ECHA
EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 2 June 2021

Addressees
Registrants of DASA_2HT as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
11 April 2019

Registered substance subject to this decision ("the Substance")
Substance name: Amines, di-C16-18-alkyl
EC number: 629-721-4
CAS number: 308062-60-4

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 ***9 December 2024***.

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

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

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

2. Long-term toxicity testing on aquatic invertebrates also requested below (triggered by Annex VII, Section 9.1.1., column 2)

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

4. Ready biodegrability (Annex VII, Section 9.2.1.1.; test method: OECD TG 301B/C/D/F or OECD TG 310)

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

1. Long-term toxicity testing on fish also requested below (triggered by Annex VIII, Section 9.1.3., column 2)

2. Soil simulation testing also requested below (triggered by Annex VIII, Section 9.2.)

3. Sediment simulation testing also requested below (triggered by Annex VIII, Section 9.2.)

4. Identification of degradation products also requested below (triggered by Annex VIII, Section 9.2.)

5. Bioaccumulation in aquatic species also requested below (triggered by Annex I, Sections 0.6.1. and 4; Annex XIII, Section 2.1.)

C. Information required from all the Registrants subject to Annex IX of REACH

1. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211)

2. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: OECD TG 210)

3. Soil simulation testing (Annex IX, Section 9.2.1.3.; test method: EU C.23./OECD TG 307) at a temperature of 12°C. Non-extractable residues (NER) must be quantified and a scientific justification of the selected extraction procedures and solvents must be provided.

4. Sediment simulation testing (Annex IX, Section 9.2.1.4.; test method: EU C.24./OECD TG 308) at a temperature of 12°C. Non-extractable residues (NER) must be quantified and a scientific justification of the selected extraction procedures and solvents must be provided.

5. Identification of degradation products (Annex IX, 9.2.3.; test method: OECD TG 307/308)

6. Bioaccumulation in aquatic species (Annex IX, Section 9.3.2; test method: OECD TG 305)

D. Information required from all the Registrants subject to Annex X of REACH

1. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: OECD TG 443) by oral route, in rats, specified as follows:
   a) Ten weeks premating exposure duration for the parental (P0) generation;
   b) Dose level setting shall aim to induce systemic toxicity at the highest dose level;
   c) Cohort 1A (Reproductive toxicity);
   d) Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

Reasons for the request(s) are explained in the following appendices entitled "Reasons to request information required under Annexes VII to X of REACH", respectively.

Information required depends on your tonnage band

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

- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is

provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

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

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is an information requirement under Annex VII to REACH (Section 8.4.1.).

You have provided the following information:

i. a key study according to OECD TG 471 on the Substance and with the following strains, TA98, TA100, TA1535, TA1537 and TA1538 ([redacted], 1987)

We have assessed this information and identified the following issue:

A. To fulfil the information requirement, the study has to meet the requirements of OECD TG 471 (1997) (ECHA Guidance R.7a, Table R.7.7-2). Therefore the following specifications must be met:
   a) the test must be performed with 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101);
   b) the maximum dose tested must induce a reduction in the number of revertant colonies per plate compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test dose must correspond to 5 mg/plate or 5 ml/plate;
   c) one positive control must be included in the study. The positive control substance must produce a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control;
   d) the number of revertant colonies per plate for the concurrent negative control must be inside the historical control range of the laboratory;
   e) the mean number of revertant colonies per plate must be reported for the treated doses and controls.

   Your registration dossier provides an OECD TG 471 showing the following:
   a) the results from an appropriate 5 strains (i.e. *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101)) are not provided;
   b) the maximum dose tested was 1500 µg/plate (hence below 5 mg/plate). You stated that in a range-finding study "the test substance was toxic at 5000 µg/plate". However, you have not indicated if the highest dose selected for the main test (*i.e.* 1500 µg/plate) led to a reduction in the number of revertant colonies compared to the negative control, the precipitation of the test material or limiting cytotoxicity;
   c) you have not provided information whether the positive control produced a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control;
   d) you have provided no information whether the negative controls were inside the historical control range of the laboratory;
   e) data on the number of revertant colonies per plate for the treated doses and the controls are not reported.

   Based on the above,
   - there are critical methodological deficiencies resulting in the rejection of the study. More specifically:
     - the study does not provide information on the required fifth strain (*i.e.*, S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101));
     - you have not provided adequate justification for the selection of the highest

dose tested, except for strain TA 100.

- the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically you have not provided supporting information on the historical control range of the laboratory and adequate reporting of the study results including results of the positive control, the treated plates and the controls.

Therefore, the specifications of OECD TG 471 are not met.

On this basis, the information requirement is not fulfilled.

Study design

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

2. Long-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.). Long-term toxicity testing on aquatic invertebrates must be considered (Section 9.1.1., Column 2) if the substance is poorly water soluble.

You have adapted the information requirement for short-term toxicity testing on aquatic invertebrates under Annex VII, Section 9.1.1., Column 2 with the following justification: the substance is poorly water soluble and a long-term toxicity study on aquatic invertebrates is available.

We have assessed this information and identified the following issue:

A. Poorly water soluble substances require longer time to reach steady-state conditions. As a result, the short-term tests does not give a true measure of toxicity for this type of substances and the long-term test is required. A substance is regarded as poorly water soluble if, for instance, it has a water solubility below 1 mg/L or below the detection limit of the analytical method of the test material (ECHA Guidance R.7.8.5).

   In Section 4.8. of your registration dossier, you provide a water solubility study based on a method derived from OECD TG 123. The water solubility of the Substance is reported as < 20 µg/L.

   Therefore, the Substance is poorly water soluble and information on long-term toxicity on aquatic invertebrates must be provided.

B. Under Annex VII, Section 9.1.1., Column2, second indent, a study may be omitted if a reliable long-term toxicity study on aquatic invertebrates is available.

   In Section 6.1.4. of your registration dossier, you provided a long-term toxicity on aquatic invertebrates for the Substance.

   However, for the reasons explained under Appendix C.1., this study does not meet the information requirement. Therefore, your adaptation is rejected.

On this basis, the information requirement is not fulfilled.

The examination of the information provided on long-term toxicity on aquatic invertebrates,

as well as the selection of the requested test and the test design are addressed under section C.1.

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 provided the following information:

i. An OECD TG 201 key study on the Substance ([redacted], 2010)

We have assessed this information and identified the following issues:

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

In Section 1.2. of your technical dossier, you indicate that the Substance may contain [redacted]% w/w of dioctadecylamine, [redacted]% w/w 1-octadecylamine, n-hexadecyl and [redacted]% w/w of dihexadecylamine. The Substance also contains a number of minor primary and secondary amine constituents of shorter C-chain length.
For study i. above, you have identified the test material as "*Amines, di-C16-18 (even numbered) alkyl with CAS 308062-60-4*" (*i.e.* the Substance). You have provided the following information on the composition of the test material: "*C12 part: < 1 area %; C14 part: 2 area %; C16 part: 29 area %; C17 part: 1 area %; C18 part: 67 area %; C20 part: 1 area %*".

The information provided on the test material does not allow verifying if the test material contains representative amounts of the constituents of the Substance (as reported in Section 1.2 of your technical dossier) as you only report the fraction of the test material corresponding to various C-chain lengths and no information on the chemical identity of these constituents. On this basis, you have not demonstrated that the test material is representative for the Substance and the information is rejected.

B. To fulfil the information requirement, a study must comply with OECD TG 201 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 specifications must be met:

Characterisation of exposure

- an adequate and reliable analytical method for the quantification of the test material in the test solutions must be available.
- the concentrations of the test material are measured at least at the beginning and end of the test. For volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24 hour intervals is required.
- 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;

Additional requirements applicable to difficult to test substances

- for adsorbing test chemical, dissolved total organic carbon concentrations (other than that due to the test chemical) must be maintained in all test solutions at or below 2 mg/L;

Other considerations

- Algal biomass is determined based on dry weight per volume, or alternatively as cell counts or biovolume using microscopy or an electric particle counter. If an alternative method is used (*e.g.* flow cytometry, *in vitro* or *in vivo* fluorescence, or optical density), a satisfactory correlation with biomass must be demonstrated over the range of biomass occurring in the test.

Your registration dossier provides a key study showing the following:

Characterisation of exposure

- on the preparation of samples prior the determination of exposure concentration, you report that "*to prevent adsorption of the test item on the glass wall during sample storage all test item concentrations and the control were diluted 1:2 with acetonitrile containing 0.02 mol/L trifluoroacetic acid followed by UPLC-MS/MS analysis*";
- you report that the limit of quantification (LOQ) of the analytical method was 10 µg/L. You also report that at the end of the test, exposure concentrations were mostly below the LOQ;
- the concentration of the test material was determined only at the beginning and end of the test (i.e. t = 0 and t =72h). The test material is highly adsorbing and measured concentrations at t = 72h were mostly below the limit of quantification of the analytical method (i.e. 10 µg/L). You have not reported the results of the required additional samplings at 24 hour intervals;
- you expressed the results based on measured initial concentration.

Additional requirements applicable to difficult to test substances

- you report that the test medium was prepared using natural river water filtered at 0.45 µm with a DOC concentration of 3.9 mg/L;

Other considerations

- biomass was measured using *in vivo* fluorescence. No justification is provided that *in vivo* fluorescence was adequate for the determination of biomass (*e.g.* evidence of correlation between the measured parameter and dry weight for both control and treated groups).

Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the results of this study. More specifically, you have not demonstrated that exposure was satisfactorily maintained during the experiment as:
  - you have not justified that the preparation of samples does not bias the estimation of truly dissolved concentration of the Substance. In the absence of this information, the reliability of the analytical method is uncertain;
  - you have not provided any justification as to why an analytical method with greater sensitivity could not be developed. In this absence of this information you have not demonstrated that the analytical method is adequate;
  - the concentration in DOC is higher than the maximum value allowed by OECD GD 23 (i.e. 2 mg/L) and may have reduced exposure to the dissolved fraction of the Substance;

    In your comments on the draft decision, you explain that the studies with natural river water were performed in line with the Bulk approach. You consider that studies conducted according to the Bulk approach are more adequate and reliable for risk assessment than tests performed under standard test conditions when evaluating sorbing cationic surfactants. You

acknowledge that the Bulk approach test are less adequate for Classification and labelling purposes as these studies do not allow the quantification of the intrinsic toxicity. However, you consider the approach acceptable as:

a) you have applied a correcting factor of 10 to the effect data assuming that 90% of the substance would be sorbed and you consider that it therefore provides a worst-case estimate of the intrinsic properties of the Substance;
b) the Bulk approach has been accepted by the Technical Meetings (TM's) for the EU risk assessments of e.g. DODMAC and primary alkyl amines category (COM070_410_412_429_430_env);
c) No comments on the use of this approach for risk assessment have yet been received for other dossiers which were subjected to compliance checks under REACH;
d) under the principle of legitimate expectations, ECHA cannot renege a method which was endorsed by EU Member States, especially shortly before (2002) but also after REACH regulation entered into force (2008) and induced the registrants to take a course of action (i.e. continue river water testing) during REACH registration.

ECHA disagrees with your conclusion for the following reasons:

a) with regard to point a. above, under section 5.4.1. of your registration dossier you report you report Kd values generated based on OECD TG 106 in three soil types ranging from 2100 to 56000 L/kg. This indicates that the adsorption potential of the substance may vary greatly depending on the nature of the sorbent matrix. Furthermore, it cannot be excluded that the fraction of the substance that would be adsorbed may be lower in low DOC and low suspended solid waters (e.g. pristine waters). Therefore, you have not provided a valid scientific justification that the proposed correction factor of 10 may be considered as a realistic worst case to correct effect values based on 'bulk' concentrations.
b) with regard to point b. above, the EU RAR reports cited by you did not conclude that classification and labelling can be based on effect values based on 'bulk' concentrations as (i) the EU RAR on DODMAC (EC Number 203-508-2) does not discuss the classification of the substance and (ii) the Draft EU RAR on Primary Alkyl Amines has not been endorsed by the European Commission (as clearly specified in the foreword section of the document). On the latter, ECHA points out that adopted RAC Opinions are available on the individual substances originally included in the Draft EU RAR on Primary Alkyl Amines (i.e. EC No. 204-015-5, EC No. 204-695-3, EC No. 262-977-1, EC No. 263-125-1, EC No. 262-976-6). RAC concluded that, for studies conducted with a dilution water containing a high level of suspended matter and humic acid, nominal concentrations do not represent truly dissolved concentrations and that such study has limited usefulness for the purposes of classification.
c) with regard to point c. above, as already explained above, RAC concluded on the inadequacy of data generated using the bulk approach for classification and labelling. In addition, we disagree with your comment that the inadequacy of the bulk approach has never been raised in any compliance check decision (see for example, communication number CCH-D-2114476324-47-01/F on Quaternary ammonium compounds, di-C12-18-alkyldimethyl, chlorides with EC number 269-924-1).
d) with regard to point d. above, the Guidance on Application of CLP Criteria, Section 1.1.3., clarifies that classification must be based on

intrinsic hazards, i.e. the basic properties of a substance as determined in standard tests or by other means designed to identify hazards. As the CLP Regulation is hazard-based, the data on intrinsic properties must not take exposure into consideration. Therefore, the bulk approach which aims at mimicking exposure under "more environmentally realistic" conditions must not be used for classification and labelling. As already explained above, this conclusion was confirmed by RAC, among other cases, for primary alkyl amines. Similar considerations apply for the PBT assessment. As per Annex XIII of REACH, the PBT assessment should be based on data generated under 'relevant conditions', i.e. those conditions that allow for an objective assessment of the PBT/vPvB properties of a substance and not the PBT/vPvB properties of a substance in particular environmental conditions. This has been also confirmed by the Board of Appeal in its Decision of 7 December 2016 in case A-013-2014. Based on the above, ECHA's conclusion that the bulk approach is not suitable to generate information on intrinsic properties does not breach of the principle of legitimate expectations.

- the concentration of the test material was not satisfactorily maintained throughout the test. Therefore, results cannot be expressed based on measured initial concentrations.

  In your comments on the draft decision, you disagree that the results of a study based on OECD TG 201 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. Your refer to paragraph 40 of OECD TG 201 and state that results can be based on measured initial concentration there is no decrease in growth inhibition during the test.

  ECHA acknowledges that OECD TG 201 states that, especially for adsorbing substances tested at low concentrations, the actual exposure concentrations may be difficult to define. In such case, disappearance of the test substance from solution by adsorption to the increasing algal biomass does not mean that it is lost from the test system. When the result of the test is analysed, it should be checked whether a decrease in concentration of the test substance in the course of the test is accompanied by a decrease in growth inhibition. If this is the case, application of a suitable model describing the decline of the concentration of the test substance may be considered. If not, it may be appropriate to base the analysis of the results on the initial (nominal or measured) concentrations.

  However, as already discussed above, loss by adsorption has likely occurred through adsorption to suspended solid and DOC introduced as a result of the use of natural water. For this specific study it cannot be argued that reduction in dissolved concentrations was solely due to adsorption to the algal biomass. Therefore, the provisions specified under paragraph 40 of OECD TG 201 do not apply. Regardless of this deficiency, it should also be noted that the guideline requires to verify whether or not a decrease in concentration of the test substance in the course of the test is accompanied by a decrease in growth inhibition to justify that results can be based on the initial concentrations. We note that you have not provided such justification.

- the reporting of the studies is not sufficient to conduct an independent assessment

of its reliability. More specifically as you have not provided any supporting information to demonstrate that *in vivo* fluorescence provides an adequate determination of algal biomass, it is not possible to verify that the study is reliable. The physiological status of algal cells is known to impact the efficiency of the non-photochemical quenching (NPQ) of fluorescence and differences in physiological status between treatments may bias the relationship between re-emitted fluorescence and biomass. You have not provided such supporting information.

In your comments on the draft decision, you state that this information is standard information generated by a GLP lab performing tests according to OECD 201. You have not provided such supporting information to demonstrate that *in vivo* fluorescence provides an adequate determination of algal biomass as part of your comments on the draft decision but you state that you intend to add this information to the robust study summary of study i.

Therefore, this study does not meet the specifications of OECD TG 201 in conjunction with OECD GD 23.

On this basis, the information requirement is not fulfilled.

Study design

The Substance is difficult to test due to the low water solubility (< 20 µg/L) and adsorptive properties (high log Kow, high measured log Koc and the Substance is ionisable). OECD TG 201 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 201. 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.

4. Ready biodegradability

Ready biodegradability is an information requirement in Annex VII to REACH (Section 9.2.1.1.).

Your registration dossier provides:

i. an OECD TG 301D supporting study on the Substance ([redacted], 1993)
ii. a reference to a publication on the biodegradation pathways of cationic surfactants (van Ginkel et al., 2004);
iii. a reference to a publication reporting biodegradation of long chain linear alkylamines by an isolated strain identified as Pseudomonas putida (Yoshimura et al., 1980);
iv. a reference to a publication reporting biodegradation, in a closed bottle test, of analogue substances including Dodecylamine, didodecylamine, dodecyl-dimethylamine and didodecylmethylamine (van Ginkel et al., 1995);
v. a reference to a publication reporting results of degradation studies based on modified OECD TGs for some fatty amine derivatives (van Ginkel et al., 2008);
vi. a study according to OECD TG 301D with the analogue substance N-octadecyloctadecan-1-amine, EC No. 204-020-2 ([redacted], 2005; Report no. [redacted]

[redacted]);

vii. a study according to OECD TG 301D with the analogue substance N-octadecyloctadecan-1-amine, EC No. 204-020-2 ([redacted], 2005; Report no. [redacted]);

viii. a study according to OECD TG 301D with the analogue substance dicoco alkylamine, EC No. 263-086-0 ([redacted], 1992).

While you have not claimed explicitly such adaptation, we understand that you attempt to demonstrate that all fatty amines are readily biodegradable under a weight of evidence approach (Annex XI, Section 1.2) and we assessed this information on that basis.

We identified the following issues:

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

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

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

However, 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 investigated by the required study.

ECHA takes note of your comments on the draft decision, that "*where possible* [you] *will update the sources of information in order to provide a (better) explanation why they can be used and provide weight of evidence for our grouping and read-across approach*".

Irrespective of the above-mentioned deficiencies on the documentation, which in itself could lead to the rejection of the adaptation, ECHA has assessed the provided sources of information.

To fulfil the information requirement ready biodegradability, normally a study performed according to 301 or 310 must be provided. OECD TG 301 or 310 require to investigate the following key investigation: the ultimate aerobic biodegradation (as measured by parameters such as DOC removal, CO_2 production and oxygen uptake) of the test material under low inoculum concentration (with a non-adapted inoculum representing a mixed bacterial community) and measured at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

The sources of information (ii.) and (iii.) do not provide relevant information in support of your adaptation as:

- source of information (ii.) is limited to discussing biodegradation pathways of cationic surfactants and does not provide any information on the above key investigation.

In your comments on the draft decision, you agree with ECHA's assessment.

- source of information (iii.) refers to information obtained from a specific microbial isolate and does not inform on biodegradation by a mixed bacterial community.

  In your comments on the draft decision, you disagree with ECHA's assessment. You state that the biodegradation tests in this publication were performed using activated sludge from a domestic wastewater treatment plant as inoculum according a test method comparable to the OECD 301 F. You specify that if this information is missing you intend to update the robust study summary for this study.

  However, we note that, in your dossier, you have only provided the following brief executive summary for this study: "*Morphological and physiological propemes of isolated bacterium were identical to those of Pseudomonas putida as shown in Table V. This isolated strain could degrade PA12 and other primary alkylamines, but not secondary akylamines, tertiary alkylarnines or cationic surfactants (Table VI). Furrhermore, this isolated strain could use PA1 2 as the sole carbon and nitrogen source for its growth. This result suggests the possibility that primary'alkylamines are biodegraded through : (a) oxidative dearnination by amine oxidase to give the corresponding fatty acid and ammonia*". Therefore, the information in your dossier on this study does not mention anywhere testing according to a test method comparable to the OECD 301 F.

The sources of information (i.) and (iv.) to (viii.) listed above provide relevant information on the above key investigation. However, the reliability of these sources of information to inform on the properties of the Substance is significantly affected by the following deficiencies:

Source of information (i.) performed on the Substance

In that study, the percentage biodegradation of the test material was determined to be 4% after 28 days and 17% after 140 days leading to the conclusion that the test material is not readily biodegradable. Furthermore, we have identified the following deficiencies with this study:

A. To inform on the intrinsic properties of a substance, the test material in a study must be representative of that substance (Article 10 and Recital 19 of REACH; ECHA Guidance R.4.1).

   In Section 1.2. of your technical dossier, you indicate that the Substance may contain [redacted]% w/w of dioctadecylamine, [redacted]% w/w 1-octadecylamine, n-hexadecyl and [redacted]% w/w of dihexadecylamine. The Substance also contains a number of minor primary and secondary amine constituents of shorter C-chain length. You do not report the presence of tertiary amines in the composition of the Substance.
   For study i. above, you have identified the test material as "*Amines, di-C16-18 (even numbered) alkyl with CAS 308062-60-4*" (*i.e.* the Substance). You have provided the following information on the composition of the test material: "*tertiary amine* [redacted]%".

   The composition of the test material is not consistent with the information reported for the Substance in Section 1.2. of your technical dossier as tertiary amines are not part of its composition. Therefore, the test material is not representative for the Substance.

   In your comments on the draft decision, you state that you will "*remove the study as supporting study for the test substance*".

B. To inform on ready biodegradability, a study must provide equivalent information to a ready biodegradability study described in any of the OECD TG 301 or 310 test methods. Therefore, for a study claimed to be conducted according to OECD TG 301D, the following key specifications are normally expected to be met:

Technical specifications impacting the sensitivity/reliability of the test
- test solutions are prepared using an appropriate nutrient medium, which includes ammonium chloride;
- a dilute inoculum without sludge flocs is used. The inoculum is normally derived from the secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage;
- the concentration of the inoculum is set to reach a bacterial cell density of 10^4 to 10^6 cells/L in the test vessel. The concentration of added inoculum is ≤ 5 mg/L;

Reporting of the methodology and results
- the inoculum concentration in the test vessel is reported as cells/L in the test vessel;
- the results of measurements at each sampling point in each replicate is reported in a tabular form;
- the calculation of the ThOD is described and justified;
- for nitrogen-containing test materials, correction for nitrification is applied on the theoretical oxygen demand (*i.e.* $ThOD_{NO3}$) unless it can be demonstrated that nitrification did not occur (*e.g.* by monitoring changes in concentrations in nitrite and nitrate).

Your registration dossier provides a study claimed to be conducted according to OECD TG 301D (study i. listed above) showing the following:
- the test material reached 4% biodegradation after 28 days and 17% after 140 days;
- you report that "*Ammonium chloride was omitted from medium to prevent nitrification*";
- activated sludge was used as an inoculum and you have not reported any procedure to would allow to ensure that no flocs were present in the test system;
- you have not reported inoculum concentration in the test vessel in cells/L;
- you have not reported the results of measurements at each sampling point in each replicate;
- you report that the calculated theoretical oxygen demand (ThOD) of the test material is 3.3 mg/L.

Based on the above,
- there are critical methodological deficiencies impacting the overall reliability of the study results. More specifically,
  - you have not used a standard test medium as you report that Ammonium chloride was omitted from the test medium. This deviation is no considered acceptable as it may artificially reduce oxygen consumption and lead to underestimating respiration in the inoculum blank (i.e. one of the validity criteria of OECD TG 301D);
  - you have not reported any procedure to removes flocs from the inoculum as required by OECD TG 301D;

In your comments on the draft decision, you provided a justification on the above deficiencies in relation to the studies vi. to viii. on the selected analogue substances. However, for the reasons explained under B.3, ECHA maintains that

you have not demonstrated that these deviations did not impact the reliability of the study.

- the reporting of the study is not sufficient to fully assess its reliability. More specifically:
  - as you have not reported inoculum concentration in the test vessel in cells/L, it is not possible to verify if the inoculum density was low enough to be consistent with the specifications of OECD TG 301D;
  - as you have not provided an adequate reporting of the study results, it is not possible to verify if validity criteria consistent with the specifications of OECD TG 301D were met;
  - you have not specified of ThOD was estimated and, as the test material is a nitrogen-containing substance, that the calculated ThOD takes into account oxygen consumption through nitrification (or alternatively supporting information that nitrification did not occur).

On the basis of the above, study (i) does not indicate that the test material used to conduct this study is readily biodegradable. In addition, this source of information is affected by key deficiencies impacting its reliability. Accordingly, this study provides little support to your weight of evidence adaptation under Annex XI, Section 1.2 to conclude that the Substance is readily biodegradable.

Sources of information (iv.) to (viii.) performed on analogue substances

As all the other sources of information submitted refer to information on analogue substances, ECHA understand that you intend to rely on a read-across justification to consolidate your weight of evidence adaptation. ECHA has therefore assessed the scientific and regulatory validity of the proposed grouping and read-across approach with regard to the requirement of Annex XI, Section 1.5.

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 (addressed under 'Scope of the grouping'). 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 R.6 and related documents.

A. Scope of the grouping

i. Description of the grouping

In your registration dossier you have formed a group (category) of 'dialkylamines'. You have provided a read-across justification document in Section 4.1.2.1.3. of your CSR.

For the purpose of this decision, the following abbreviations are used for the group members:
[1] Dodecylamine or DDA (primary amine) (No EC or CAS No. provided);
[2] Didodecylamine or DiDDA (No EC or CAS No. provided);
[3] Dodecyldimethylamine or DDMA (No EC or CAS No. provided);
[4] Didodecylmethylamine or DiDDMA (tertiary amine) (No EC or CAS No. provided);
[5] Dioctadecylamine or DiODA (EC No. 204-020-2); and

[6] Dicoco alkylamine or DiCocoA (EC No. 263-086-0).

You provide the following reasoning for the grouping the substances: "*Based on the broad substrate specificity of micro-organisms degrading fatty acids with respect to the alkyl chain length it is unlikely that the biodegradability of dialkylamines differs significantly with varying alkyl chain lengths".*

You define the the structural basis for the grouping as "[substances with] *two alkyl chains linked directly to a nitrogen atom. The alkyl chains may be derived from different sources like dodecyl, coco, or tallow*". ECHA understands that this is the applicability domain of the grouping and will assess your predictions on this basis.

ii. Assessment of the grouping

ECHA notes the following shortcomings with regards to your grouping approach.

1) Applicability domain of the category

According to the ECHA Guidance, a category (grouping) hypothesis should address "*the set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members for the given endpoint"* (ECHA Guidance Section R.6.2.4.1.). Particularly, "*the applicability domain of a (sub)category would identify the structural requirements and ranges of physico-chemical* [and] *environmental fate* [...] *properties within which reliable estimations can be made for the (sub)category members*" (ECHA Guidance R.6.2.1.2.). Therefore, to reliably predict properties within a category the applicability domain should be described including the borders of the category, for which chemicals the category does not hold and a justification for the inclusion and/or exclusion rules.

You describe the applicability domain of the substances covered by the grouping as: "[substances with] *two alkyl chains linked directly to a nitrogen atom. The alkyl chains may be derived from different sources like dodecyl, coco, or tallow*"

This applicability domain does not introduce unambiguous inclusion/exclusion criteria which would identify the structural requirements (in particular, in terms of alkyl chain length and degree of unsaturation and branching) and ranges of physico-chemical properties within which reliable estimations can be made for the (sub)category members.

In your comments on the draft decision, you explain that the "*applicability domain, the description of which substances are included in the group used for read across, will be updated*".

B. Predictions for ready biodegradability

You have provided the following reasoning for the prediction of toxicological properties:

- Similar biodegradation is expected due to the "*broad substrate specificity of micro-organisms degrading fatty acids*";
- "*The valid ready biodegradability test results obtained with didodecylamine and dioctadecylamine, and the scientific evidence that fatty amine derivative degrading bacteria degrade these substances though b-oxidation lead to the conclusion that all dialkylamines are readily biodegradable".*
- "*The low biodegradability* [in some] *tests should be attributed to the limited bioavailability under the stringent test conditions and should consequently be ignored"*

and "adequate availability of the test substance to microorganisms is key for demonstrating the true biodegradability of dialkylamines";

- The substances from the group are expected to be biodegraded through a similar metabolic pathway, i.e. cleavage of the alkyl-N bond, liberation of the alkyl chains as alkanals, enzymatic transformation of the alkanal to fatty acid and finally degradation of the fatty acid by β-oxidation. To support similar metabolism, you refer to van Ginkel *et al.* 2004 (i.e. source of information ii listed above)

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 predictions of toxicological properties.

1) *Read-across hypothesis*

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical and environmental fate 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 (read-across approach).
A read-across hypothesis needs to be provided, establishing why a prediction for a toxicological or ecotoxicological property is reliable. This hypothesis should be based on recognition of the structural similarities and differences between the source substance(s) and your Substance (ECHA Guidance R.6). It should explain why the differences in the chemical structures should not influence environmental fate properties or should do so in a regular pattern.

Your read-across hypothesis is that the similarity in chemical structure and in biodegradation metabolic pathways between the category members is a sufficient basis for predicting the properties of the Substance for other endpoints.

Similarity in chemical structure and in biodegradation metabolic pathway does not necessarily lead to predictable or similar environmental fate properties. You have not provided a well-founded hypothesis to establish a reliable prediction for ready biodegradability, based on recognition of the structural similarities and differences between the category members.

In your comments on the draft decision, you explain that your read-across hypothesis does not predict fate properties but predicts the biodegradation potential of a chemical in the environment.

Ready biodegradability is a key process driving the environmental fate of a substance. In the above assessment, environmental fate is used as a generic term and we agree that your intention was to predict ready biodegradability.

2) *Missing supporting information on source of information (iv. and v.)*

Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide, in particular, robust study summary(ies) of the source study(ies) (ECHA Guidance R.6.2.6.2.).

For the studies iv. and v., you have only provided a brief statement summarising the outcome. You have not provided robust study summaries for these studies.

In the absence of such documentation, this information is disregarded as it is not possible to assess its reliability.

In your comments on the draft decision, you explain that studies iv. and v. are publication from peer review literature. You intend to provide robust study summaries for these studies. However, you acknowledge that it is unlikely that all information required for regulatory compliance will be available in the original publications.

3) *Adequacy and reliability of the source studies (vi) to (viii.)*

Under Annex XI, Section 1.5., if grouping concept is applied then in all cases, the results must, in particular, provide an adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3).

Therefore for studies vi. to viii., which are claimed to be conducted according to OECD TG 301D, the following key specifications are normally expected to be met:

Technical specifications impacting the sensitivity/reliability of the test

- test solutions are prepared using an appropriate standard nutrient medium, which includes ammonium chloride.

  For studies vi. to viii., you report that "*Ammonium chloride was omitted from medium to prevent nitrification*". Therefore, none of these studies used a standard test medium as ammonium chloride was omitted. This deviation is not considered acceptable as it may artificially reduce oxygen consumption and lead to underestimating respiration in the inoculum blank (i.e. one of the validity criteria of OECD TG 301D).

  In your comments on the draft decision, you state that ammonium chloride is added under the assumption that there is a nitrogen limitation in ready biodegradability tests. You provide the following justification for the omission of ammonium chloride from the test medium:

  a) You consider that the results using the reference substance indicates that the omission of ammonium chloride did not result in nitrogen limitation in the OECD TG 301D.

     However, ECHA notes that you have not provided an reference substance control with addition of ammonium chloride in the test medium. Therefore, it is not possible to evaluate the impact of the presence or absence of ammonium chloride on the degradation rate of the reference substance in these studies. Furthermore, this issue does not address the issue identified above.

  b) You explain that that nitrifying bacteria utilizing ammonium as energy source and carbon dioxide as carbon source (autotrophic growth) and are not involved in the biodegradation of organic substances. Therefore you consider that it did not impact the stringency of the test.

However, we disagree that nitrifying bacteria are restricted to autotrophic mode of growth. Heterotrophic nitrification do also occur among denitrifiers commonly found in the environment as acknowledged by numerous publications available in the peer review literature. Therefore, it cannot be excluded that the omission of ammonium chloride may have impacted to some extent the composition or relative abundance of competent micro-organisms.

c) You consider that the impact of omitting ammonium chloride on the inoculum blank respiration will not affect the reliability of the study as a reduced background respiration would ensure the accuracy of the measured oxygen consumption by the test substance.

We agree that lower inoculum blank respiration induced by ammonium chloride omission will likely favour better accuracy of measurement of the oxygen consumption resulting from the biodegradation of the test material. However, as explained further below, this may also lead to bias in the estimation of the true biodegradation rate of the test material. The conditions of the OECD TG 301D are already set to provide appropriate accuracy for determining oxygen consumption originating from the test material degradation. Therefore, this does not constitute on its own a valid reason to modify the test medium composition.

d) You argue that nitrifying bacteria are sensitive and relative slow growing bacteria. Low bacteria numbers at the start of the tests or an initial delay in growth by toxic effects is therefore not easily overcome over a 28 days test period. A test substance that is (slightly) toxic to nitrifying bacteria will delay or stop the growth of nitrifying bacteria in the test bottles. In such a case, the inoculum blank (with no hampering of the growth of nitrifying bacteria) will overestimate the background respiration.

However, we note that the OECD TG 301D includes a toxicity control to determine if reduced degradation may originate from inoculum toxicity. Furthermore, you have provided no justification to support that significant toxicity towards nitrifying bacteria would occur at the test concentration used to conduct these tests.

e) You acknowledge that the analysis of formed nitrate and nitrite in the OECD 301D test and control bottles allows a correction for the additional oxygen consumption by the nitrification process. However, you consider that these analyses will however also introduce analytical inaccuracy and hence an increased variation (inaccuracy) of the final calculated test substance biodegradation.

On the potential inaccuracy originating from correcting additional oxygen consumption from the nitrification process, we note that the omission of ammonium chloride may also have led to bias that may overestimate biodegradation, and among others:

- when environmental C:N ratios are high, heterotrophic bacteria would be subject to some degree of N limitation. Under such circumstances, heterotrophic bacteria are expected to outcompete nitrifying bacteria due to their higher ammonium scavenging efficiency. Therefore, by inducing N limitation, the omission of ammonium chloride may artificially increase the relative abundance of competent bacteria;

- the respiration in the inoculum blank also provides some information about inoculum activity. Respiration in the inoculum blank depends on the bacterial density of the inoculum as well as from the concentration of exogenous compounds that are introduced with the inoculum. High inoculum blank respiration (i.e. above the validity criteria of OECD TG 301D) could indicate that the inoculum density and/or the inorganic matter introduced with the inoculum was too high. This could indicate that the conditions of the test were too favourable. By omitting ammonium chloride a direct comparison with the OECD TG 301D limit value for inoculum blank respiration is no longer possible.

On that basis, ECHA maintains that you have not provided a valid justification as to why the omission of ammonium chloride did not affect the reliability of these studies.

- a dilute inoculum without sludge flocs is used. The inoculum is normally derived from the secondary effluent of a treatment plant or laboratory-scale unit receiving predominantly domestic sewage.

  For studies vi. and vii., activated sludge was used as an inoculum. However, you have not reported any procedure to would allow to ensure that no flocs were present in the test system. The extent to which this deviation may have impacted the bioavailability of the test materials used in these studies is not addressed.

  In your comment on the draft decision, you agree with the above assessment. You explain that for studies vi. and vii., the preconditioned sludge was homogenized (removal of larger flocs) by pressing it through a sterile needle with a syringe. You intend to update the robust study summaries of these studies accordingly.

- the concentration of the inoculum is set to reach a bacterial cell density of 10^4 to 10^6 cells/L in the test vessel. The concentration of added inoculum is ≤ 5 mg/L.

  For studies vi. to viii., you have not reported inoculum concentration in the test vessel in cells/L. Therefore, it is not possible to verify if the inoculum density was low enough to be consistent with the specifications of OECD TG 301D;

  In your comments on the draft decision, you acknowledge that this information is not available.

- the concentration of the test material is in the range of 2-10 mg/L, corresponding to 5 to 10 mg ThOD/L.

  For studies vi. and vii., your report that the test material concentration was 0.5 mg/L. As the test material concentration was below the minimum test concentration of 2 mg/L specified in OECD TG 301D, the test conditions are considered too favourable which may lead to an overestimation of the biodegradation potential of the test materials used in these studies.

  In your comments on the draft decision, you acknowledge that the test concentrations in these studies were below the minimum concentration specified in the OECD TG 301D.

Reporting of the methodology and results

- the results of measurements at each sampling point in each replicate is reported in a tabular form.

  For studies vi. to viii., you have not reported the results of measurements at each sampling point in each replicate. As you have not provided an adequate reporting of the study results, it is not possible to verify if validity criteria consistent with the specifications of OECD TG 301D were met.

  In your comments on the draft decision, you specified that this information is available and that you intend to provide it through a dossier update.

- the calculation of the ThOD is described and justified. For nitrogen-containing test materials, correction for nitrification is applied on the theoretical oxygen demand (*i.e.* $ThOD_{NO3}$) unless it can be demonstrated that nitrification did not occur (*e.g.* by monitoring changes in concentrations in nitrite and nitrate).

  For studies vi. and vii., the reported ThOD of the test material does not take into account nitrification and you have not provided any justification that nitrification did not occur during the test and that correction for nitrification is not required. For study viii., you report that the calculated theoretical oxygen demand (ThOD) of the test material is 3.3 mg/L. You have not specified how this value was obtained and if it takes into account oxygen consumption through nitrification.

  Therefore, you have not demonstrated that the reported ThOD values provide a reliable basis to determine biodegradation kinetics in these studies.

  In your comments on the draft decision, you specify that all calculated biodegradation percentages are based on the $ThOD_{NH3}$ and will be recalculated to $ThOD_{NO3}$.

Due to these significant deficiencies, none of the source studies (vi) to (viii.) provide an adequate and reliable coverage of the key parameters addressed in OECD TG 301D. Therefore, the reliability of such information in support of your weight of evidence adaptation under Annex XI, Section 1.2 is considered low.

In your comments on the draft decision, you recognise the fact that there are some significant deficiencies that may question the reliability of the information available in the dossier.

4) *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 R.6, Section R.6.2.2.1.f 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 category members. 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 category members cause the same type of effect(s).

While study (i.) has deficiencies which may have led to an overestimation of the biodegradation of the test material, it shows very limited biodegradation after 28 days. Similarly, while study (iv.) also has deficiencies, you state in relation to it that "*demonstration of the ready biodegradability of the water-insoluble dioctadecylamine under the prescribed standard conditions is almost impossible due to the limited bioavailability of this compound*".

The available set of data on the category members does not support similar environmental fate properties. This contradicts your read-across hypothesis whereby the structurally similar category members cause the same type of effect(s). Therefore you have not demonstrated and justified that the properties of the category members are likely to be similar despite the observation of these differences.

In your comments on the draft decision, you state that the similar environmental fate properties of category members will be better substantiated with old/and or new biodegradation data.

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

As explained above, you have not established that relevant properties of the Substance can be predicted from data on the analogue substance. Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. and therefore it cannot be regarded as a reliable justification that all dialkylamine, including the Substance, can be considered readily biodegradable.

Conclusion on the weight-of evidence

On the basis of the information provided above, 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 a ready biodegradability study. Therefore, your adaptation is rejected.

On this basis, the information requirement is not fulfilled.

In your comments on the draft decision, you agreed with the above assessment and you specify that you intend to improve the data for the grouping of the category members. The envisaged grouping approach will not be changed. You also acknowledge the need to provide valid ready biodegradation test data as endpoints to be used for read-across. Therefore, you specify that you intend "*to re-test a longer (C16-C18) and a short (C10-C12) dialkyl chain amine in the OECD 301D test*". You specify that you believe that the omission of ammonium chloride is an acceptable deviation and therefore you plan to use this deviation in new tests.

However, for the reasons already explained above, ECHA notes that you have not provided a valid justification that this deviation from the test specifications of OECD TG 301D is acceptable.

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

1. Long-term toxicity testing on fish

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

You have provided a short-term toxicity study on fish (OECD TG 203) on the Substance but no information on long-term toxicity on aquatic invertebrates for the Substance.

We have assessed this information and identified the following issue:

A. Poorly water soluble substances require longer time to reach steady-state conditions. As a result, the short-term tests do not give a true measure of toxicity for this type of substances and the long-term test is required. A substance is regarded as poorly water soluble if, for instance, it has a water solubility below 1 mg/L or below the detection limit of the analytical method of the test material (ECHA Guidance R.7.8.5).

   As already explained in Appendix A.3., the Substance is poorly water soluble and information on long-term toxicity on fish must be provided.

The examination of the information provided, as well as the selection of the requested test and the test design are addressed under Appendix C.2.

2. Soil simulation testing

and

3. Sediment simulation testing

and

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).

You have not submitted in your dossier any further degradation testing on the Substance. You have provided two OECD TG 303A on Quaternary ammonium compounds, di-C12-18-alkyldimethyl, chlorides (EC No. 269-924-1) and 2,2'-(octadec-9-enylimino)bisethanol (EC No. 246-807-3). You also provided a reference to a publication by van Ginkel *et al.* (2003) which states that a bacterial isolate capable of utilising some quaternary ammonium substances as sole source of carbon and energy. This information is considered irrelevant to cover the information requirement for the Substance as i) no read-across justification is provided and ii) the sources of information listed above do not inform on biodegradation under relevant environmental conditions and therefore cannot be used to conclude whether or not a substance meets the P and/or vP criteria (ECHA Guidance R.11.4.1.1.).

This information requirement is triggered in case the chemical safety assessment (CSA) indicates the need for further degradation investigation (Annex I, Section 4; Annex XIII, Section 2.1), such as if the substance is a potential PBT/vPvB substance (ECHA Guidance R.11.4.). This is the case if the Substance itself or any of its constituent or impurity present

in concentration ≥ 0.1% (w/w) or relevant transformation/degradation product meets the following criteria:

- it is potentially persistent or very persistent (P/vP) as:
  - it is not readily biodegradable (*i.e.* <60/70% degradation in an OECD 301 or 310 study, and
- it is potentially bioaccumulative or very bioaccumulative (B/vB) as:
  - it has a high potential to partition to lipid storage (*e.g.* log K_{ow} > 4.5);
  - for some groups of substances (e.g. organometals, ionisable substances, surfactants) other partitioning mechanisms may drive bioaccumulation (e.g. binding to protein/cell membranes) and high potential for bioaccumulation cannot be excluded solely based on its potential to partition to lipid;
- it meets the T criteria set in Annex XIII: NOEC or EC_{10} < 0.01 mg/L or classification as carc. 1A or 1B, muta. 1A or 1B, repro. 1A, 1B or 2, or STOT RE 1 or 2.

The information in your dossier is currently incomplete and therefore:

- it is not possible to conclude on the persistence potential of the Substance (see Appendix A.4. of this decision), and
- it is not possible to conclude on the bioaccumulation potential of the Substance (see Appendix C.6. of this decision), and
- it is not possible to conclude on the toxicity of the Substance see Appendices A.2. to A.3., C.1. to C.2. and D.1. of this decision).

The information above indicates that the Substance may be a potential PBT/vPvB substance. The Substance has low water solubility (< 20 µg/L), high partition coefficient, and high adsorption coefficient (log $K_{oc,soil}$ up to 30) and it is ionisable, indicating high potential to adsorb to soil and sediment.

Therefore, the chemical safety assessment (CSA) indicates the need for further degradation investigation. Based on the adsorptive properties of the Substance, soil represents a relevant environmental compartment.

The examination of the available information or adaptations, as well as the selection of the requested tests and the tests design are addressed respectively in Appendices C.3. to C.5.

5. Bioaccumulation in aquatic species

Bioaccumulation in aquatic species is required for the purpose of PBT/vPvB assessment (Annex I, Sections 0.6.1 and 4 to REACH).

You have not submitted in your dossier any testing on bioaccumulation in aquatic species.

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

As already explained above under Appendices B.2. to B.4., available information indicates that the Substance may be a potential PBT/vPvB substance.

Therefore, the chemical safety assessment (CSA) indicates the need for further investigation on bioaccumulation in aquatic species.

The examination of the available information or adaptations, as well as the selection of the requested test and the test design are addressed in Appendix C.6.

Appendix C: Reasons to request information required under Annex IX of REACH

1. Long-term toxicity testing on aquatic invertebrates

Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

You have provided the following information:

i. A long-term toxicity on aquatic invertebrates (OECD TG 211) on the Substance ([redacted], 2010)

We have assessed this information and identified the following issues:

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

In Section 1.2. of your technical dossier, you indicate that the Substance may contain [redacted]% w/w of dioctadecylamine, [redacted]% w/w 1-octadecylamine, n-hexadecyl and [redacted]% w/w of dihexadecylamine. The Substance also contains a number of minor primary and secondary amine constituents of shorter C-chain length.
For study i. above, you have identified the test material as "*Amines, di-C16-18 (even numbered) alkyl with CAS 308062-60-4*" (*i.e.* the Substance). You have provided the following information on the composition of the test material: "*C12 part: < 1 area %; C14 part: 2 area %; C16 part: 29 area %; C17 part: 1 area %; C18 part: 67 area %; C20 part: 1 area %*".

The information provided on the test material does not allow verifying if the test material contains representative amounts of the constituents of the Substance (as reported in Section 1.2 of your technical dossier) as you only report the fraction of the test material corresponding to various C-chain lengths and no information on the chemical identity of these constituents. On this basis, you have not demonstrated that the test material is representative for the Substance and the information is rejected.

B. To fulfil the information requirement, a study must comply with the 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 specifications must be met:

Characterisation of exposure

- an adequate and reliable analytical method for the quantification of the test material in the test solutions must be available.
- the test media prepared specifically for analysis of exposure concentrations during the test is treated identically to those used for testing;

[Technical specifications impacting the sensitivity/reliability of the test]

- the test medium fulfils the following condition(s): total organic carbon (TOC) ≤ 2 mg/L;

Additional requirements applicable to difficult to test substances

- for adsorbing test chemical, dissolved total organic carbon concentrations (other than that due to the test chemical) must be maintained in all test solutions at or below 2 mg/L;

Your registration dossier provides a key study showing the following:

Characterisation of exposure

- On the preparation of sample prior the determination of exposure concentration, you report that "*to prevent adsorption of the test item on the glass wall during sample storage all test item concentrations and the control were diluted 1:2 with acetonitrile containing 0.02 mol/L trifluoroacetic acid followed by UPLC-MS/MS analysis*";
- You report that "*for the analyses of the old media separate replicates without algae and test organisms will be prepared and stored under test conditions*";

Additional requirements applicable to difficult to test substances

- the test material is a highly adsorbing test chemical. You report that the test medium was prepared using natural river water filtered at 0.45 µm with a DOC concentration of 3.9 mg/L.

Based on the above, there are critical methodological deficiencies resulting in the rejection of the results of this study. More specifically, you have not demonstrated that exposure was satisfactorily maintained during the experiment as:

- you have not justified that the preparation of samples does not bias the estimation of truly dissolved concentration of the Substance. In the absence of this information, the reliability of the analytical method is uncertain;
- the samples used for analysis of exposure concentrations during the test were not treated identically to those used for testing and you have not demonstrated that the measured values provide a realistic estimate of the exposure to the test material
- the concentration in DOC is higher than the maximum value allowed by OECD GD 23 (i.e. 2 mg/L) and may have reduced exposure to the dissolved fraction of the Substance.
- the concentration of the test material was not satisfactorily maintained throughout the test. Therefore, results cannot be expressed based on measured initial concentrations

  Therefore, this study does not meet the specifications of OECD TG 211 in conjunction with OECD GD 23.

On this basis, the information requirement is not fulfilled.

Study design

OECD TG 211 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.3.

2. Long-term toxicity testing on fish

Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

You have provided the following information:

i. a justification to omit the study which you consider to be based on Annex IX, Section 9.1., Column 2. In support of your adaptation, you provided the following justification: "*The safety assessment according to Annex 1 does not indicate the need to investigate further the effects on aquatic organisms. Therefore no chronic fish testing is considered*

to be required".

We have assessed this information and identified the following issue:

A. Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on long-term toxicity to fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

   Your adaptation is therefore rejected.

On this basis, the information requirement is not fulfilled.

Study design

OECD TG 210 specifies that for difficult to test substances OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design' under Appendix A.3.

3. Soil simulation testing

Soil simulation testing is an information requirement under Annex IX to REACH (Section 9.2.1.3.) for substances with a high potential for adsorption to soil.

The Substance has low water solubility (< 20 µg/L), high partition coefficient, and high adsorption coefficient (log $K_{oc,soil}$ up to 30) and it is ionisable and therefore has high potential for adsorption to soil.

You have provided the following information:

i. an adaptation under Annex IX, Section 9.2.1.3., Column 2 with the following justification: *"The results of the Ready Biodegradability tests indicate that dialkylamines are readily biodegradeable, thus there is no requirement for performing a soil biodegradation test".*

We have assessed this information and identified the following issue:

A. Under Annex IX, Section 9.2.1.3., Column 2, first indent, a study may be omitted if the substance is readily biodegradable.

   For the reasons explained under Appendix A.4., the information requirement on ready biodegradability is not met. Therefore, you have not demonstrated that the Substance is readily biodegradable, and your adaptation is rejected.

On this basis, the information requirement is not fulfilled.

In your comments on the draft decision, you state that *"derivation of compartment-specific degradation half-lives for sediment and water from OECD 308 data alone is highly uncertain and not recommended. DT50,w and DT50,sed values are confounded by phase transfer processes and should not be used for comparison to persistence cut-off values or for exposure modelling"*. Therefore, you intend to conduct a soil simulation study only after ECHA will have evaluated the results of the sediment simulation study requested under Appendix C.4 and if it is concluded that the Substance is not persistent in the sediment compartment.

ECHA emphasizes that the requested soil simulation study is a standard information requirement under Section 9.2.1.3. of Annex IX to REACH. Therefore, under Article 41 to REACH, ECHA is evaluating whether your dossier complies with the requirements set out under Article 40(1). In this context, if you decide this information is not needed, you may submit an adaptation based on the specific adaptation rules specified of the second column of Section .2.1.3. of Annex IX or the general adaptation of Annex XI to REACH. ECHA will evaluate your adaptation once the deadline specified in this decision to submit the requested information has passed.

Study design

Simulation degradation studies must include two types of investigations (ECHA Guidance R.7.9.4.1):

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

In accordance with the specifications of OECD TG 307, you must perform the test using at least four soils representing a range of relevant soils (*i.e.* varying in their organic content, pH, clay content and microbial biomass).

The required test temperature is 12°C, which corresponds to the average environmental temperature for the EU (ECHA Guidance R.16, Table R.16-8) and is in line with the applicable test conditions of the OECD TG 307.

In accordance with the specifications of OECD TG 307, non-extractable residues (NER) must be quantified. The reporting of results must include a scientific justification of the used extraction procedures and solvents (ECHA Guidance R.7.9.4.1.). 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.11.4.1.1.3.). Further recommendations may be found in the background note on options to address non-extractable residues in regulatory persistence assessment available on the ECHA website.

Relevant transformation/degradation products are at least those detected at ≥ 10% of the applied dose at any sampling time or those that are continuously increasing during the study even if their concentrations do not exceed 10% of the applied dose, as this may indicate persistence (OECD TG 307; ECHA Guidance R.11.4.1.).

4. Sediment simulation testing

Sediment simulation testing is an information requirement under Annex IX to REACH (Section 9.2.1.4.) for substances with a high potential for adsorption to sediment.

As described in section C.3 above, the Substance has high potential for adsorption to sediment.

You have provided the following information:

i. an adaptation under Annex IX, Section 9.2.1.4., Column 2 with the following justification: "*the study does not need to be conducted because the substance is readily biodegradable*".

We have assessed this information and identified the following issue:

A. Under Annex IX, Section 9.2.1.4., Column 2, first indent, a study may be omitted if the substance is readily biodegradable.

   For the reasons explained under Appendix A.4., the information requirement on ready biodegradability is not met. Therefore, you have not demonstrated that the Substance is readily biodegradable, and your adaptation is rejected.

On this basis, the information requirement is not fulfilled.

Study design

Simulation degradation studies must include two types of investigations (ECHA Guidance R.7.9.4.1.):

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

In accordance with the specifications of OECD TG 308, you must perform the test using two sediments. One sediment should have a high organic carbon content (2.5-7.5%) and a fine texture, the other sediment should have a low organic carbon content (0.5-2.5%) and a coarse texture. If the Substance may also reach marine waters, at least one of the water-sediment systems should be of marine origin.

The required test temperature is 12°C, which corresponds to the average environmental temperature for the EU (ECHA Guidance R.16, Table R.16-8) and is in line with the applicable test conditions of the OECD TG 308.

In accordance with the specifications of OECD TG 308, non-extractable residues (NER) must be quantified. The reporting of results must include a scientific justification of the used extraction procedures and solvents (ECHA Guidance R.7.9.4.1.). 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.11.4.1.1.3.). Further recommendations may be found in the background note on options to address non-extractable residues in regulatory persistence assessment available on the ECHA website.

Relevant transformation/degradation products are at least those detected at ≥ 10% of the applied dose at any sampling time or those that are continuously increasing during the study even if their concentrations do not exceed 10% of the applied dose, as this may indicate persistence (OECD TG 308; ECHA Guidance R.11.4.1.).

In your comments on the draft decision, you explain that in order to quantify NER and mass balance, a ^{14}C labeled test material will be needed. You state that from past experience you found that the radiostability of dialkylamines was shown to be very poor unless a hydrochloride salt of the amine is used. You consider that, as the biodegradation rate of a substance is determined by its structure and bioavailability and taking into account the composition of the Substance, dioctadecylamine can be considered a reasonable worst case for the Substance. You justify the selection of dioctadecylamine with the following arguments:

- along with dihexadecylamine, dioctadecylamine is the constituent of the substance showing the highest Kdsoil (28500 L/kg and 21000 L/kg for dihexadecylamine and dioctadecylamine, respectively). Based on higher sorption, the dissolved fraction of dioctadecylamine is expected to have among the lowest bioavailability and consequently among the lowest degradation rate for the constituents of the Substance;
- the undissolved fraction of the Substance (i.e. unprotonated) will sorb based on hydrophobic interaction and therefore dioctadecylamine will be a reasonable worst case for the Substance.

ECHA understands that you intend to conduct this study with an analogue substance (i.e. the hydrochloride salt of dioctadecylamine). Based on the provisions of Annex XI, Section 1.5., you will need to document and justify the approach in your registration dossier. In particular, you will need to justify that the hydrochloride salt and the free base of dioctadecylamine have similar sorption behaviour. ECHA acknowledges that the proposed approach has merit and that dioctadecylamine may be considered a reasonable worst-case for the Substance. ECHA also acknowledges the complexity of conducting a study according to OECD TG 308 using a UVCB as test material.

5. Identification of degradation products

Identification of degradation products is an information requirement under Annex IX to REACH (Section 9.2.3.).

As explained under section B.4. you have not provided any relevant information on the identity of transformation/degradation products for the Substance.

Therefore, this information requirement is not met.

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

On this basis, the information requirement is not fulfilled.

Study design

Regarding the selection of appropriate and suitable test method(s), the method(s) will have to be substance-specific. 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, log K_{ow} and potential toxicity of the transformation/degradation may need to be investigated. You may obtain this information from the degradation studies requested in Appendices C.3. and C.4. or by some other measure. If any other method is used for the identification of the transformation/degradation products, you must provide a scientifically valid justification for the chosen method.

To determine the degradation rate of the Substance, the requested studies according to OECD TG 308 and 307 (Appendices C.3. and C.4.) must be conducted at 12°C and at test material application rates reflecting realistic assumptions. However, to overcome potential analytical limitations with the identification and quantification of major transformation/degradation products, you may consider running a parallel test at higher temperature (but within the frame provided by the test guideline) and at higher application rate (*e.g.* 10 times).

6. Bioaccumulation in aquatic species

Bioaccumulation in aquatic species is a standard information requirement under Annex IX to REACH (Section 9.3.2.).

You have provided the following information:

i. an adaptation under Annex XI, Section 2 ('Testing is technically not feasible') with the following statement: "*Standard OECD 305 tests are technically not feasible with these strongly sorbing hydrolytically unstable substances. In addition is the route of exposure in an standard OECD 305 test unrealistic for these substances because the substance will either be sorbed or (bio)degraded*";
ii. an adaptation under Annex IX, Section 9.3.2., Column 2 with the following justification: the Substance has low potential for bioaccumulation.

We have assessed this information and identified the following issues:

A. Under Annex XI, Section 2, a study may be omitted if it is technically not possible to conduct the study as a consequence of the properties of the substance. The guidance given in the test methods referred to in Article 13(3), in this case OECD TG 305, more specifically on the technical limitations of a specific method, must always be respected.

OECD TG 305 acknowledges that, for strongly hydrophobic substances (log Kow > 5 and a solubility below ~ 0.01-0.1 mg/L), testing via aqueous exposure may become increasingly difficult. The technical guideline specifies that a test via aqueous exposure must be conducted unless it can be demonstrated that a stable and fully dissolved concentration of the test substance in water cannot be maintained within ± 20% of the mean measured value or the highest achievable concentration is less than an order of magnitude above the limit of quantification (LoQ) of a sensitive analytical method. If it can be demonstrated that these conditions cannot be met, the technical guideline gives the option to conduct a test using a dietary approach.

In your justification, you consider that the study is technically not possible as the Substance is "*strongly sorbing hydrolytically unstable substances*". Under Section 5.2.1. of your technical dossier, you have adapted the information requirement for hydrolysis. As part of your justification you state that "*Dialkylamines do not contain hydrolysable covalent bonds*".

Firstly, we agree with the statement provided under Section 5.2.1. of your technical dossier that the Substance is unlikely to be subject to fast hydrolysis. Therefore, your claim that hydrolytic instability would make a study according to OECD TG 305 not technically feasible is not considered plausible.
Additionally, you have not provided any justification as to why neither the aqueous approach or the dietary approach described in OECD TG 305 are not technically feasible due to the low solubility of the Substance.
Therefore, you have not provided adequate justification as to why it is technically not possible to conduct a study according to OECD TG 305 with the Substance and your adaptation is rejected.

B. Under Section 9.3.2., Column 2, first indent of Annex IX to REACH, the study may be omitted if the Substance has a low potential for bioaccumulation (for instance a log Kow ≤ 3) or is unlikely to cross biological membranes. ECHA Guidance R.7.8.5. explains that there is no scientific basis to define molecular characteristics that would render a substance unlikely to cross biological membranes. In this context, the indicators used for low likelihood of a high bioaccumulation potential (ECHA Guidance R.11, Figure R.11-4) must be considered, including:
   - physico-chemical indicators of hindered uptake due to large molecular size (*e.g.*

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

Your registration dossier provides:
- under Section 4.7. of your technical dossier, a QSAR prediction based on KOWWIN v1.68 (EPI Suite v4.11) for the one of the constituent of the Substance (*i.e.* di-C18). The log Kow of this constituent was predicted to be 16.52;

Furthermore, in your justification:
- you claim that based on this predicted log Kow value, a calculated BCF of 3.162 L/kg was obtained. You have not provided an endpoint study record to report this prediction nor have you specified how this value was obtained;
- you claim that the substance is readily biodegradable and therefore has a low potential for bioaccumulation.

Based on the information you provided in support of your adaptation we note the following:
- the predicted log Kow value you have reported relates to the neutral form of the di-C18 constituent of the Substance. The Substance is ionised under relevant environmental pH and therefore this value does not provide a reliable estimate of the potential of this constituent to partition to lipids under environmentally relevant pH. Furthermore, you have not provided any information on the log Kow of the other constituents of the Substance. Therefore, you have not demonstrated that all the constituents of the Substance show reliable log Kow values that are consistently above 10;
- the substance is ionisable and therefore other partitioning mechanisms may drive bioaccumulation (e.g. binding to protein/cell membranes). For this reason log Kow is not considered a valid descriptor of the bioaccumulation potential for such substances (ECHA Guidance R.7c, Appendix R.7.10-3);
- you have not provided any other information on physico-chemical parameters that would be indicative of hindered uptake;
- existing information on the Substance from 28-day and 90-day studies via oral route in rats shows evidence of granulomatous inflammation in the mesenteric lymph nodes in males and females. Severity and incidence of granulomatous inflammation increases with dose and no reversibility can be seen at the high dose level in the 42 days recovery group. In addition, dose-dependent decrease of lymphocytes is observed in female rats. The effects observed with the Substance therefore contradict your hypothesis that the Substance may have a low potential to cross biological membranes;
- a non-documented calculated BCF for a constituent of the Substance or a claim that the Substance is readily biodegradable are not relevant pieces of information to support an adaptation under Section 9.3.2., Column 2, first indent of Annex IX to REACH.

Based on the above, you have not provided a valid justification that the Substance, including all its constituents, have a low potential for bioaccumulation or is unlikely to cross biological membranes. Therefore your adaptation is rejected.

On this basis, the information requirement is not fulfilled.

In your comments on the draft decision, you explain that you will perform the study only in case the substance under consideration is concluded to be persistent in either the sediment

or soil simulation testing.

Study design

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

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

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

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

Appendix D: Reasons to request information required under Annex X of REACH

1. Extended one-generation reproductive toxicity study

The basic test design of an Extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is an information requirement under Annex X to REACH (Section 8.7.3.). Furthermore, Column 2 of Section 8.7.3. defines when the study design needs to be expanded.

You have adapted this information requirement under Annex XI, Section 3 (Substance-tailored exposure-driven testing). You provided the following justification for the adaptation: *"Based on the low exposure indicated from the modelled exposure assessment on Amines, di-C16-18 (even-numbered) alkyl (CAS No. 308062-60-4), the possibility to perform substance-tailored exposure-driven testing, as described in Annex XI Section 3 and the outcome from the 90 day study (OECD 408), it is not considered scientifically justifiable to perform an extended one-generation reproductive toxicity study (OECD 443), which is the recommended test on this endpoint. Waiving of this study will ensure that any unnecessary animal testing is avoided."*

We have assessed this information and identified the following issue:

A. Under Annex XI, Section 3, this information may be omitted based on the exposure scenario(s) developed in the Chemical Safety Report. The justification must be based on a rigorous exposure assessment in accordance with Annex I, Section 5 and, for an adaptation under Annex XI, Section 3.2(a) or (b), it must meet, among others, the following criteria::
   - the second criterion 3.2 (a)(ii) requires that the manufacturer or importer demonstrates and documents that a suitable DNEL or a PNEC can be derived from results of available test data for the Substance taking full account of the increased uncertainty resulting from the omission of the information requirement, and that DNEL or PNEC is relevant and appropriate both to the information requirement to be omitted and for risk assessment purposes.
   - 3.2 (b) where the substance is not incorporated in an article the manufacturer or the importer must demonstrate and document for all relevant scenarios that throughout the life cycle strictly controlled conditions as set out in Art 18(4)(a) to (f) apply.

   The worker long-term systemic DNEL, which you have derived, is based on a sub-chronic 90-days study (OECD TG 408) with the Substance.

   Information from an OECD TG 408 study is not relevant nor appropriate to derive a DNEL for toxicity to reproduction as such study does not investigate effects on mating, fertility, pregnancy, lactation and postnatal developments of the fully exposed F1 generation up to the adulthood as an extended one-generation reproductive toxicity study (OECD TG 443).

   In addition, the RCRs do not demonstrate in all relevant exposure scenarios for the combined routes, systemic long-term, strictly controlled conditions as per Annex XI, section 3.2(b). In particular, the condition set out in 3.2 (b) as set out in Article 18(4) does not appear to be fulfilled because it has not been demonstrated that the substance is rigorously contained by technical means during its whole lifecycle.

   Therefore, your adaptation does not meet the conditions specified in Annex XI, Section 3.2 (a)(ii). and (b)) and it is rejected.

On this basis, the information requirement is not fulfilled.

Study design:

Premating exposure duration and dose-level setting

The length of premating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required to obtain results adequate for classification and labelling and /or risk assessment. There is no substance specific information in the dossier supporting shorter premating exposure duration.[1]

Therefore, the requested premating exposure duration is ten weeks.

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no relevant data to be used for dose level setting, it is recommended that range-finding results are reported with the main study.

You have to provide a justification with your study results that demonstrates that the dose level selection meets the conditions described above.

Cohorts 1A and 1B

Cohorts 1A and 1B belong to the basic study design and must be included.

Species and route selection

The study must be performed in rats with oral administration (ECHA Guidance R.7.6.2.3.2.).

Further expansion of the study design

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and/or Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during the conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Annex X, Section 8.7.3., Column 2. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance R.7.6.

Appendix E: 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[2].

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 the careful identification and description of the characteristics of the Tests Materials in accordance with OECD GLP (ENV/MC/CHEM(98)16) and EU Test Methods Regulation (EU) 440/2008 (Note, Annex), namely all the constituents must be identified as far as possible as well as their concentration. Also any constituents that have harmonised classification and labelling according to the CLP Regulation must be identified and quantified using the appropriate analytical methods.

With that detailed information, ECHA can confirm 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[3].

[2] https://echa.europa.eu/practical-guides
[3] https://echa.europa.eu/manuals

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

A. Strategy for the PBT/vPvB assessment

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

You are advised to consult 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. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in ECHA Guidance R.11 (Section R.11.4.2.2), you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the "known constituents approach" (by assessing specific constituents), or
- the "fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the "whole substance approach", or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

Appendix G: 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 24 March 2020.

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

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

> The timeline indicated in the draft decision to provide the information requested is 33 months from the date of adoption of the decision. In your comments to the draft decision, you requested an extension of the timeline to 39 months. You justified your request with following statement "*considering the high workload at the different CRO's, taking into account sequential performance of the tests listed above as described in the text in bold and the fast decay of the radiopurity observed for alkylamines plus the interpretation of the result of the OECD 308 by ECHA (assuming that ECHA requires about 3 months for the evaluation), the initially generous appearing 33 months from the date of decision as deadline is expected to be too tight to finalize the BCF study and update the dossier accordingly in time. It is therefore proposed to increase this deadline to 39 months*".
>
> ECHA acknowledges that you agreed to conduct the requested environmental fate studies using a radiolabeled test material and note the technical issues related to radiostability of ^{14}C alkylamines. In addition, you provided documentary evidence from the CRO to support your request to extend the deadline. On this basis, we granted the deadline extension and updated the deadline to submit the requested information to 39 months.

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 H: List of references - ECHA Guidance[4] 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)[5]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[5]

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[6]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

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

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

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

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

Appendix I: 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.