~~Confidential~~

Helsinki, 17 September 2021

Addressees
Registrant(s) of JS_Basic_Green_4_ox as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
28/11/2019

Registered substance subject to this decision ("the Substance")
Substance name: Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate
EC number: 241-922-5
CAS number: 18015-76-4

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 ***22 June 2023***.

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. Transgenic rodent somatic and germ cell gene mutation assay (Annex VII, Section 8.4., column 2; test method: OECD TG 488 from 2020[1]) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach. Duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

   OR

   In vivo mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum

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);

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

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

[1] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66.

Information required depends on your tonnage band

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

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa)

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". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

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

Failure to comply

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

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

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

Appendix on Reasons common to several requests

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

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

1. Genetic toxicity in vivo
2. Short-term toxicity testing on aquatic invertebrates
3. Growth inhibition study aquatic plants

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

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

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

In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation. 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. The common deficiencies are set out here, while the specific ones are set out under the information requirement concerned in the Appendices below.

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

Uncertainty on reliability of studies for aquatic toxicity

OECD TGs 201 and 202 include the following conditions:

- the concentrations of the test material are measured as required in the respective TGs;
- the results can be based on nominal or measured initial concentration only if the concentration of the test material has been maintained within 20% of the nominal or measured initial concentration throughout the test;

No information on analytical verification of exposure conducted in any of these studies is reported in the registration dossier or in the new information provided in the comments on the initial draft decision. Thus, you have not demonstrated that (no-)effect values can reliably be based on nominal test material concentrations and there is no underestimation of the effects.

Therefore, the reporting of the studies is not sufficient to conduct an independent assessment of its reliability.

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

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

1. **Transgenic rodent somatic and germ cell gene mutation assay**

   OR

   ***In vivo* mammalian alkaline comet assay**

Under Annex VII Section 8.4., column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria which raise the concern for gene mutation.

Also, your dossier contains the following *in vivo* studies:

i. WoE study (1984) mammalian bone marrow chromosome aberration test according to OECD TG 475 with the Substance.
ii. WoE study (2004) chromosome aberration test in rat according to "standard three-exposure protocol as described in detail by Shelby et al. (1993)" with Malachite green chloride (EC no. 209-322-8).
iii. WoE study (2004) chromosome aberration test in mouse according to "assay presented by MacGregor et al. (1990)" with Malachite green chloride (EC no. 209-322-8).
iv. WoE study (2004) chromosome aberration test in mouse, dietary study, no guideline, with Malachite green chloride (EC no. 209-322-8).
v. WoE study (2004) in vivo mammalian gene mutation study in mouse, according to "lymphocyte Hprt mutant analyses conducted as described by Meng et al. and modified by Dobrovolsky et al." with Malachite green chloride (EC no. 209-322-8).
vi. WoE study (2004) in vivo mammalian gene mutation study in mouse, "liver cII mutant frequency determination and characterization of mutations" with Malachite green chloride (EC no. 209-322-8).

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

As explained under Appendix on Reasons 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.

Relevance of provided information

Relevant information that can be used to support weight of evidence adaptation for information requirement of Column 2 of 8.4. under Annex VII includes similar information that is produced by the OECD TG 488/489, i.e. on key investigation:

- Gene mutation *in vivo*

The *in vivo* studies i. to iv. provided are not addressing this key investigation; only *in vivo* studies v. to vi. do.

Reliability of provided information

A. Read-across

You have adapted this information requirement by using a Grouping of substances and read-across approach under Annex XI, Section 1.5 using the following source substance:

1. Malachite green chloride (EC no. 209-322-8)

In your comments on the initial draft decision, you provide a read-across justification and you state that "*It has to be underlined that only Malachite Green Chloride and analogous salts (Acetate, hydrogen sulphate....) salts can be considered "Source substances"*. ECHA has therefore deleted the references to Leucomalachite green from this decision.

ECHA notes that the triggering of the request for an *in vivo* genotoxicity assay stems from positive results with the Substance (OECD TG 471, EC no 241-922-5, 1984). Therefore, the triggering remains valid even if Leucomalachite results are not taken into account.

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

Read-across hypothesis contradicted by existing data

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances. The ECHA Guidance[3] indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance. The observation of differences in the toxicological properties between the source substance(s) and the Substance would contradict the hypothesis that the properties of the Substance can be predicted from the data on the source substances. An explanation why such differences do not affect the read-across hypothesis needs to be provided and supported by scientific evidence.

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

The results of the information on mutagenicity obtained with the source substance vary. Specifically, positive results are observed in the *in vitro* gene mutation study in bacteria conducted with the Substance (OECD TG 471, 1984) while negative results are reported for equivalent studies conducted for the source substance Malachite green chloride (OECD TG 471, 2004).

You have not provided any justification for that contradiction for the intrinsic property concerned and why it would not have any impact on the prediction.

The negative results obtained with Malachite green oxalate in another *in vitro* gene mutation study in bacteria (OECD TG 471, 1999), and reported in your dossier and in Annex II to your comments, cannot be considered as representative of the properties of your Substance since a purity of only 70.8% was indicated for the test material in that study. This low degree of purity is below the range of concentrations indicated in your dossier for the composition of your Substance (i.e. 80-100%) and also below the reported boundary composition (75-100%). Therefore, these negative results cannot be used to overrule the positive *in vitro* gene mutation study in bacteria with the Substance (OECD TG 471, EC no 241-922-5, 1984) and for which the test material was reported with a purity higher than 90%. Therefore, the negative results obtained (OECD TG 471, 1999) does not resolve the above identified contradiction.

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

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

B. Study deficiency

OECD TGs 488/489 include the following conditions:

a) The study must include a minimum of three doses/groups of treated animals, as well as a negative control group and a positive control group.

You provided two *in vivo* gene mutation studies, studies v. to vi., with only one dose reported.

In your comments on the initial draft decision, you explain the background for dose selection for the *in vivo* genotoxicity testing but without assessing the impact on the reliability of these studies, in particular regarding interpretation of the results in the absence of dose-response information. The issue with the low number of doses remains, with only one dose tested for Malachite green chloride in the non-guideline "lymphocyte Hprt mutant analyses" (study v, 2004) and the "liver cII mutant frequency determination and characterization of mutations" (study vi), which are the studies relevant for the gene mutation concern.

Therefore the reliability of this information is significantly affected.

Conclusion on WoE

For these reasons and those developed under the Appendix on reasons common to several requests, taken together, even if some of the sources of information provide information on the key investigation, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

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

Study design

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified *in vitro*.

According to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation.

In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally.

Based on the recent update[4] of OECD TG 488, you are requested to follow the new 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals. This updated version provides for a transitional period for the new version. However, ECHA is aware that testing according to the updated OECD TG is already available from CROs and the new study design would provide meaningful germ cell data, so this decision requires the application of the new version.

According to the test method OECD TG 488, the test must be performed by analysing tissues from the liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum as rapidly proliferating tissue and site of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

In case you decide to perform the comet assay according to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from the liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Germ cells

In case you decide to perform the TGR, you may consider to collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

In case you decide to perform the comet assay, you may consider to collect the male gonadal cells collected from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells.

[4] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66

This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2. Short-term toxicity testing on aquatic invertebrates

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

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. (weight of evidence) of REACH.

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

i. Weight of evidence (WoE) 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. Reliability score 4.
ii. WoE study (scientific publication, Iannacone and Alvarino, 2007), no method specified, with analogue substance malachite green. Reliability score 4.
iii. WoE study (review article/handbook data, Burchmore and Wilkinson, 1993), no method specified, with analogue substance malachite green. Reliability score 4.
iv. WoE study (OECD TG 202 study, Daphnia sp., acute Immobilisation Test and Reproduction Test, part I, adopted April 4th, 1984), with analogue substance malachite green. Reliability score 4.
v. WoE study (Certificate of analysis, DystarColours Distribution GMBH, 1998), no method specified, with the Substance. Reliability score 4.
vi. WoE study (Certificate of analysis, DystarColours Distribution GMBH, 1998), no method specified, with analogue substance Astrazon Gruen M 01 Verde Malaquita NL Polvo (i.e. Bis[[4-[4-(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium] oxalate, dioxalate). Reliability score 4.
vii. WoE study (Certificate of analysis, DystarColours Distribution GMBH, 1998), no method specified, with analogue substance Malachite Green Hydrochloride. Reliability score 4.
viii. long-term toxicity study to aquatic invertebrates (OECD 202, Daphnia sp., acute Immobilisation Test and Reproduction Test, part II, adopted April 4th, 1984) with analogue substance malachite green. Reliability score 3 (disregarded by you due to major methodological deficiencies).

We have assessed this information and identified the following issues:

As explained under Appendix on Reasons 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.

Relevance of provided information

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 9.1.1 at Annex VII includes similar information that is produced by the OECD TG 202, i.e. on key investigation:

- the concentration leading to 50% immobilisation/mortality of aquatic invertebrates (preferably daphnids) at the end of the test is estimated .

ECHA understands that all sources of information report the concentration leading to mortality (when lethal effect concentrations reported or mortality is reported as basis for estimation of effect concentration) or to immobility of 50% of various tested aquatic invertebrate species at the end of the test.

Thus, the sources of information i.-viii. provide relevant information on key investigation, but have the following deficiencies affecting their reliability.

Reliability of provided information

As explained in the Appendix on Reasons common to several requests the reporting of the studies is not sufficient to conduct an independent assessment of its reliability.

Furthermore, the use of other aquatic invertebrate species than daphnids for testing is accepted if it is justified that these species are not less sensitive than daphnids. However, you have not demonstrated that, for studies conducted with species other than daphnids, these species are not less sensitive than daphnids.

In your comments on the initial draft decision, you stated that '*simply all available data on the aquatic toxicity on invertebrates have been reported, and, independently from the species and the sensitivity, in all cases a high toxicity level has been recorded.'*. However, the information in your comments on the initial draft decision is not sufficient for ECHA to make an independent assessment, because raw data are missing to verify the validity criteria and the key parameters of the studies. Furthermore, you did not address the reliability issue of the data identified in the common Appendix, and you have not justified why 'high toxicity level' would be sufficient to establish the reliability of their studies.

The source of information (study v.) reports effect concentration after 24 hours only while standard OECD TG 202 requires the test duration to be 48 hours, therefore cannot be considered reliable. You have also identified study viii. as reliability score 3 because the validity criteria of the OECD TG 202, part II, adopted on April 4, 1984 (this TG is replaced by OECD TG 211 for the long-term Daphnia toxicity testing) are not met. ECHA agrees with your conclusion and considers this study to be not reliable.

In your comments on the initial draft decision you reconsider that the study viii. is reliable and adequate to fulfil the information requirement, and you submitted a full study report (Annex II of your comments). However, also in this study report there is still no information on the analytical verification of exposure conducted as required by OECD TG 202 and 211. Thus, you have not demonstrated that (no-)effect values can reliably be based on nominal test material concentrations and there is no underestimation of the effects.

For these reasons and those developed under the Appendix on reasons common to several requests, taken together, even if these sources of information provide information on the key investigation, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

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

Study design

The Substance is difficult to test due to ionisable, hydrolysable and photodegradable properties of the Substance, and the Substance is a coloured dye. Indeed, based on the information in your registration dossier, 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. Furthermore, the Substance undergoes photolytic reaction leading to a large number of transformation products.

OECD TG 202 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented.

In addition, if it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 202. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

3. Growth inhibition study aquatic plants

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

You have adapted this information requirement according to Weight of Evidence under Annex XI, Section 1.2. of REACH.

You have provided the following information;

i. Weight of evidence (WoE) study (Certificate of analysis, DystarColours Distribution GMBH, 1998), no method specified with the Substance. Reliability score 4.
ii. WoE evidence study (Certificate of analysis, DystarColours Distribution GMBH, 1998), no method specified with analogue substance malachite green dioxalate, Reliability score 4.

We have assessed this information and identified the following issues:

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.

Relevance of provided information

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 9.1.2 at Annex VII includes similar information that is produced by the OECD TG 201. The key investigation investigated by this test is growth rate of algal cultures and the following information should be provided:

- The concentrations of the test material leading to a 50 % (EC50) and 0% (or 10%) (EC0 or EC10) inhibition of growth at the end of the test are estimated.

Relevance of provided information

Both sources of information you provided investigate the growth rate of algae species *Scenedesmus subspicatus* and report EC 50 (based on growth rate) at the end of the test. However, they do not report EC0 or EC10 at the end of the test. Therefore, they provide only partially relevant information that would contribute to the conclusion on this key investigation.

Moreover, the studies have the following deficiencies affecting their reliability.

Reliability of provided information

As explained in the Appendix on Reasons common to several requests, there is no weight of evidence justification and the reporting of the studies is not sufficient to conduct an independent assessment of its reliability.

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

The conditions of OECD TG 201 specifies that:

1. Validity criteria listed in the test guideline have to be met;
2. Information on the methodology (test design, conditions, method for determination of biomass etc.) is reported;
3. Results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form.

For both source information i) and ii), you have not provided the information listed above.

In your comments on the initial draft decision, 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 a scientific article (P. Matpang, 2017). However, the information in your comments on the initial draft decision is not sufficient for ECHA to make an independent assessment, because raw data are missing to verify the validity criteria of the studies.

Therefore, it is not possible to conduct an independent assessment of their reliability.

As a conclusion, sources of information as indicated above, provide information on the growth rate of algal cultures, but do not provide also necessary relevant information on the EC0 or EC 10 after 72 hours and it is not possible to conduct an independent assessment of their reliability.

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.

On this basis, the information requirement is not fulfilled.

Study design
OECD TG 201 specifies that for difficult to test substances OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design' under Section A.2.

Appendix B: 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[5].

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[6].

[5] https://echa.europa.eu/practical-guides
[6] https://echa.europa.eu/manuals

Appendix C: 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 19 August 2020.

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

ECHA took into account your comments and did not amend the requests 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 D: List of references - ECHA Guidance[7] 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)[8]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[9]

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[10]

[7] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[8] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[9] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316
[10] 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 E: Addressees of this decision and their corresponding information requirements

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

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

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.