~~Confidential~~

Helsinki, 15 March 2023

Addressees
Registrants of DFAS_C16-18 C18 unsat_JS as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
10/12/2013

Registered substance subject to this decision ("the Substance")
Substance name: Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, dioleates
EC/List number: 800-362-7

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 ***5 January 2026***.

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

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

1. Skin sensitisation (Annex VII, Section 8.3.) :
   i. in vitro/in chemico skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD TG 442D) and activation of dendritic cells (EU B.71/OECD TG 442E)(Annex VII, Section 8.3.1.); and
   ii. Only if the in vitro/in chemico test methods specified under point i.) are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment, in vivo skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429);

2. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: OECD TG 471, 2020)

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

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

5. Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: EU C.4. C/D/E/F/OECD TG 301B/C/D/F or EU C.29./OECD TG 310) on relevant constituent(s)/fraction(s) of the Substance, as described under the corresponding appendix on reasons for the request.

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

6. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.; test method: OECD TG 473) or In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487)

7. If negative results are obtained in tests performed for the information requirement of Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. then: In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490)

8. Justification for an adaptation of a Short-term repeated dose toxicity (28 days) based on the results of the Sub-chronic toxicity study (90 days) requested below (Annex VIII, Section 8.6.1.)

9. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) by oral route, in rats

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

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

11. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats

12. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit)

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

14. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210)

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

Information required depends on your tonnage band

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

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

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

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested

by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report**, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

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

Failure to comply

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

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

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

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

Appendix 1: Reasons for the decision

Contents

0. Reasons common to several requests

0.1. Assessment of the read-across approach

1 You have adapted the following standard information requirements by using grouping and read-across approach under Annex XI, Section 1.5:

- Skin sensitisation study (Annex VII, Section 8.3.)
- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)
- Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2)

2 ECHA has considered the scientific and regulatory validity of your read-across approach(es) in general before assessing the specific standard information requirements in the following sections.

3 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

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

0.1.1. Predictions for (eco)toxicological properties

5 You provide no read-across justification document in either your CSR, IUCLID Section 13, the IUCLID section corresponding to the information requirements listed above.

6 You predict the properties of the Substance from information obtained from the following source substances:

- octadec-9-enoic acid - N-octadec-9-en-1-ylpropane-1,3-diamine (2:1), EC No. 251-846-4.
- Oleyl-diamine dioleate, EC No. 254-754-2
- N-Oleyl-1,3-diaminopropane, EC No. 230-528-9
- N-C12,14 alkyl-1,3-diaminopropane, EC No. 292-562-0

7 You provide no reasoning for the prediction of (eco)toxicological properties.

8 ECHA assumes that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source substance.

9 We have identified the following issues with the predictions of (eco)toxicological properties:

0.1.1.1. Absence of read-across documentation

10 Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must include an explanation why the properties of the Substance may be predicted from information on the source substance(s).

11 You have provided robust study summary(ies) for study(ies) conducted with other substances than the Substance in order to comply with the REACH information requirements. However, you have not provided documentation as to why this information is relevant for the Substance and thus why the properties of the Substance may be predicted from information on the source substance(s).

12 In the absence of such documentation, the properties of the Substance cannot be reliably predicted from the data on the source substances.

13 In your comments on the draft decision, you acknowledge that "*the complete lack of appropriate read-across documentation* [prevents] *to make apt and informed decisions on structural similarity and biological relevance of the proposed substances which endpoints are utilized*".

14 You propose to "*remedy this short-coming in read-across documentation by constructing and providing appropriate read-across documentation for category members*". ECHA understands that you intend to revise and improve an earlier read-across justification document to address the above issue. In the absence, yet, of such updated documentation, ECHA is not in a position to assess it and therefore take it into account for this decision.

0.1.1.2. Missing supporting information

15 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 supporting information to scientifically justify the read-across explanation for prediction of properties. The set of supporting information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6, Section R.6.2.2.1.f.).

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

17 As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substances cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm that both substances cause the same type of effects. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

18 For the source substances, you provide the studies used in the prediction in your registration dossier. You do not provide any bridging studies of comparable design and duration for the Substance. You registration dossier does not contain any bridging information for long-term toxicity to aquatic invertebrates. Therefore, it is not possible to compare the properties of the Substance and the source substances in order to confirm that both substances cause the same type of effects.

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

20 In your comments to the draft decision, you "*agree that there is at this moment still insufficient reliable bridging data in the DFAS category* […] *for the three requested aquatic ecotoxicity tests. The three requested aquatic ecotoxicity studies will therefore be performed according to the mentioned guidelines as far as possible in agreement with OECD Guidance document 23. A read across document according to the RAAF document (ECHA 2017) will be generated for the DFAS category with detailed compositional information after the requested studies have been performed, where endpoints which may not be covered by actual testing can be addressed*".

21 ECHA understands from your comments that you will no longer rely on a read-across adaptation to meet the information requirement for Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2), and that you may submit adaptations for other information that is not compliant with the REACH requirements. In any case, this does not change the fact that you have not provided supporting information justifying the available read-across adaptation, as explained above.

0.1.1.3. Characterisation of the source substance(s) used for predictions of toxicological properties

22 Under Annex XI Section 1.5, Structural similarity for UVCB substances (Unknown or Variable composition, Complex reaction products or of Biological materials) must be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents. Qualitative compositional as well as quantitative characterisation of the individual constituents of these substances must be provided, to the extent that this is measurable (Guidance on IRs and CSA, Section R.6.2.5.5.).

23 You do not describe the compositional information for the Substance and the source substances (EC No. 254-754-2 and EC No. 292-562-0). The Substance and the source substances are UVCB composed of alkyl-diamines of various carbon chain lengths. No information on the variability in concentration of constituents is provided. Therefore, you do not provide information on the characterisation of these two source substance in order to enable a comparaison in the similarities and differences in concentration of specific consitutents between the Substance and these two source substances.

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

0.1.1.4. Adequacy and reliability of source studies

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

1) be adequate for the purpose of classification and labelling and/or risk assessment;
2) have adequate and reliable coverage of the key parameters addressed in the corresponding study that shall normally be performed for a particular information requirement;
3) cover an exposure duration comparable to or longer than the corresponding study that shall normally be performed for a particular information requirement if exposure duration is a relevant parameter.

26 Specific reasons why the studies on the source substances do not meet these criteria are explained further below under the applicable information requirement in sections 2 and 13. Therefore, no reliable predictions can be made for these information requirements.

0.1.2. Conclusion on the read-across approach

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

Reasons related to the information under Annex VII of REACH

1. Skin sensitisation

28 Skin sensitisation is an information requirement under Annex VII, Section 8.3. Under Section 8.3., Column 1, the registrants must submit information allowing (1) a conclusion whether the substance is a skin sensitiser and (2) whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

1.1. Information provided

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

(i) a skin sensitization test in Guinea pigs (2004) with the analogue substance Oleyl diamine, dioleate, EC number 254-754-2.

1.2. Assessment of the information provided

1.2.1. Assessment whether the Substance causes skin sensitisation

1.2.1.1. Read-across adaptation rejected

30 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

32 In your comments on the draft decision, you propose *"to update the read-across documentation to further justify the use of this key study on the test substance Oleyl diamine, dioleate (EC no. 254-754-2) as an acceptable read-across for the skin sensitization endpoint for Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, dioleates (EC no. 800-362-7)"*.

33 ECHA acknowledges your intentions to improve your read-across adaptation. This strategy relies on information that is yet to be submitted. Therefore, ECHA cannot currently assess the validity of the proposed strategy. You remain responsible for complying with this decision by the set deadline.

1.3. Specification of the study design

34 To fulfil the information requirement for the Substance, information on molecular interaction with skin proteins and inflammatory response in keratinocytes and activation of dendritic cells (OECD TG 442C and OECD TG 442D and EU B.71/OECD TG 442E) must be provided. Furthermore an appropriate risk assessment is required if a classification of the Substance as a skin sensitiser (Cat 1A or 1B) is warranted.

35 In case no conclusion on the skin sensitisation potency can be made for the Substance based on the newly generated in vitro and in chemico data, in vivo skin sensitisation study must be performed and the murine local lymph node assay (EU Method B.42/OECD TG 429) is considered as the appropriate study for the potency estimation.

2. In vitro gene mutation study in bacteria

36 In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, OECD TG 471 (2020).

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

2.1. Information provided

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

(i) an *in vitro* gene mutation study in bacteria (2003) with the analogue substance Oleyl diamine, dioleate, EC number 254-754-2;
(ii) an *in vitro* gene mutation study in bacteria (1984) with the analogue substance Oleyl diamine, dioleate, EC number 254-754-2.

2.2. Assessment of the information provided

2.2.1. Read-across adaptation rejected

39 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected. In addition, ECHA identified endpoint-specific issue addressed below.

2.2.2. Adequacy and reliability of studies on the source substances

40 Under Annex XI, Section 1.5., the results to be read across must have an adequate and reliable coverage of the key parameters addressed in the test guideline for the corresponding study that shall normally be performed for a particular information requirement, in this case OECD TG 471. Therefore, the following specifications must be met:

a) the test is 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 positive control substance produces a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control;
c) the mean number of revertant colonies per plate is reported for the treated doses and the controls.

41 The study (i) is described as an in vitro gene mutation study on bacteria.

42 However, the following is not according to the requirements of the OECD TG 471:

b) the positive control substance is not indicating that it produced a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control.

In your comments on the draft decision, you state that "*statistical analysis is not a strict requirement of OECD 471(2020)*".

However, ECHA notes that the OECD introduction to the genotoxicity test guidelines lists the relevant criteria for identification of clear positive findings, which includes (among others) that statistically significant results must be outside the distribution of the historical negative control data (e.g. 95% confidence interval). By analogy, the results of a positive control must be outside the distribution of the historical negative control data.

In any case, you propose to submit the information on concurrent negative (solvent/vehicle) and positive control data, with ranges, means and standard deviations. You propose to conduct additional statistical analysis of the data if required by ECHA. ECHA welcomes your intention to update your registration dossier with the information listed above. However, as you have not provided this information in your comments, ECHA is not in a position to assess and take into account for this decision whether the positive control provided an adequate response. In addition, as the information is currently not available in your registration dossier, the reporting issue remains. You should submit this information in an updated registration dossier by the deadline set in the decision.

c) the mean number of revertant colonies per plate for the treated doses and the controls was not reported.

In your comments on the draft decision, you propose to submit this information in an update of the registration dossier. However, as you have not provided this information in your comments, ECHA is not in a position to assess its adequacy. In addition, as the information is currently not available in your registration dossier, the reporting issue remains. You should submit this information in an updated registration dossier by the deadline set in the decision.

43 The study (ii) is described as an in vitro gene mutation study on bacteria.

44 However, the following specifications are not according to the requirements of the OECD TG 471:

a) the test was performed with the strains S. typhimurium TA 1535, TA 1537, TA1538, TA 98 and TA 100 (i.e., the strain *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101) is missing).

In your comments on the draft decision, you acknowledge that information on the fifth strain is missing.

b) the positive control substance is not indicating that it produced a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control.

In your comments on the draft decision, you provided the same comments as detailed under paragraph 42, point b). ECHA's reply equally applies to study (ii).

c) the mean number of revertant colonies per plate for the treated doses and the controls was not reported.

In the comments on the draft decision, you provided the same comments as detailed under paragraph 42, point c). ECHA's reply equally applies to study (ii).

45 The information provided does not cover the key parameter(s) required by the OECD TG 471.

2.3. Other information provided in your comments on the draft decision

46 In your comments on the draft decision, you state that the that study (ii) "*has been designated as a supporting study for this reason and is used as a weight of evidence approach with other more complete data set of study I which does include S. typhimurium*

TA 102". On this basis we understand that you intended to invoke a weight-of evidence adaptation under section 1.2 of Annex XI of REACH.

2.4. Assessment of the other information provided in your comments on the draft decision

2.4.1. Weight of evidence adaptation requires several sources of information

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

48 However, you only rely on information from an analogue substance and the read-across is rejected for the reasons specified under Section 0.1. Therefore, you have not provided adequate information to support your conclusion on the information requirement.

2.4.2. Lack of documentation justifying the weight of evidence adaptation

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

50 However, you have not included a justification for your weight of evidence adaptation, which would include an adequate and reliable documentation as to why the sources of information provide sufficient weight to conclude on the information requirements under consideration.

51 Therefore, while you claim you intend to use the information currently in your registration dossier as a weight of evidence, the requirement of Annex XI, Section 1.2 are currently not met.

52 Therefore, the information requirement is not fulfilled.

2.5. Specification of the study design

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

3. Long-term toxicity testing on aquatic invertebrates

54 Short-term toxicity testing on aquatic invertebrates is an information requirement under Column 1 of Annex VII to REACH (Section 9.1.1.). However, long-term toxicity testing on aquatic invertebrates must be considered (Section 9.1.1., Column 2) if the substance is poorly water soluble.

3.1. Triggering of the information requirement

55 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 (Guidance on IRs and CSA, Section R.7.8.5).

56 You have provided an OECD TG 123 on the analogue substance octadec-9-enoic acid - N-octadec-9-en-1-ylpropane-1,3-diamine (1:1) (CAS RN 40027-38-1 / EC 254-754-2) (2011). On this basis, you consider that the saturation concentration of the Substance in water is c.a. 0.005 mg/L.

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

3.2. Information provided

58 You have provided an OECD TG 211 study (2011) on the analogue substance octadec-9-enoic acid - N-octadec-9-en-1-ylpropane-1,3-diamine (2:1) (CAS RN 34140-91-5 / EC 251-846-4).

3.3. Assessment of the information provided

59 The examination of the information provided, as well as the selection of the requested test and the test design are addressed under Request 13.

4. Growth inhibition study aquatic plants

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

4.1. Information provided

61 You have provided an OECD TG 201 study on the Substance (2012)

4.2. Assessment of the information provided

4.2.1. The provided study does not meet the information requirement

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

63 Technical specifications impacting the sensitivity/reliability of the test

a) one of the two alternative growth media (*i.e.* the OECD or the AAP medium) is used. Any deviations from recommended test media must be described and justified;

64 Additional requirements applicable to difficult to test substances

b) 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;
c) where loses due to e.g. adsorption potentials are anticipated samples for analysis are to be taken at the beginning of the test, and 24-hour intervals throughout the test in order to obtain the mean measured concentrations;

65 Reporting of the methodology and results

d) the method for determination of biomass and evidence of correlation between the measured parameter and dry weight are reported. Algal biomass is normally 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;

e) when a decrease in concentration of the test substance in the course of the test is accompanied by a decrease in growth inhibition, the use of a suitable model describing the decline of the concentration of the test substance needs to be considered.

66 Your registration dossier provides an OECD TG 201 study showing the following:

67 Technical specifications impacting the sensitivity/reliability of the test

a) you specify that water from the river Innerste was used and that "[a]*dditionally 50 % of the components concentrations of the dilution water (total application volume 6.5 mL/L) acc. to the guideline was added to enable a sufficient growth of algae*". You justify this deviation from the test guideline requirements by referring to the bulk approach (ECETOC Technical Report no. [redacted], 2003) and you consider that "*the results of these bulk approach tests are therefore much easier and more realistic, and if compared to PECbulk clearly provide a more appropriate assessment of risks for the environment*".

68 Additional requirements applicable to difficult to test substances

b) you report that the TOC content of the water taken from the Innerste river on 20 March 2012 was 3.66 mg/L.

c) you report that analytical exposure of exposure concentration was conducted at in new medium (0h) and old medium (72h) only. The percentage recovery ranged from 92-110% at t=0h and dropped to 8-19% at t=72h.

69 Reporting of the methodology and results

d) the method for determination of biomass was in vivo chlorophyll a fluorescence. You have provided no information to demonstrate a satisfactory correlation with biomass over the range of biomass occurring in the test;

e) as explained under c) above a sharp decrease in exposure concentrations was observed by the end of the test. In parallel, for nominal test concentrations ranging from 0.178 to 0.562 mg/L, after a marked growth rate reduction observed at 0 to 24 hours, a decreased in growth inhibition was observed after 24 hours. At a nominal concentration of 0.562 mg/L, growth rates were determined to be 0.35, 0.56 and 1.07 at t=0-24h, t=24-48h and t=48-72h, respectively. At nominal concentrations of 0.178 and 0.316 mg/L, after a marked reduction in growth rate observe din the first 24 hours, growth rates were similar to those in the control. Despite these observations, you have not reported the use of a suitable model describing the decline of the concentration of the test substance over the exposure period.

70 Based on the above, there are critical methodological deficiencies resulting in the rejection of the study results. More specifically,

- the study was conducted with river water with an organic carbon content above the maximum acceptable value.
- you justify the use of natural water by referring to the bulk approach. However, information on intrinsic properties should be generated independently from exposure considerations (e.g., decision of the Board of Appeal of 11 December 2018 in case A-006-2017, para. 133-135). The bulk approach which aims at mimicking exposure under "*more environmentally realistic*" conditions must not be used for classification and labelling or PBT assessment.

- you have not provided any supporting information to demonstrate that in vivo fluorescence provides an adequate determination of algal biomass, therefore 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. Further, river water does contain natural algal populations and you have not justified that it did not affect the sensitivity of the test.

  In your comments to the draft decision, you provide the parameters of the calibration curve used to convert fluorescence into cell numbers. However, you fail to provide information on how this calibration curve was obtained and whether the calibration curve holds for both control and treated algal cells. Therefore, its adequacy cannot be assessed. Further, you state that "[t]*he natural river water is stored frozen as this was found to be suitable to minimize the content of vital natural algae cells of the water as well as to reduce microbial (bacterial) activity"*. You state that the calibration curve was obtained using the dilution water to determine the background signal. However, you provide no information on how viable algal cells from the dilution water may have biased the determination of algal growth over the course of the experiment.

- the sampling frequency for the determination of exposure concentration was too low to adequately characterise losses of the test substance from the exposure medium. Further, you have not used an adequate model to derive effects values.

71 Therefore, the requirements of OECD TG 201 are not met.

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

73 In your comments to the draft decision, you agree that "*the bulk approach test results are less suitable for quantifying the intrinsic toxicity of cationic surfactants*" and "*for Classification and Labelling as they use non-standard test medium*". Therefore, you agree to conduct the requested study.

74 However, you note that "*the WAF approach does not resolve the analytical problems which means that the quantification of the truly dissolved fraction of the test substance remains difficult in a system where algae cells are present.* […] *The analytical results are due to low analytical recoveries considered to be of poor reliability and therefore less suitable for deriving the real intrinsic toxicity. It is therefore questioned if C&L based on mean-measured concentrations for UVCB's at this low concentration level are more reliable than the currently used classification based on the Bulk-approach test results applying an additional safety factor of 10 to compensate for the potential reduction of the bioavailability*".

75 ECHA acknowledges the technical challenges in conducting adequate analytical monitoring of exposure for cationic surfactants such as the Substance. However, your justification relies solely on the fact that by using the bulk approach, "*the two main weaknesses in the calculation of the environmental risk to aquatic organisms which are the quantification of the exposure concentrations during testing and the calculation of the dissolved concentration for the PECwater are elegantly eliminated from the RCR equation*". It does not address to what extent the presence of high(er) TOC/DOC mitigates the intrinsic toxicity of the Substance. ECHA further notes that the "*additional safety factor of 10*" does not rely on any scientific justification and therefore the validity of such approach is not demonstrated.

4.3. Study design and test specifications

76 The Substance is difficult to test due to the low water solubility (c.a. 0.005 mg/L by analogy with octadec-9-enoic acid - N-octadec-9-en-1-ylpropane-1,3-diamine (1:1) (CAS RN 40027-38-1 / EC 254-754-2)). 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.

77 In your comments to the draft decision, you agree to perform the new study "*in agreement with OECD GD 23*". You raise technical difficulties in relation to the analytical monitoring of exposure concentration. In particular, you state that "[t]*o be able to quantify the truly dissolved concentration algae* […] *need to be removed from the sample.* […] *Due to the extreme low water solubility and/or relatively high toxicity of the test substance very low test concentrations are used. The amount of test substance remaining in the aqueous phase is due to this too low to allow pre-saturation of filter or centrifuge tube*".

78 As already stated above, ECHA acknowledges the technical challenges in conducting adequate analytical monitoring of exposure for cationic surfactants such as the Substance. You are advised to document the methodology employed (including any pre-tests) in order for ECHA to assess its adequacy and that reasonable efforts have been employed to obtain reliable results.

79 For multi-constituents/UVCBs, the analytical method must be adequate to monitor qualitative and quantitative changes in exposure to the dissolved fraction of the test material during the test (e.g. by comparing mass spectral full-scan GC or HPLC chromatogram peak areas or by using targeted measures of key constituents or groups of constituents).

80 If you decide to use the Water Accommodated Fraction (WAF) approach, in addition to the above, you must:

- use loading rates that are sufficiently low to be in the solubility range of most constituents (or that are consistent with the PEC value). This condition is mandatory to provide relevant information for the hazard and risk assessment (Guidance on IRs and CSA, Appendix R.7.8.1-1, Table R.7.8-3);
- provide a full description of the method used to prepare the WAF (including, among others, loading rates, details on the mixing procedure, method to separate any remaining non-dissolved test material including a justification for the separation technique);
- prepare WAFs separately for each dose level (i.e. loading rate) and in a consistent manner.

5. Ready biodegradability

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

5.1. Information provided

82 You have provided a study according to OECD TG 301D on the Substance (2010).

5.2. Assessment of the information provided

5.2.1. Test material not representative of the Substance

83 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; Guidance on IRs and CSA, Section R.4.1.

84 The provided study was conducted with N-(tallow-alkyl)-1,3-propanediamine oleates, EC No. 263-186-4. On the test material, you specify that the purity is "[redacted]% (active)". You have provided no further information on the composition of the test material.

85 In the absence of adequate composition information on the test material, the identity of the test material and its impurities cannot be assessed, and you have not demonstrated that the test material is representative for the Substance.

86 In your comments to the draft decision, you provide detailed information on the composition of the test material which confirms that it corresponds to the Substance. However, as the information is currently not available in your registration dossier, the issue remains. You should submit this information in an updated registration dossier by the deadline set in the decision.

5.2.2. Ready biodegradation tests are normally intended for pure substances

87 The revised introduction to the OECD Guidelines For Testing Of Chemicals, Section 3 Part I states that ready biodegradability tests are intended for pure substances but may also be relevant, on a case-by-case basis, to mixtures of structurally similar chemicals (i.e. which are composed of constituents expected to show similar degradation kinetics). However, such tests are not generally applicable for complex mixtures or substances (i.e. UVCB or multi-constituent substances) containing different types of constituents. For complex substances, a single ready biodegradability test does not allow to conclude on the ready biodegradability of all constituents and therefore, does not fulfil the information requirement.

88 You have provided a study conducted on a test material claimed to be representative of the Substance as a whole. In Section 1.1. of your dossier you describe the Substance as UVCB. In Section 1.2, you describe the substance as a mixture of:

- N-C16-18-alkyl-(even numbered) C18 unsaturated) propane-1,3-diamine dioleate;
- Amines, C16-18 and C18-unsaturated Alkyl oleate;
- Reaction product of oleic acid and N-C16-18-alkyl-(even numbered) C18 unsaturated) propane-1,3-diamine
- (9Z)-octadec-9-enoic acid.

89 The Substance is a complex substance and contains constituents with significant structural differences described above. Therefore, the provided study does not provide unequivocal conclusion that all constituents can safely be regarded as readily biodegradable.

90 In your comments to the draft decision, you explain that the "*substance will dissociate under neutral conditions*". Therefore, upon dissociation, the Substance will release "*three types of constituents with varying chain lengths which to* [your] *interpretation have only a "limited structural difference" i.e. Oleic acid which is a fatty acid known to be readily biodegradable, a C16-18 and C18 unsaturated alkyl amine which is shown to be readily biodegradable (new biodegradability studies with the UVCB (EC no. 268-219-6) are currently running) with and a C16-18 and C18 unsaturated alkyl-1,3-diaminopropane (CAS*

no.: 1219010-04-4) which is also shown to be readily biodegradable (see Appendix 2 for more details)". You also state that "[n]*ot considered yet is the influence of the varying chain lengths of the three types of constituents on the biodegradability potential*". However, you consider that "*it is unlikely that the biodegradability (the potential for biodegradation) of these substances differs significantly with varying chain lengths*".

91 ECHA notes that, as explained by you, the Substance, upon dissociation, will release three types of constituents (i.e. fatty acid, alkyl amine, alkyl diaminopropane) with varying carbon chain length (mainly C16-C18 but, according to the compositional information provided in your comments, the substance includes constituents varying from C12 to C20) and degree of unsaturation. Therefore, ECHA maintains that it contains constituents with significant structural differences and that a test on the whole substance does not allow concluding whether or not all constituents are readily biodegradable.

92 You claim that all three types of constituents (i.e. fatty acid, alkyl amine, alkyl diaminopropane) are readily biodegradable and that carbon chain length will not have a significant impact on the conclusion. However, you failed to provide reliable experimental evidence in support of your conclusions. You refer to ongoing studies on EC no. 268-219-6 but you did not provide any information on these studies. You also refer to some publications (Annex 2 of your comments on the draft decision) where dodecylamine was found to be degraded by some isolated bacterial strains. It remains unclear to what extent this information is relevant to conclude that this Substance should be regarded as readily biodegradable under the conditions specified in ready biodegradability test guidelines. Furthermore, you provide statements that all alkylamines from C8 to C18 should be regarded as readily biodegradable, but you do not provide any description of the supporting experimental evidence allowing to reach this conclusion.

5.2.3. The provided study does not meet the specifications set out in the applicable test guideline

93 To fulfil the information requirement, a study must comply with the OECD TG 301 or 310 (Article 13(3) of REACH). Therefore, for a study according to OECD TG 301D, the following requirements must be met:

94 Technical specifications impacting the sensitivity/reliability of the test

a) test solutions are prepared using an appropriate nutrient medium, which includes ammonium chloride;

95 Reporting of the methodology and results

b) the inoculum concentration in the test vessel is reported as cells/L in the test vessel and as volume of added inoculum (for OECD TG 301D, 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 mL);
c) the results of measurements at each sampling point in each replicate is reported in a tabular form;
d) the calculation of the ThOD is described and justified;
e) 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).

96 Your registration dossier provides a study claimed to be conducted according to OECD TG 301D showing the following:

97 Technical specifications impacting the sensitivity/reliability of the test

a) you report that "Ammonium chloride was omitted from medium to prevent

nitrification". You justify the deviation by stating that "the omission does not result in nitrogen limitation as shown by the biodegradation of the reference compound";

98 Reporting of the methodology and results

b) you have not reported inoculum concentration in the test vessel in cells/L nor the volume of added inoculum;
c) you have not reported the results of measurements at each sampling point in each replicate;
d) you report that the ThOD of the test material is 2.9 mg/mg. However, you have not described and justified the ThOD calculation;
e) you have not reported whether a correction for nitrification was applied on the theoretical oxygen demand.

99 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). The lack of nitrogen limitation in the positive control does not address the above issue as it does not provide additional information with regard respiration in the inoculum blank.
- the reporting of the study is not sufficient to fully assess its reliability. More specifically:
  i. 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. In your comments to the draft decision, you acknowledge that the viable number of bacteria in the inoculum was not quantified. You specify that "[f]*or new tests the number of bacteria in the inoculum will be quantified*";
  - 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 to the draft decision, you provide this information. However, as the information is currently not available in your registration dossier, the issue remains. You will have to submit this information in an updated registration dossier by the deadline set in the decision.
  - 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).
    In your comments on the draft decision, you explain that "*the basic information for the calculation of the ThOD is confidential information as it gives information about the recipe for the manufacturing of the test substance. For this reason, this information was not included in the report*". You provide an average molecular formula used for the calculation of the ThOD.

100 On this basis, the requirements of OECD 301 D are not met, and the information requirement is not fulfilled.

101 In your comments on the draft decision, you state that "*if the endogenous respiration would use more oxygen there is less oxygen available to assess the biodegradation of the test substance resulting in a less accurate biodegradation assessment*". Furthermore, you state

that "*by adding the ammonium chloride to the medium there is a high chance of failing the endogenous respiration validity criteria. This means the test validity criterion might be failed because of the oxygen consumption by the nitrification of the ammonium added to the test medium. Not passing the endogenous validity criteria as a result of adding the ammonium chloride to the test medium might be used by ECHA as an indication of a too high bacterial density*".

102 ECHA notes that the validity criteria of the OECD TG 301D were set based on the use of a test medium that does contain ammonium chloride and that the method was validated through ring testing. Furthermore, while ECHA agrees that low respiration in the inoculum blank ensures that sufficient oxygen remains available in the test system for biodegradation assessment, this parameter also provides some information about inoculum activity (and not only bacterial density). 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.

103 In your comments, you consider that that tests with omission of ammonium chloride from the test medium should be accepted. You claim that this conclusion was supported in a previous compliance check decision (e.g. CCH-D-2114522376-51-01/F, page 14).

104 ECHA considers that there were case specific considerations which explain why this deviation was considered of secondary importance in the earlier compliance check decision that you are referring to. In particular, the respiration in the inoculum blank after 28 days was well below the cut-off value of 1.5 mg O2/L in the corresponding studies (i.e., 0.5 mg O_2/L) and it can be reasonably assumed that it would have still remained under that value in the presence of ammonium chloride. However, in the provided study, the respiration in the inoculum blank after 28 days was already close to the cut-off value (i.e. 1.4 mg O_2/L) in the absence of ammonium chloride. As stated by you "*assuming 100% nitrification this will result in an additional 0.6 mg/L additional oxygen consumption*". Therefore, higher uncertainty exists as to whether it would have remained below 1.5 mg/L if a standard test medium had been used.

5.3. Study design and test specification

105 For the reasons provided above, testing on the Substance as a whole does not fulfil the information requirement. For the generation of information on ready biodegradability, you must consider the level of information required for the purposes of classification and labelling and, if applicable to your registration, the PBT/vPvB assessment and the exposure assessment/risk characterisation. In order to conclude on which of constituents of the Substance are and which are not readily biodegradable, you may have to consider conducting more than one study using selected individual constituents and/or fractions. If you choose to test one (or more) fraction(s) of the Substance, you must provide a justification that their constituents within chosen fraction(s) are similar enough so that similar degradation kinetics can be assumed. If you decide to conduct a single study in order to prove that all constituents of the Substance are readily biodegradable, you must provide a justification that the selected constituent/fraction can be considered a reasonable worst-case for the Substance as a whole in terms of degradation kinetics.

106 Justification for selection of relevant constituent and/or fractions for the testing, must consider degradation kinetics of constituents of the Substance based, as minimum, on the similarity/differences of the chemical structures and the physico-chemical properties of

constituents of the Substance. For that purpose, tools and approaches mentioned in Guidance on IRs and CSA, Sections R.7b and R.11 should be considered.

Reasons related to the information under Annex VIII of REACH

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

107 An in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is an information requirement under Annex VIII, Section 8.4.2.

6.1. Information provided

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

(i) an *in vitro* cytogenicity study (2012) with the analogue substance Oleyl diamine, dioleate EC number 254-754-2

6.2. Assessment of the information provided

6.2.1. Read-across adaptation rejected

109 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

111 In your comments on the draft decision, you "*propose to ECHA to update the read-across documentation to further justify the use of this key study on the test substance Oleyl diamine, dioleate (EC no. 254-754-2) as an acceptable read-across for the In vitro cytogenicity study in mammalian cells or In vitro micronucleus study endpoint for Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, dioleates (EC no. 800-362-7)*".

112 ECHA acknowledges your intentions to improve your read-across adaptation. This strategy relies on information that is yet to be submitted. Therefore, ECHA cannot currently assess the validity of the proposed strategy. You remain responsible for complying with this decision by the set deadline.

6.3. Specification of the study design

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

7. In vitro gene mutation study in mammalian cells

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

7.1. Triggering of the information requirement

115 Your dossier contains an adaptation for an in vitro gene mutation study in bacteria, and an adaptation for an in vitro cytogenicity study in mammalian cells or in vitro micronucleus study.

116 The information for the in vitro gene mutation study in bacteria and for the in vitro cytogenicity study in mammalian cells or in vitro micronucleus study provided in the dossier are rejected for the reasons provided in requests 2 and 6.

117 The result of the requests for an in vitro gene mutation study in bacteria and for an in vitro cytogenicity study in mammalian cells will determine whether the present requirement for an in vitro mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

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

7.2. Information provided to meet the information requirement

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

(i) in vitro gene mutation study in mammalian cells with the analogue substance Oleyl-diamine dioleate, EC No. 254-754-2

7.2.1. Read-across adaptation rejected

120 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

122 In your comments on the draft decision, you "*propose to ECHA to update the read-across documentation to further justify the use of this key study on the test substance Oleyl diamine, dioleate (EC no. 254-754-2) as an acceptable read-across for the in vitro gene mutation study in mammalian cells endpoint for Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, dioleates (EC no. 800-362-7)*".

123 ECHA acknowledges your intentions to improve your read-across adaptation. This strategy relies on information that is yet to be submitted. Therefore, ECHA cannot currently assess the validity of the proposed strategy. You remain responsible for complying with this decision by the set deadline.

7.3. Specification of the study design

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

8. Justification for an adaptation of a Short-term repeated dose toxicity (28 days) based on the results of the Sub-chronic toxicity study (90 days)

125 A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII, Section 8.6.1. This information may take the form of a study record or a valid

adaptation in accordance with either a specific adaptation rule under Column 2 of Annex VIII or a general adaptation rule under Annex XI.

8.1. Information provided

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

(i) a repeated dose 28-day oral toxicity study (2012) with the analogue substance Oleyl diamine, dioleate EC number 254-754-2
(ii) a repeated dose 28-day oral toxicity with 14day recovery (2010) with the analogue substance N-Oleyl-1,3-diaminopropane, EC No. 230-528-9
(iii) a repeated dose 28-day oral toxicity with 14day recovery (2010) with the analogue substance N-C12,14 alkyl-1,3-diaminopropane, EC No. 292-562-0

8.2. Assessment of the information provided

8.2.1. Read-across adaptation rejected

127 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

8.3. In your comments on the draft decision, you "agree with ECHA and will propose a justification waiver for the short-term repeated dose toxicity (28 days) pending the outcome of information used to fulfil the formal requirements for the subchronic toxicity study (90 days) study as well as provide other supporting evidence to assist in interpretation of the endpoint".Specification of the study design

129 Annex VIII, Section 8.6.1., Column 2 provides that an experimental study for this information requirement is not needed if a reliable sub-chronic (90 days) or chronic toxicity study is available.

130 The present decision requests the registrants concerned to generate and submit a reliable sub-chronic toxicity study (90 days) (see request 11). According to Annex VIII, Section 8.6.1., Column 2 and to prevent unnecessary animal testing, a short-term toxicity study (28 days) does not therefore need to be conducted.

131 Because you still must comply with the information requirement in Annex VIII, Section 8.6.1., you are requested to submit a justification for the adaptation provided in Column 2 of that provision.

9. Screening for reproductive/developmental toxicity

132 A screening for reproductive/developmental toxicity study (OECD 421 or OECD 422) is an information requirement under Annex VIII, Section 8.7.1., if there is no evidence from analogue substances, QSAR or in vitro methods that the substance may be a developmental toxicant.

9.1. Information provided

133 You have not provided any information on the screening study. Instead, you have provided the following information on the waiving of the two-generation reproduction study: "*No*

adverse effects on reproductive organs were identified in the 28-day study on Oleyl-diamine dioleate, nor in the various studies available on Oleyl-diamine and C12-14-diamine, including the 90-day study on C12-14-diamine. Based on the read-across from these studies, it can be expected that Tallow-diamine dioleate will also not have an impact on reproduction organs. In accordance with Section 8.7.3 of column 1, Annex IX of REACH, no further two-generation reproduction study is therefore indicated".

9.2. Assessment of the information provided

9.2.1. Your justification has no legal basis

134 A registrant may only adapt this information requirement based on the specific rules of Annex VIII Section 8.6.1, Column 2 or the general rules set out in Annex XI.

135 Your justification to omit this information does not refer to any legal ground for adaptation under Annex VIII, Section 8.6.1, Column 2 or Annex XI to REACH.

136 Therefore, you have not demonstrated that this information can be omitted.

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

138 In the comments to the draft decision, you agree to perform the requested study.

9.3. Specification of the study design

139 A study according to the test method EU B.63/OECD TG 421 or EU B.64/OECD TG 422 must be performed in rats.

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

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

10. Long-term toxicity testing on fish

142 Short-term toxicity testing on fish is an information requirement under Column 1 of Annex VIII to REACH (Section 9.1.3.). However, long-term toxicity testing on fish must be considered (Section 9.1.3., Column 2) if the substance is poorly water soluble.

10.1. Triggering of the information requirement

143 As already explained under Request 2, the Substance is concluded to be poorly water soluble. Therefore, the Substance is poorly water soluble and information on long-term toxicity on fish must be provided.

10.2. Information provided

144 You have omitted the information requirement on long-term toxicity to fish with a justification you consider to be based on Annex IX, Section 9.1., Column 2.

10.3. Assessment of the information provided

145 The examination of the information provided, as well as the selection of the requested test and the test design are addressed under Request 14.

Reasons related to the information under Annex IX of REACH

11. Sub-chronic toxicity study (90-day)

146 A sub-chronic toxicity study (90 day) is an information requirement under Annex IX, Section 8.6.2.

11.1. Information provided

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

(i) a sub-chronic toxicity study (2010) with the analogue substance N-C12,14 alkyl-1,3-diaminopropane, EC No. 292-562-0

11.2. Assessment of the information provided

11.2.1. Read-across adaptation rejected

148 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

150 In your comments on the draft decision, you propose "*to update the read-across documentation to further justify the use of this key study on the test substance Oleyl diamine, dioleate (EC no. 254-754-2) as an acceptable read-across for the sub-chronic toxicity study (90 day) endpoint for Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, dioleates (EC no. 800-362-7)*".

151 ECHA acknowledges your intentions to improve your read-across adaptation. This strategy relies on information that is yet to be submitted. Therefore, ECHA cannot currently assess the validity of the proposed strategy. You remain responsible for complying with this decision by the set deadline.

11.3. Specification of the study design

152 Following the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity of the Substance; Guidance on IRs and CSA, Section R.7.5.6.3.2.

153 According to the OECD TG 408, the rat is the preferred species.

154 Therefore, the study must be performed in rats according to the OECD TG 408 with oral administration of the Substance.

12. Pre-natal developmental toxicity study in one species

155 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX, Section 8.7.2.

12.1. Information provided

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

(i) a developmental toxicity study (2010) with the analogue substance N-C12,14 alkyl-1,3-diaminopropane, EC No. 292-562-0

12.2. Assessment of the information provided

12.2.1. Read-across adaptation rejected

157 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

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

159 In your comments on the draft decision, you propose "*to update the read-across documentation to further justify the use of this key study on the test substance Oleyl diamine, dioleate (EC no. 254-754-2) as an acceptable read-across for pre-natal developmental toxicity (PNDT) study endpoint for Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, dioleates (EC no. 800-362-7)*".

160 ECHA acknowledges your intentions to improve your read-across adaptation. This strategy relies on information that is yet to be submitted. Therefore, ECHA cannot currently assess the validity of the proposed strategy. You remain responsible for complying with this decision by the set deadline.

12.3. Specification of the study design

161 A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species.

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

163 Therefore, the study must be conducted in rats or rabbits with oral administration of the Substance

13. Long-term toxicity testing on aquatic invertebrates

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

13.1. Information provided

165 You have adapted this information requirement by using a Grouping of substances and read-across approach under Annex XI, Section 1.5 of REACH. In support of your adaptation, you provided the following information:

(i) a study according to OECD TG 211 on octadec-9-enoic acid - N-octadec-9-en-1-ylpropane-1,3-diamine (2:1) with EC No. 251-846-4 (2012)

13.2. Assessment of the information provided

13.2.1. Your read-across adaptation is rejected

166 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected. In addition, ECHA identified endpoint-specific issue addressed below.

13.2.2. Adequacy and reliability of the study on the source substance

167 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), in this case the OECD TG 211 and the requirements of OECD GD 23 if the substance is difficult to test. Therefore, the following specifications must be met:

168 Technical specifications impacting the sensitivity/reliability of the test

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

169 Your registration dossier provides an OECD TG 201 study showing the following:

170 Technical specifications impacting the sensitivity/reliability of the test

a) you report that the study was conducted using natural water from Innerste river. The TOC content of the water on 20 March 2012 was 3.66 mg/L. You also report that the test was conducted from 10 July September 2012 to 02 August 2012.

171 Based on the above, there are critical methodological deficiencies resulting in the rejection of the study results. More specifically,

- the study was conducted with river water with an organic carbon content above the maximum acceptable value. the DOC/TOC values reported in the robust study summary may also not provide a representative estimate for the water used to conduct the test considering the high temporal variation of this parameter (e.g. due to seasonal variation, run-offs etc.). As a result, it may be that the actual DOC/TOC content of the test water was higher than the reported values (as DOC/TOC of river waters is usually higher in summer compared to winter). For the reasons already explained under Request 4, conducting testing with high DOC/TOC water does not permit to generate adequate information for the purpose of classification and labelling and of the PBT assessment.

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

173 In your comments to the draft decision, you agree that "*the bulk approach test results are less suitable for quantifying the intrinsic toxicity of cationic surfactants*" and "*for Classification and Labelling as they use non-standard test medium*". Therefore, you agree to conduct the requested study. You raise the same technical challenges already explained in the reasoning for Request 4 above. ECHA's reply equally applies to this information requirement.

13.3. Study design and test specifications

174 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 and test specifications' under Request 4.

14. Long-term toxicity testing on fish

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

14.1. Information provided

176 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided following justification: "*the CSA does not indicate the need for further testing of vertebrates. Moreover, the low bioaccumulative potential does not trigger the need for long-term testing and the acute aquatic toxicity data indicate that fish is less sensitive to ethoxylated quaternary ammonium compounds than algae and daphnia. Therefore long-term toxicity testing with fish is waived in order to avoid unnecessary vertebrate testing*".

14.2. Assessment of the information provided

177 We have assessed this information and identified the following issue:

14.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

178 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).

179 Your adaptation is therefore rejected.

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

181 In your comments to the draft decision, you agree to conduct the requested study.

14.3. Study design and test specifications

182 To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

183 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 and test specifications' under Request 4.

References

The following documents may have been cited in the decision.

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

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

Guidance on data-sharing; ECHA (2017).

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

Read-across assessment framework (RAAF)

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

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

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

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

Appendix 2: Procedure

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

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

The compliance check was initiated on 07 December 2021.

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

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

In your comments on the draft decision, your requested an extension of the deadline from 18 months to 24 months. To justify your request, you mention CROs availability. You also explain that "*if ECHA finds that our read-across proposal will still not meet the formal requirements for the 90-day repeat dose toxicity test we would like to progress by integrating the testing strategy to combine with an OECD 422 guideline for combine repeat-dose toxicity test. The combination of a 408 and a 422 will allow for less animals to be used [but it] adds approximately a total of 10 weeks to the premating period to the animals and thus increases the likely hood of exceeding the 18 months deadline for completion*".

The deadline of the draft decision was set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account your comments and also the currently longer lead times in contract research organisations.

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 3: Addressees of this decision and their corresponding information requirements

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

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

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

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

Appendix 4: Conducting and reporting new tests for REACH purposes

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

1.1. Test methods, GLP requirements and reporting

(1) Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

(2) Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[2].

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

1.2. Test material

The Test Material used to generate the new data must be selected taking into account the following:

a) the variation in compositions reported by all members of the joint submission,
b) the boundary composition(s) of the Substance,
c) 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.

Information on the Test Material needed in the updated dossier

a) 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.
b) 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,

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

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 (https://echa.europa.eu/manuals).

2. General recommendations for conducting and reporting new tests

2.1. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in Guidance on IRs & CSA, 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.

References to Guidance on REACH and other supporting documents can be found in Appendix 1.