~~Confidential~~

Helsinki, 17 September 2021

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

Date of submission of the dossier subject to this decision
24/02/2020

Registered substance subject to this decision ("the Substance")
Substance name: [4-[a-[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 below, by the deadline of ***2 January 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. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202);

2. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201 // EU C.26./OECD TG 221).

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

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

2. Adsorption/ desorption screening (Annex VIII, Section 9.3.1.; test method: (1) OECD TG 121 in case an appropriate buffer with a pH in the range of 5.5 to 7.5 is used or (2) OECD TG 106);

3. 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;

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

5. 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) with, to the extent technically feasible, analytical monitoring of all transformation/degradation products identified in the study requested under B.4 above.

Reasons for the request(s) are explained in the following appendix/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 Annex VII to REACH, for registration at 1-10 tpa;
- 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 the Grouping of substances and read-across approach under Annex XI, Section 1.5.**

You seek to adapt the information requirements for the following standard information requirements by grouping substances in the category and applying a read-across approach in accordance with Annex XI, Section 1.5:

- 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.)
- Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2)
- Long-term toxicity testing on fish (Annex VIII, Section 9.1.3, column 2)
- Bioaccumulation in aquatic species (Annex I, sections 0.6.1. and 4.; Annex XIII, Section 2.1.)

ECHA has considered the scientific and regulatory validity of your grouping and read-across approach 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 ecotoxicological and environmental fate properties

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

- Malachite green ([redacted], no EC/CAS numbers provided)
- Malachite Green (Active ingredient: bis-(p-dimethylaminophenyl) phenylmethane treated with HCl, no EC/CAS numbers provided);
- Malachite Green (Basic Green 4, no EC/CAS numbers provided)
- Malachite Green kit ([redacted]) containing Malachite Green Oxalate ([redacted])
- 4-[P-(dimethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-l-ylidene dimethyl-ammonium chloride (Malachite Green Chloride, no EC/CAS numbers provided)
- Malachite Green Chloride (Malachitgruen TR.([redacted]) Hydrochlorid, CAS: 569-64-2

[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

- Malachite Green Oxalate (CAS: 18015-76-4)
- Malachite Green dioxalate ([redacted], CAS: 2437-29-8)

In your comments on the initial draft decision you provided an updated read-across justification document (Annex I of [redacted]) providing more information on the source substances and on the composition of the test material used to generate the source data.

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 ecotoxicological and environmental fate properties.

A-1 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); and
- 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.

A-1.1 Further deficiencies (for aquatic toxicity endpoints, only)

According to Annex XI, Section 1.5., if the grouping concept is applied then a source study must have adequate and reliable coverage of the key parameters of the corresponding test method, in this case OECD TG 201/TG 221/202/203. Therefore, the following requirements must be met:

1. The analytical measurement of test concentrations is conducted (validity criterion OECD TG 203);
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;

In all the aquatic toxicity studies mentioned below, you have not demonstrated that the concentration of the test material has been maintained within 20% of the nominal or measured initial concentration throughout the test.

Despite this, you reported the result based on the nominal concentration.

In your comments you state that: *'For most information requirements related to the environmental fate and toxicity, no GLP or original reports of studies have been presented from the source substances, but the different forms of the substance have been extensively tested and when the group concept is applied, substances shall be classified and labelled on this basis. Many companies, universities, organizations around the worlds, have researched on them using different methods, different conditions, different species and a wealth of literature data is available from their results Although for none of those data the analytical identification of the substance is reported, are highly consistent and the conclusion of the*

hazard for the endpoint is always the same, putting the substance in the highest toxicity category for the environment.'

The information in your comments is not sufficient for ECHA to make an independent assessment, because, regardless of whether there is high consistency in the information, raw data are missing to verify the validity criteria and the key parameters of the studies. Furthermore, 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.

Therefore, the validity criterion of OECD TG 203 is not met for the short-term fish studies (listed in the Appendix B-1 below) and long-term fish studies (also listed in Appendix B-1 below), and there are critical methodological deficiencies affecting the reliability of the test results for short-term and long-term daphnia and algae studies.

There are additional endpoint specific reasons which are explained further below under the relevant information requirement section(s) A.1, A.2, B.1. and B.5.

B. Conclusions on the grouping of substances and read-across approach

As explained above, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. Therefore, your adaptation is rejected.

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

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

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

1. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2)
2. Long-term toxicity testing on fish (Annex VIII, Column 2, Section 9.1.3)
3. Bioaccumulation in aquatic species (Annex I, sections 0.6.1. and 4.; Annex XIII, Section 2.1.)

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 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.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of 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 of the information for the given regulatory information requirement. Subsequently, relevance, reliability, 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 adaptation.

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 and identified the following issues. These issues identified below are essential for all the information requirements in which you invoked a weight of evidence.

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

A. Reliability of source studies

A-1 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.

A-2 Assigned reliability of studies

To fulfil an information requirement or be appropriate for an adaptation, a study must be reliable, i.e. assigned with a Klimisch score of 1 or 2 (ECHA Guidance R.4).

Following submitted studies have been given a reliability score of 4 (non-assignable) by you with limited reporting and no further justification.

- Certificate of analysis ([redacted], 1998) with analogue substance malachite green oxalate, used to cover the requirement for algae;
- Certificate of analysis ([redacted], 1998) with analogue substance malachite green dioxalate, used to cover the requirement for algae;
- Scientific publication (Adeyemo *et al.*, 2011) with analogue substance malachite green, used to cover the requirement for long-term fish, GLP compliance not specified;
- Scientific publication (El-Neweshy and Abou, 2011) with analogue substance malachite green, used to cover the requirement for long-term fish, GLP compliance not specified;
- Scientific publication ([redacted] 1990) with analogue substance malachite green oxalate, used to cover the requirement for bioaccumulation;
- Certificate of analysis ([redacted], 1998) with analogue substance malachite green dioxalate, used to cover the requirement for bioaccumulation;
- Certificate of analysis ([redacted], 1998) with analogue substance malachite green chloride, used to cover the requirement for bioaccumulation.

In your comments you state that: *'All this information cannot be scored either Klimish 1 or 2, but they constitute many pieces of information which design a general well supported reliable weight of evidence.'*

As explained in Section 1.A above, it is still not possible to verify whether validity criteria are met and to determine whether and to what extent the tested organisms were exposed to the test material.

Therefore, the studies cannot be regarded as reliable.

A-3 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 with GLP compliance not specified:

- Scientific publication (Adeyemo *et al*., 2011) with analogue substance malachite green, used to cover the requirement for long-term fish;
- Scientific publication (El-Newshy and Abou, 2011) with analogue substance malachite green, used to cover the requirement for long-term fish;
- Scientific publication (Biliandzic *et al*.,2012) with analogue substance malachite green oxalate, used to cover the requirement for bioaccumulation.

Therefore the studies cannot be regarded as reliable.

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

In your comments you state that: '*Those data have been considered and accepted as valid by EFSA, in the Scientific opinion on Malachite green in food in 2016*'.

EFSA, however, conducts a different assessment than ECHA under compliance check and a consideration of a study by EFSA does not mean that it complies with REACH, which a registrant must still demonstrate.

As explained by the Board of Appeal (BoA) in the BoA decision A-006-2018: *In the compliance check decision-making procedure, the Agency (ECHA) does not assess the risks that exposure to the substance poses to human health and to the environment. Instead, the Agency verifies whether the information requirements set out in the testing Annexes are fulfilled in the lead registrant's dossier.*

You also refer to Article 13(1) of REACH and Annex VI, step 4, concluding that a scientific approach must be taken in the evaluation of all existing data, and the contribution of every piece of information, even if not complete, on the general risk assessment has to be taken into account.

The pieces of evidence, however, have significant deficiencies addressed above.

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. You have not provided such assessment taking into account these significant deficiencies.

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

1. 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.).

In addition, column 2 states that the study does not need to be conducted if a long-term aquatic toxicity study is available (Section 9.1.1., Column 2).

You have provided the following information:

i. key study (Scientific publication, Bill et al., 1977) conducted according to methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the protocol described by Marking (1975) with analogue substance malachite green chloride;
ii. supporting study: OECD TG 202 ([redacted], 1988) with analogue substance malachite green;
iii. Supporting study (review article/handbook data, Burchmore and Wilkinson, 1993), no method specified, with analogue substance malachite green.

In addition, you have provided a long-term toxicity study (key study: OECD TG 211, 1988) with analogue substance malachite green.

In your comments to this draft decision you submitted a full study report for this study.

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. OECD TG 202 study

To fulfil the information requirement, a study must comply with OECD TG 202 and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test (Article 13(3) of REACH). The validity criteria of the OECD TG 202 include:

1. the percentage of immobilised daphnids is ≤ 10% at the end of the test in the controls (including the solvent control, if applicable)

Your registration dossier provides an OECD TG 202 (study ii, supporting study) showing the following:

EC50(21d)=ca. 0.81 mg/L based on measured geometric mean.

However, you have indicated that the validity criterion specified above was not fulfilled and concluded that *"[t]The study is invalidated because it did not satisfy the validity criteria for OECD 202 "In the control, including the control containing the soluble agent, not more than 10% of the daphnids should been immobilised.", the result shows that 11.03% of the animals died in the control."*

Based on the above, the validity criteria of OECD TG 202 are not met.

Therefore, the requirements of OECD TG 202 are not met.

C. Use of existing data

Although you do not explicitly claim an adaptation, ECHA understands that the information provided was submitted in order to meet the required information by way of adaptation under Annex XI, Section 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular:

1) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case an OECD TG 202 which include the following conditions:
    1. the concentration of the test material leading to the immobilisation of 50% of daphnids at the end of the test is estimated (key parameter of the TG 202);
    2. the percentage of immobilised daphnids is ≤ 10% at the end of the test in the controls (including the solvent control, if applicable);
    3. the dissolved oxygen concentration is ≥ 3 mg/L in all test vessels at the end of the test;
    4. *Daphnia magna* (or other suitable *Daphnia* species) is used as test species;

Regarding point 1, key study (study i. above) provides information on the concentration of the test material leading to mortality of 50% of ostracods (Cypridopsis) and prawns (*Palaemonates kadiakensis*). Thus, this study does not provide adequate and reliable coverage of the key parameter foreseen to be investigated in an OECD TG 202.

Supporting study (study iii. above) provide information on EC_{50}(24-72 hours) for daphnids (*Daphnia magna*) among other fresh water and salt water aquatic invertebrates organisms. However, you did not specify on what key parameter (e.g. immobility) the effect concentrations are based on.

Regarding point 2 and 3 (i.e. validity criteria of OECD TG 202), both study i. and iii. does not provide information on dissolve oxygen. Study i. does not investigate immobility of daphnids and study iii. does not include the information on the percentage of immobilised daphnids at the end of the test in the controls.

Regarding point 4, as already stated, the study i. the test was conducted on ostracods (Cypridopsis) and Prawns (*Palaemonates kadiakensis*) and not on *daphnia magna*.

Furthermore, for both studies (i) and (iii) you have not specified whether the analytical monitoring was performed nor provided information on the analytical method used. For study iii, information on pH is not reported. Information on these points are important because, as stated below under *"study design"*, the Substance exists as both malachite green and malachite green carbinol in solution and the relative portion depends on pH.

Based on the above,
- the key parameter of OECD TG 202 is not covered by both study i. and iii.);
- the validity criteria of OECD TG 202 are not met (by both study i. and iii.)
- there are critical methodological deficiencies resulting in the rejection of all the study results. More, specifically information on analytical monitoring, and test conditions including pH.
- the Substance is difficult to test (The Substance and source substances are ionisable, hydrolysable, photodegradable, and coloured dye) and there are critical

methodological deficiencies resulting in the rejection of all the study's results. More, for all the studies, specifically no information on analytical method is provided and chemical species for which effect concentrations are based is not specified.

Therefore, your adaptation does not have adequate and reliable coverage of the key parameters of OECD TG 202, it is rejected and the information requirement is not fulfilled.

D. OECD TG 211 study

To fulfil the information requirement, a study must comply with OECD TG 211 and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test (Article 13(3) of REACH). Therefore, the following requirements must be met:

1. the concentrations of the test material leading to no observed effect (NOECs) on the following parameters are estimated:
   1) the reproductive output of *Daphnia* sp. expressed as the total number of living offspring produced at the end of the test, and
   2) the survival of the parent animals during the test, and
   3) the time to production of the first brood.

Regarding point 1 above (i.e. key parameters of OECD TG 211), in your long-term toxicity study (key study: OECD TG 211, 1988), you have reported effect concentrations (EC_{50} and EC_{100} (21day)) based on the immobility of daphnids. NOEC is not reported. In the conclusion section, you have stated that "*at each test concentration clear toxic effects were observed, at the lowest test concentration of 0.0081 mg/L effects on reproduction occurred, while at higher test concentrations adults did not survive to reproduce*".

However to determine whether the study fulfil the above key parameters.

Point 1: it is not possible for ECHA to derive NOEC based on the information provided in the dossier and you have not demonstrated that the key parameters of OECD TG 211 are covered;

Furthermore, in your comments you state that: '*Analytical verification has been performed to* validate the study'. However, in the report study (report in German at page 75 of your comments) it is stated: '*No suitable analytical method for monitoring was available.*'. Therefore, the issue of analytical monitoring indicated in Appendix on Reasons common to several requests has not been addressed.

Therefore, the requirements of OECD TG 211 are not met.

E. Overall conclusion

Therefore, your adaptations are rejected and the information requirement for OECD 202 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 coloured 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 and the information provided in your dossier, 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 reach 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.

2. Growth inhibition study aquatic plants

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

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. Weight of evidence: Method not specified (1998) with analogue substance malachite green oxalate, reliability 4
ii. Weight of evidence: Method not specified (1998) with analogue substance malachite green dioxalate, reliability 4

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 specifies that:

1. exponential growth in the control cultures is observed over the entire duration of the test;
2. at least 16-fold increase in biomass is observed in the control cultures by the end of the test;
3. the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is ≤ 35%;
4. the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is ≤ 7% in tests with [*Pseudokirchneriella subcapitata* / *Desmodesmus subspicatus*]. For other less frequently tested species, the value is ≤ 10%;
5. three replicates at each test concentration and at least three replicates for controls (including solvent controls, if applicable) are included;
6. at least 6 treatment replicates are included if a limit test (at 100 mg/L or at the limit of solubility of the test substance) is conducted;
7. one of the two alternative growth medium (*i.e.* the OECD or the AAP medium) is used. Any deviations from recommended test media must be described and justified;
8. the pH of the control medium does not increase by > 1.5 units;
9. the test concentrations are arranged in a geometric series with a spacing factor ≤ 3.2, unless a higher factor is justified by a flat concentration response curve;
10. if a solvent is used, its concentration is ≤ 100 µg/L;
11. a reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (*i.e.* detection and quantification) and working range must be available.
12. the test media prepared specifically for analysis of exposure concentrations during the test is treated identically to those used for testing (*i.e.* inoculated with algae and incubated under identical conditions);

For both source information i) and ii), you have provided only the test substance name (with CAS number), name of the test organisms, test duration and effect concentration.

No further information is available in the technical dossier such as the test method used, the key parameters and validity criteria.

You have not indicated whether the analytical monitoring was performed, nor what the effect concentration is based (i.e. nominal or measured concentrations). In addition, as stated in the Appendix A-1 under "test design" section , the Substance exists as both malachite green and malachite green carbinol in solution at this pH range. You did not provide information on the pH of the test solution nor specify which chemical species the effect concentration is based on.

In your comments you confirm that 'For both (studies) no further data are available except the test substance name (with CAS number), name of the test organisms, test duration and effect concentration, no further information have been recovered or the original data.

In addition you also state that new information is available from scientific articles (P. Matpang, 2017; Fitzgerald G.P., 1952; Ericson J.W., 1977) confirming the level of toxicity for aquatic plants reported. However, the information in your comments is not sufficient for ECHA to make an independent assessment, because you have not provided raw data to verify the conditions of exposure of OECD 201 (as listed above).

In your comments to the draft decision you also state that: '*In all cases algae seems not to be the most sensitive species compared to fish, even if effect values are in the order of magnitude of the milligrams, therefore no further information on the risk assessment for aquatic compartment will be obtained performing a new test.*'

However, you do not refer to any legal basis for adaptation under Column 2 or Annex XI; for example you do not claim that aquatix toxicity is unlikely. Therefore, your comment is rejected.

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.

C. Overall conclusion

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.1.

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

1. Short-term toxicity testing on fish

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

In addition, column 2 states that the study does not need to be conducted if a long-term aquatic toxicity study is available (Section 9.1.3., Column 2).

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. key study (Scientific publication, Bill et al., 1977) conducted according to methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the protocol described by Marking (1975) with analogue substance malachite green chloride;
ii. supporting study (Scientific publication, Wayne, 1966) conducted according to static bioassay by Lennon and Walker (1964) and LC50 calculated as described by Litchfield and Wilcoxon (1949) with analogue substance malachite green (Active ingredient: bis-(p-dimethylaminophenyl) phenylmethane treated with HCl);
iii. supporting study (Scientific publication, Little and Lamb, 1972) conducted according to Standard Methods of the Examination of Water and Wastewater (APHA et al. 1971) with analogue substance Basic Green 4, Malachite Green;
iv. supporting study (Scientific publication, Bills et al., 1993) conducted according to Static toxicity test procedures described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and ASTM, and and LC50 calculated as described by Litchfield and Wilcoxon (1949) with analogue substance Malachite Green;
v. supporting study (Handbook data, Burchmore and Wilkinson 1993), method not specified, with analogue substance Malachite Green;

In addition, you have provided following two sources of information on long-term toxicity to fish as a weight of evidence.

vi. Weight of evidence: scientific publication (Adeyemo et al., 2011), GLP not specified, reliability 4, conducted according to standard testing guideline, with analogue substance malachite green;
vii. Weight of evidence: scientific publication (El-Weshy and Abou 2011), GLP not specified, reliability 4, conducted according to standard testing guideline, with analogue substance malachite green.

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.

In addition, we have identified following additional issues:

B. Study deficiencies of short-term studies

Under Anenx XI, Section 1.5, study results should have:

1) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case an OECD TG 203, which include the following conditions:

Key parameter to be measured

1. the concentration of the test material leading to the mortality of 50% of the juvenile fish at the end of the test is estimated;

Validity criteria

2. mortality in the control(s) is ≤ 10% (or one fish, if fewer than 10 control fish are tested) at the end of the test (validity criterion);
3. the dissolved oxygen concentration is ≥ 60% of the air saturation value in all test vessels throughout the exposure (validity criterion);

Technical specifications impacting the sensitivity/reliability of the test

4. at least 7 fish are used at each test concentration and in the control(s);
5. at least 5 concentrations are tested;
6. the test duration is 96 hours or longer;

Regarding the point 1 above, information on the life stage and/or the age of the test organisms used is not provided for study i., ii., iii., and v.

Regarding point 2 above, for all the reported studies, mortality in the control(s) at the end of the test is not reported.

Regarding point 3 above, for study i., ii., iv., and v. information on dissolved oxygen is not provided. For study iii, the concentration range of the dissolved oxygen (in mg/l) is reported but not in % of air saturation value.

Regarding point 4 above, for study i., ii., and v. information on number of organism tested is not provided.

Regarding point 5 above, for study i. only tested concentration range for range-finding study is provided. For study ii., iv., and v. test concentrations are not reported.

Regarding point 6 above, the exposure duration is indicated as 48 hr in study ii. For study v. test duration reported is shorter than 96 hr for some of the fish species reported.

Based on the above,

- the key parameter of OECD TG 203 is not covered by the studies i., ii., iii., and v.;
- the validity criteria of OECD TG 203 are not met (in all studies)
- there are critical methodological deficiencies resulting in the rejection of all the study results. More specifically, information on number of test organism and test concentrations used.
- the Substance is difficult to test (The Substance and source substances are ionisable, hydrolysable, photodegradable, and coloured dye) and there are critical methodological deficiencies resulting in the rejection of all the study results. More specifically, no information on analytical method is provided and chemical species for which effect concentrations are based on is not specified.

Therefore the provided studies (i – v) cannot be considered equivalent to data generated by an OECD TG 203 study.

In your comments to this draft decision you state that you will provide more information to demonstrate that studies i. and iii are equivalent to an OECD TG 203 study. You also state that you will improve the reporting of the robust study summary for the other studies.

The information in your comments is not sufficient for ECHA to make an independent assessment, because you have not provided raw data to verify the issues listed above.

Furthermore, you state that the '*the Substance is one of the most widely studied on fish in all details and also in the last 8 years further tests have been conducted around the world.*' This statement does not solve the issues listed above for compliance with REACH.

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 adaptation does not provide adequate and reliable coverage of the study's key parameter, it is rejected and the information requirement is not fulfilled.

C. Long-term fish studies

C-1 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.

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.

To fulfil the information requirement, normally a study according to OECD TG 210 must be provided. The key parameters investigated by this test are:

1. Stage of embryonic development,
2. Hatching and survival of embryos and larvae,
3. Survival of juvenile fish,
4. Abnormal appearance,
5. Abnormal behaviour (e.g. hyperventilation, uncoordinated swimming, atypical quiescence and atypical feeding behaviour),
6. Weight at the end of the test,
7. Length at the end of the test.

The sources of information vi. provide information on hatching of eggs, survival of juvenile fish, abnormal appearance (shape of eggs) and histopathological changes in testes and skin of treated male organism (African Catfish *Clarias gariepinus*). The source of information vii. provides information on the pathological and haematological effects of malachite green toxicity in adult fish Nile tilapia (*Oreochromis niloticus*) after chronic (6 weeks) exposure. This information may be partly relevant for the (dangerous) property as investigated by a study conducted according to OECD TG 210.

Regarding key parameters 1. stage of embryonic development, 5. abnormal behaviour 6.

Weight and 7. Length at the end of the test, none of the sources of information provided investigate these key parameters. Therefore, they do not provide information that would contribute to the conclusion on these key parameters.

Regarding key parameter 2. Hatching and survival of embryos and larvae and 3. Survival of juvenile fish, source of information vi. may provide relevant information as you concluded that egg treated with malachite green did not hatch. However, the reliability of these sources of information is significantly affected by the deficiencies identified in in Section 2 of the Appendix on Reasons common to several requests.

In addition, the reliability of the source of information vii. is also affected by the following issue.

The conditions of exposure in OECD TG 210 specifies that the test should start as soon as possible after the eggs have been fertilised until species-specific time period that is necessary for the control fish to reach a juvenile life-stage (28-60-d post-hatch[5]).

In the submitted study, exposure duration was 45 minutes repeated every other day for three treatments. In addition, female and male fish, instead of fertilised eggs, were exposed to the test substance.

Therefore, the study duration is shorter than indicated, and mode of exposure is different as specified in the OECD TG 210. This condition of exposure is essential because the effects observed in a long-term study might be considerably more pronounced than over a shorter study duration, as well as, the life stage of the exposure.Therefore the provided study vi. cannot be considered a reliable source of information.

Regarding key parameter 4. abnormal appearance, information source vi. provide information on the abnormal shape of eggs and skin of male fish after the exposure to the test substance. The information source vii. provides further information on the effects on fish organs (gills, hepatopancreas, posterior kidney and spleen) as well as effects on blood parameters after 6 weeks of exposure at 0.076 mg/L of the test substance. However, information vii. does not provide any parameters related to the survival and development of fish in early life stages from the stage of fertilized egg until the juvenile life-stage as defined by OECD TG 210.

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

As a conclusion, sources of information as indicated above, provide information on long-term toxicity to fish but essential parts of information of the dangerous property is lacking (such as stage of embryonic development, length at the end of the test). Furthermore, the information provided on Hatching and survival of embryos and larvae, survival of juvenile fish, abnormal appearance 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 properties foreseen to be investigated in an OECD TG 210 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled.

D. Overall conclusion

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

[5] OECD TG 210, Annex 2

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.1.

2. Adsorption/ desorption screening

Adsorption/desorption screening is an information requirement under Annex VIII to REACH.

You have adapted this information requirement according to Annex VIII, Section 9.3.1, Column 2 with the following justification: "*the study does not need to be conducted because the substance has a low octanol water partition coefficient and the adsorption potential of this substance is related to this parameter*".

We have assessed this information and identified the following issue:

Annex VIII, Section 9.3.1., column 2 specifies that a study does not need to be conducted in case the log K_{ow} is low if lipophilicity is the sole characteristic driving the adsorption potential of a substance since, for some substances (e.g. ionisable substances, surfactants) other mechanisms than lipophilicity may drive adsorption (Guidance R.7a, Section R.7.1.15.4).

You have justified the low potential for adsorption because the partition coefficient value (log K_{ow}) was determined to be 0.9 based on OECD TG 107.

However, based on the structure of the Substance and as explained in 'Study design' under Section A.1., the Substance is ionised at environmentally relevant pH.

Therefore, log Kow is not a valid descriptor for assessing the adsorption potential of the Substance and your adaptation is rejected.

In your comments to this draft decision you have agreed to perform a new test. You also wonder whether would be more appropriate to perform an OECD TG 121 study. This method (OECD 121) is applicable to ionisable substances if an appropriate buffer with a pH in the range of 5.5 to 7.5 is used.

Therefore, the information requirement is not fulfilled.

3. 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 CSA 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, 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;

The information provided in your dossier indicates that:

1. the Substance is potentially P/vP since there is no reliable ready biodegradation studies on the Substance, and you have concluded that the Substance is not readily biodegradable;
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 to be Repro tox 2

In your comments you state that: '*Nevertheless, regarding this endpoint, Annex VIII point 9.2 states that further degradation testing shall be considered if the chemical safety assessment according to Annex I indicates the need to investigate further the degradation of the substance.The chemical safety assessment for this substance, being classified for the environment, takes into consideration the exposure as well as hazard. The substance has a so high classification for the environment according to CLP, that a use can be allowed only if the substance does not reach the environment in any possible case. Therefore, no exposure is expected for the substance and CSA is supporting the waiving.*'

However, this information does not allow to conclude on whether the Substance is PBT/vPvB under Section 4 of Annex I and, therefore, information is still needed to complete the PBT/vPvB assessment in the chemical safety assessment.

As an aside, Annex XIII, section 2.1 of REACH, states that where the process and use conditions of the substance meet the conditions as specified in Section 3.2(b) or (c) of Annex XI the additional information may be omitted, and subsequently the substance is considered as if it is a PBT or vPvB in the registration dossier.

You have not claimed that, and in the CSR, you have not proven that conditions of Annex XI, sections 2.3(b) and (c) are met. You did not provide information proving that the Substances is used under strictly controlled conditions and/or incorporated in article or matrix permanently through all life stage, with no release potential throughout whole life cycle of the substance.

Therefore, further information on biodegradation must be provided.

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., and qualitative or quantitative structure-activity relationshop ((Q)SAR) under Annex XI, Section 1.3., of REACH.

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

i. Weight of evidence: Certificate of analysis on OECD TG 301C and 302B ([redacted], 1998), with analogue substance Basic Green Chloride/ Malachitegruen TR. ([redacted]) Hydrochlorid (CAS No. 569-64-2), reliability 4, showing 0.3 % degradation after 14 days (OECD TG 301C) and 82 % degradation (OECD TG 302B, no information on the test duration provided);
ii. Weight of evidence: Certificate of analysis on OECD TG 301C and 302B ([redacted], 1998), with analogue substance malachite green dioxalate (CAS no. 2437-29-8), reliability 4, showing 0.3 % degradation after 14 days (OECD TG 301C) and 82 % degradation

(OECD TG 302B, no information on the test duration provided);

iii. a QSAR prediction (2012) EpiSuite 4.1 on Malachite green in neutral form (i.e Leucomalachite green), reliability 4, predicted to be not readily biodegradable;

iv. Weight of evidence: ECHA Registered substances database information (OECD TG 301F) on analogue substance Crystal violet, reliability 4, showing the analogue substance is not readily biodegradable.

Based on above, you have concluded that the Substance is not readily biodegradable.

A. Read-across

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

B. Weight of evidence

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

For this endpoint your study needs to have adequate and reliable coverage of the key parameter foreseen to be investigated in an OECD TG 301/310 study, which is the ultimate aerobic biodegradation of the test material.

The provided sources of information investigate aerobic biodegradation. 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, all sources of information (i.e. study i., ii., and iv.) have been given a reliability score of 4 by you (not assignable), with limited reporting and ECHA agrees that these sources studies are not reliable.

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

C. QSAR

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

1. the results are adequate for classification and labelling and/or risk assessment.

In the technical dossier you stated that the prediction was performed on the neutral form of the Substance, leucomalachite green and you have provided SMILES code used for the prediction as CN(C)c1ccc(cc1)[C@@H](c2ccccc2)c3ccc(cc3)N(C)C.

You have provided a QSAR prediction on leucomalachite green and concluded that the SMILES code you have used is not correct for the neutral form of the Substance and it refers to a different substance. The correct SMILES for the Substance is CN(C)c1ccc(cc1)C(c1ccccc1)=C1C=CC(C=C1)=[N+](C)C.

As incorrect SMILES is used for the prediction, the outcome of the prediction is not reliable. Hence, it is not adequate for the classification and/or risk assessment.

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

D. Overall conclusion

The available screening information is not sufficient to conclude on the P/vP properties of the Substance. In addition, available literature[6] indicates that the Substance is not readily biodegradable and that it may persist in the environment.

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 5 below of this decision), and
- it is not possible to conclude on the toxicity of the Substance see Appendix A, Sections 1-2 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.

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).

4. 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 CSA 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

[6] 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.

the case if the substance, a constituent, an impurity or a transformation/degradation product meets the PBT/vPvB criteria.

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

In your comments you state that: 'This endpoint will be covered in connection with the simulation test study. Since the simulation test study can be waived according to Chemical safety Assessment result, this part will not need to be covered.'

As explained already (section B-3 above), the the Substance may have PBT or vPvB properties and therefore further information on biodegradation must be provided.

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-5 below of this decision) and toxicity (see Appendix A, Sections 1-2 and Appendix B, Section 1 of this decision) of the Substance.

In your comments to the initial draft decision, you also provide new information on photodegradation products. According to ECHA Guidance R.11, p. 43: Data derived from other abiotic studies (e.g. photodegradation, oxidation, reduction) cannot be used on their own within persistence assessment, but may be used as part of a Weight-of-Evidence approach. Due to the large variation in the light available in different environmental compartments, the use of photolysis data is not generally recognised for persistence assessment.

You have not submitted a weight-of-evidence approach nor explained why the use of photolysis data would be sufficient.

Based on the above this information requirement is triggered and information must be provided.

Study selection and design

You must obtain this information while performing the simulation study requested in this decision (request B-3 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.

5. Bioaccumulation in aquatic species

This information requirement is triggered in case results from screening tests or other information indicate that the substance is a potential PBT or vPvB (Annex I, Section 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-3 above.

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

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. Weight of evidence: Scientific publication (Bilandzic et. al., 2012), not GLP compliant, with analogue substance malachite green oxalate, reliability 2, investigating the concentrations of malachite green in muscle tissue of fish (*Cyprinus carpio*) determined by the immunoassay method according to the criteria of Commission Decision 2002/657/EC, showing that the concentrations of malachite green were below the minimum required performance limit (MRPL) of 2 µg/kg in food of animal origin, as per Commission Decision 2004/25/EC;
ii. Weight of evidence: Scientific publication (Schuetze et. al., 2008), with analogue substance malachite green, reliability 2, investigating bioaccumulation and margin of exposure of malachite green and leucomalachite green in detected in wild eels;
iii. Weight of evidence: Scientific publication (Plakas et. al., 1995), with analogue substance malachite chloride, reliability 2, investigating the disposition and half-life of malachite green and its metabolites in channel catfish (*Ictalurus punctatus*), showing rapid metabolic reduction of malachite green to leucomalachite green, and its subsequent slow elimination from the tissue, reported half-life of malachite green 67 h.;
iv. Weight of evidence: Scientific publication ([redacted], 1990), with analogue substance malachite chloride, reliability 4, investigating the concentrations of malachite green oxalate residues in muscles, eggs and frys of Atlantic salmon (Salma solar) and chinook salmon (Oncorhynchus tshawytscha);
v. Weight of evidence: Certificate of analysis ([redacted], 1998), with analogue substance malachite dioxalate, reliability 4, reporting BCF >36-<96 in fish (*Cyprinus carpio*), concluding that "*bioaccumulation is not expected*";
vi. Weight of evidence: Certificate of analysis ([redacted], 1998), with analogue substance malachite chloride, reliability 4, reporting BCF >36-<96, concluding that "*bioaccumulation is not expected*".

We have assessed this information and identified the following issue:

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 305 must be provided. The key parameters foreseen to be investigated by this test are:

1. the uptake rate constant (k1) and loss rate constants including the depuration rate constant (k2), and/or
2. the steady-state bioconcentration factor (BCFSS), and/or
3. the kinetic bioconcentration factor (BCFK), and/or
4. the biomagnification factor (BMF).

The sources of information i. to iv (listed above) you provided the bioaccumulation/detection of malachite green and its residues in fish. However, you have not reported any measurement performed (eg, BCF) for studies i. to iv above. So, it is not possible to assess whether any of these studies cover the above key parameter(s).

Therefore, you have not demonstrated that they provide the information that would contribute to the conclusion on this key parameter(s).

The sources of information v. and vi. provide information on BCF. This information may be relevant for the (dangerous) property as investigated by a study conducted according to OECD TG 305, but only partly because you have not reported the measurement used (eg, BCFSS or BCFK).

In addition, the reliability of these two 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 validity criteria of OECD TG 305 specifies that:

1. Similar growth in the control and in the test vessel is observed;
2. The water temperature variation is ≤ ± 2°C;
3. The concentration of dissolved oxygen is ≥ 60% saturation;
4. The stock population of fish is acclimated for at least two weeks. Only batches showing mortalities below 5% of the population in seven days and with no diseases or abnormalities are used;
5. Mortality or other adverse effects/disease in both control and test group fish is ≤ 10% at the end of the test;

For both source information v. and vi., you have provided only the test substance name (with CAS number), name of the test organism (only source information for study v.), uptake duration and BCF.

No further information is available in the technical dossier for all studies, such as the test concentrations used and information needed to verify the validity criteria listed above.

As a conclusion, sources of information as indicated above, partly provide information on bioaccumulation in fish but essential parts of information of the dangerous property is lacking. Furthermore, even the information provided on bioaccumulation in fish is not reliable.

In your comments you provided new information on bioaccumulation from scientific articles ([redacted], 1997; [redacted], 1988; [redacted], 1994; [redacted], 1994) and a MITI study (Japan database MITI, 1992). However, the information in your comments is

not sufficient for ECHA to make an independent assessment, because you have not provided raw data to verify the validity criteria listed above.

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 properties foreseen to be investigated in an OECD TG 305 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled.

C. Overall conclusion

Thus, the available screening information is not sufficient to conclude on the B/vB properties of the Substance. In addition, although it is not considered equivalent to OECD TG 305 study, the source information i.-iv. partly show that the Substance and its metabolite leucomalachite green are detected in various fish (i.e. tissues, eggs and frys).

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 malachite green carbinol, leucomalachite green[7].

Therefore, the study must monitor not only the Substance (i.e. malachite green) , but also and any other relevant transformation/degradation products identified under the request in Appendix B-4 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.

[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 (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[8].

B. Test material

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

1. Selection of the Test material(s)

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

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

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

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[9].

[8] https://echa.europa.eu/practical-guides
[9] 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 07 June 2019.

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 and the deadline.

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[10] 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)[11]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[11]

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[12]

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

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

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

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

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

Appendix 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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

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