. Read-across adaptation rejected

125 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

127 In the comments to the draft decision you agree with the request.

5.4. Specification of the study design

128 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.

5.5. Possibility for data sharing

129 The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[4].

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

130 A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII, Section 8.6.1.

6.1. Information provided

131 You have adapted this information requirement by using weight of evidence and a Grouping of substances and read-across approach based on the following experimental data:

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

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).

6.2. Assessment of the information provided

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

6.2.1. Missing documentation of the weight of evidence

133 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

134 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.

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

136 However, the reliability of these studies are significantly affected by the deficiencies identified in the Section 0.1 Reasons common to several requests..

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

138 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

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

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

141 As a conclusion, sources of information as indicated above, provide information on repeated dose toxicity but the information provided is not reliable.

142 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.

6.2.2. Read-across adaptation rejected

143 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

145 In the comments to the draft decision you agree with the request.

6.3. Specification of the study design

146 When there is no information available neither for the 28-day repeated dose toxicity (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the

reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the same time fulfil the information requirement of REACH Annex VIII, Section 8.6.1 and that of REACH Annex VIII, Section 8.7.1. (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

147 For information on the study design see request for OECD TG 422 below.

6.4. Possibility for data sharing

148 The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[5].

7. Screening for reproductive/developmental toxicity

149 A screening for reproductive/developmental toxicity study (OECD 421 or OECD 422) is an information requirement under Annex VIII, 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.

7.1. Information provided

150 You have adapted this information requirement by using weight of evidence and a Grouping of substances and read-across approach based on the following experimental data:

> 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.

7.2. Assessment of the information provided

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

7.2.1. Missing documentation of the weight of evidence

152 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

153 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.

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

155 However, the reliability of the sources of information is significantly affected by the deficiencies identified in Section 0.1 Reasons common to several requests..

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

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

157 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.

158 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.

7.2.2. Read-across adaptation rejected

159 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

161 In the comments to the draft decision you agree with the request.

7.3. Specification of the study design

162 A study according to the test method EU B.64/OECD TG 422 must be performed in rats.

163 The study must be conducted with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

164 Therefore, the study must be conducted in rats with oral administration of the Substance.

7.4. Possibility for data sharing

165 The other registrants of the joint submission relied on an adaptation to meet this information requirement. You may consider sharing this information[6].

8. Simulation testing on ultimate degradation in surface water

166 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).

167 This information requirement is triggered in case the chemical safety assessment (CSA) indicates the need for further degradation investigation (Annex I, Section 4; Annex XIII, Section 2.1), such as if the substance is a potential PBT/vPvB substance (Guidance on IRs and CSA, Section R.11.4.). This is the case if the Substance itself or any of its constituent or impurity present in concentration ≥ 0.1% (w/w) or relevant transformation/degradation product meets the following criteria:

- it is potentially persistent or very persistent (P/vP) as:
  - it is not readily biodegradable (*i.e.* <60/70% degradation in an OECD 301D), and;
- it is potentially bioaccumulative or very bioaccumulative (B/vB) as:
  - for some groups of substances (e.g. organometals, ionisable substances, surfactants) other partitioning mechanisms may drive bioaccumulation (e.g. binding to protein/cell membranes) and high potential for bioaccumulation cannot be excluded solely based on its potential to partition to lipid;
- it meets the T criteria set in Annex XIII: NOEC or EC_{10} < 0.01 mg/L or classification

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

as carc. 1A or 1B, muta. 1A or 1B, repro. 1A, 1B or 2, or STOT RE 1 or 2.

8.1. Information provided

168 Your registration dossier provides the following:

- the Substance is not readily biodegradable (38% degradation after 35 days in OECD TG 301D);
- the Substance is ionisable and therefore high potential for bioaccumulation cannot be excluded based on available information;
- the Substance meets the T criteria: currently self-classified in the technical dossier as Repro tox 2.

169 Furthermore, the information in your dossier is currently incompliant and therefore:

- it is not possible to conclude on the bioaccumulation potential of the Substance (see Request 10 of this decision), and
- it is not possible to conclude on the toxicity of the Substance see sections 1-7 of this decision).

170 Under section 2.3 of your IUCLID dossier ('PBT assessment'), you conclude that the Substance is not P/vP, B/vB. In support of your conclusion you provide the following additional information: you conclude that the Substance is inherently biodegradable based on the key study (2018) on ready biodegradability and that the Substance is not B based on the estimated BCF value.

171 However, this conclusion is not supported by the reported results of the key study on ready biodegradability. The key study indicates that the Substance is not readily biodegradable and that it 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 QSAR prediction you provide for biodegradation in water indicates that the Substance is P (estimated half life of the Substance in water was 60 days).

172 Furthermore the QSAR prediction you provide to support the conclusion for not B or vB is not reliable for the reasons provided under Section 10.

173 Therefore, the additional information from your PBT assessment is not adequate to conclude that the Substance is not a potential PBT/vPvB substance.

174 Based on the above, the available information on the Substance indicates that it is a potential PBT/vPvB substance. Further, the additional information from your PBT assessment is not adequate to conclude on the PBT/vPvB properties of the Substance.

8.2. Information provided

175 You have provided a QSAR prediction under Annex XI, Section 1.3 of the REACH Regulation.

176 We have assessed this information and identified the following issues:

8.2.1. (Q)SAR results are not sufficient to conclude on P/vP properties

177 Under Section 1.3., first paragraph, third indent of Annex XI to REACH, a study may be omitted if QSAR results are adequate for the purpose of classification and labelling and/or risk assessment, including PBT assessment. Results obtained from biodegradation (Q)SAR models are only regarded as screening information on P/vP properties (Annex XIII, Section 3.1.). As further explained in Guidance on IRs and CSA, Section R.11.4.1.1.4., such information is not considered sufficient on its own to conclude on non-persistence and must be supported by additional information (e.g. test data information, read-across).

178 You have provided the following QSAR prediction[s] in your dossier:

- EPISUITE prediction on the Substance, the half-life period of the Substance in water is estimated to be 60 days (1440 hrs). The half-life (60 days estimated by EPI suite) indicates that the chemical is persistent in water.

179 Based on these QSAR results, you conclude that the Substance meets the P/vP criteria. You have not provided additional information to support this conclusion.

180 As explained above, the provided QSAR results alone does not provide a robust approach to conclude that the Substance does or does not meet the P/vP criteria and thus are not adequate for PBT assessment. Therefore, you adaptation is rejected.

181 On this basis, the information requirement is not fulfilled.

182 In the comments to the draft decision you agree with the request.

8.3. Study design and test specifications

183 Simulation degradation studies must include two types of investigations (Guidance on IRs and CSA, Section R.7.9.4.1.):

1) a degradation pathway study where transformation/degradation products are quantified and, if relevant, are identified, and
2) a kinetic study where the degradation rate constants (and degradation half-lives) of the parent substance and of relevant transformation/degradation products are experimentally determined.

184 You must perform the 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) (Guidance on IRs and CSA, Section R.11.4.1.1.3.).

185 The required test temperature is 12°C, which corresponds to the average environmental temperature for the EU (Guidance on IRs and CSA, Table R.16-8) and is in line with the applicable test conditions of the OECD TG 309.

186 As specified in Guidance on IRs and CSA, Section R.7.9.4.1., the organic carbon (OC) concentration in surface water simulation tests is typically 2 to 3 orders of magnitude higher than the test material concentration and the formation of non-extractable residues (NERs) may be significant in surface water tests. Paragraph 52 of the OECD TG 309 provides that the "*total recovery (mass balance) at the end of the experiment should be between 90% and 110% for radiolabelled substances, whereas the initial recovery at the beginning of the experiment should be between 70% and 110% for non-labelled substances*". NERs contribute towards the total recovery. Therefore, the quantity of the (total) NERs must be accounted for the total recovery (mass balance), when relevant, to achieve the objectives of the OECD TG 309 to derive degradation rate and half-life. The reporting of results must include a scientific justification of the used extraction procedures and solvents.

187 For the persistence assessment by default, total NERs is regarded as non-degraded Substance. However, if reasonably justified and analytically demonstrated a certain part of NERs may be differentiated and quantified as irreversibly bound or as degraded to biogenic NERs, such fractions could be regarded as removed when calculating the degradation half-life(s) (Guidance on IRs and CSA, Section R.11.4.1.1.3.). Further recommendations may be found in the background note on options to address non-extractable residues in regulatory persistence assessment available on the ECHA website (NER - summary 2019 (europa.eu)).

188 Relevant transformation/degradation products are at least those detected at ≥ 10% of the applied dose at any sampling time or those that are continuously increasing during the study even if their concentrations do not exceed 10% of the applied dose, as this may indicate persistence (OECD TG 309; Guidance on IRs and CSA, Section R.11.4.1.).

9. Identification of degradation products

189 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).

190 This information requirement is triggered in case the chemical safety assessment (CSA) indicates the need for further degradation investigation (Annex I, Section 4; Annex XIII, Section 2.1), such as if the substance is a potential PBT/vPvB substance (Guidance on IRs and CSA, Section R.11.4.).

191 As already explained in Request 8, the Substance is a potential PBT/vPvB substance.

192 Therefore, the chemical safety assessment (CSA) indicates the need for further degradation investigation.

9.1. You have provided no information

193 You have provided no information on the identity of transformation/degradation products for the Substance.

194 Therefore, this information requirement is not met.

195 This information is required for the purpose of the PBT/vPvB assessment (Annex I, Section 4) and the risk assessment (Annex I, Section 6) of the Substance.

196 In the comments to the draft decision you agree with the request.

9.2. Study design and test specifications

197 To determine the degradation rate of the Substance, the requested study according to OECD TG 309 (Request 8) must be conducted at 12°C and at a test concentration < 100 µg/L. However, to overcome potential analytical limitations with the identification and quantification of major transformation/degradation products, you may consider running a parallel test at higher temperature (but within the frame provided by the test guideline, e.g. 20°C) and at higher application rate (i.e. > 100 µg/L).

10. Bioaccumulation in aquatic species

198 Under Annex VIII, Section 9.3., Column 2, further information on bioaccumulation or further testing as described in Annex IX must be generated if the chemical safety assessment (CSA) in accordance with Annex I indicates the need to investigate further the bioaccumulation properties of the substance.

10.1. Triggering of the information requirement

199 Therefore, this information requirement is triggered in case if for example additional information on bioaccumulation as set out in Annex XIII, point 3.2.2, is required to assess PBT or vPvB properties of the substance in accordance with subsection 2.1 of that Annex.

200 As already explained in Request 8, the Substance is a potential PBT/vPvB substance.

10.2. Information provided

201 You have adapted this information requirement by using weight of evidence, and a Grouping of substances and read-across approach based on the following information:

(i) Weight of evidence: QSAR (OPERA, 2018) on the Substance

(ii) Weight of evidence: OECD TG 305 C study (2001), GLP compliance not specified, with analogue substance N, N-dimethylaniline (EC 204-493-5)

(iii) Weight of evidence: OECD TG 305 C study from Japanese database (2020), GLP compliance not specified, with analogue substance [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)

10.3. Assessment of the information provided

202 We have assessed this information and identified the following issue[s]:

10.3.1. Missing documentation of the weight of evidence

203 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

204 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex IX, Section 9.3.2 includes similar information that is produced by the OECD TG 305. OECD TG 305 requires the study to investigate the following key elements:

205 Key parameters

a) the study covers the following key parameters:
- the uptake rate constant (k_1) and loss rate constants including the depuration rate constant (k_2), and/or
- the steady-state bioconcentration factor (BCF_{SS}), and/or
- the kinetic bioconcentration factor (BCF_K), and/or
- the biomagnification factor (BMF).

206 The sources of information (i)-(iii)may provide relevant information on the key parameters.

207 However, the reliability of these sources of information is significantly affected by the deficiencies identified in Section 0.1. and by the following deficiency:

10.3.1.1. The prediction is not adequate due to low reliability

208 Under ECHA Guidance R.6.1.3.4 a prediction is adequate for the purpose of classification and labelling and/or risk assessment when the model is applicable to the chemical of interest with the necessary level of reliability.

209 Your registration dossier provides the following information from study (i):

210 The BCF model in OPERA to predict the bioaccumulation of the Substance, obtaining a result of 356. The prediction is based on the BCF values of the most similar substances in the training set of the model (i.e. it is a nearest neighbour type of model). Further, the model calculates two applicability domains: the global and the local ones. The global domain takes into account the whole training set, while the local applicability domain index depends on the degree of similarity of the neighbours.

211 The model considers the substance to be within the global domain, and assigns a score of 0.429 for the local applicability domain index, on a scale from 0 to 1.

212 The prediction is based on nearest neighbours, but the identified neighbours have significant dissimilarities. More precisely, the nearest neighbours have hydroxy and carbonyl functionalities, while the Substance has an amine and an ammonium functional group. Further, the cation part of the Substance is positively charged (i.e. a cation), while the neighbours are neutral. This may have a significant impact on the reliability of the prediction but was not addressed.

213 In your comments you do not address the reasons for low reliability.

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

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

216 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.

10.3.1.2. Read-across adaptation rejected: studies (ii) and (iii)

217 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

10.4. Study design and test specification

218 Bioaccumulation in fish: aqueous and dietary exposure (Method EU C.13 / OECD TG 305) is the preferred test to investigate bioaccumulation (Guidance on IRs and CSA, Section R.7.10.3.1.). Exposure via the aqueous route (OECD TG 305-I) must be conducted unless it can be demonstrated that:

- a stable and fully dissolved concentration of the test material in water cannot be maintained within ± 20% of the mean measured value, and/or
- the highest achievable concentration is less than an order of magnitude above the limit of quantification (LoQ) of a sensitive analytical method.

219 This test set-up is preferred as it allows for a direct comparison with the B and vB criteria of Annex XIII of REACH.

220 You may only conduct the study using the dietary exposure route (OECD 305-III) if you justify and document that testing through aquatic exposure is not technically possible as indicated above. You must then estimate the corresponding BCF value from the dietary test data according to Annex 8 of the OECD 305 TG and OECD Guidance Document on Aspects of OECD TG 305 on Fish Bioaccumulation (ENV/JM/MONO(2017)16).

221 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[7]. Available literature shows that the Substance is metabolically transformed by fish

[7] 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 (2004/25/EC; see also Regulation 2021/80, Article 1).

into leucomalachite green; it has been shown in recent studies[8] that both the Substance (i.e. malachite green) and leucomalachite green are detected in fish and aquaculture products within the EU.

222 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.

223 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.

[8] 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.

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; (ECHA 2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).

All Guidance on REACH is available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF), ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs), ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: 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 23 November 2021.

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

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

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

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

Following the Board of Appeal's decision in case A-001-2022 ECHA revised the study design specifications for meeting the information requirement for simulation testing on ultimate degradation in surface water (Annex VIII, column 2, section 9.2 and/or Annex IX, first column, section 9.2.1.2).

Appendix 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

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

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.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. 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[9].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. 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[10].

2. General recommendations for conducting and reporting new tests

2.1. Strategy for the PBT/vPvB assessment

[9] https://echa.europa.eu/practical-guides
[10] https://echa.europa.eu/manuals

Under Annex XIII, the information must be based on data obtained under conditions relevant for the PBT/vPvB assessment. You must assess the PBT properties of each relevant constituent of the Substance present in concentrations at or above 0.1% (w/w) and of all relevant transformation/degradation products. Alternatively, you would have to justify why you consider these not relevant for the PBT/vPvB assessment.

You are advised to consult Guidance on IRs & CSA, Sections R.7.9, 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.