~~Confidential~~

Helsinki, 15 March 2023

Addressees
Registrant(s) of DFAS_Di_C18unsat_JS as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
18/02/2022

Registered substance subject to this decision ("the Substance")
Substance name: Oleic acid, compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (2:1)
EC/List number: 251-846-4

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

2. 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.

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

Information required depends on your tonnage band

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

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

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes

to classification and labelling, based on the newly generated information.

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

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 request(s)
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 request(s)

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0. Reasons common to several requests

0.1. Read-across adaptation rejected

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

- Ready biodegradability (Annex VII, Section 9.2.1.1.)

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

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. Scope of the grouping of substances (category)

5 You provide a read-across justification document under the endpoint section listed above.

6 For the purpose of this decision, the following abbreviations are used for the category members:

7 Linear polyamines

a) Diamine C12/14: C12/14 propylene diamine (CAS RN 90640-43-0)

b) Diamine C: Coco propylene diamine (CAS RN 91771-18-5) also referred to as Amines, N-C12-18-alkyltrimethylenedi- (CAS RN 68155-37-3)

c) Diamine T: Tallow propylene diamine (CAS RN 61791-55-7) also referred to as N-C16-18-alkyl-(evennumbered, C18 unsaturated) propane-1,3-diamine (CAS RN 1219010-04-4)

d) Diamine HT: Hydrogenated tallow propylene diamine (CAS RN 68603-64-5) also referred to as Amines, N-C16-18-alkyl (evennumbered) propane-1,3-diamine (CAS RN 133779-11-0)

e) Diamine O: Oleyl propylene diamine (CAS RN 7173-62-8)

f) Triamine C: Coco dipropylene triamine (CAS RN 91771-18-5)

g) Triamine T: Tallow dipropylene triamine (CAS RN 61791-57-9) also referred to as N-(3-aminopropyl)-N'-C16-18 (evennumbered), C18 unsaturated alkyl -propane-1,3-diamine (CAS RN 1219458-14-6)

h) Triamine OV: Oleyl (vegetable oil) dipropylene triamine (CAS RN 28872-01-7)

i) Tetramine T: N-tallow alkyltripropylene tetramine (CAS RN 68911-79-5) also referred to as N-(3-aminopropyl)-N'-[3-(C16-18 (evennumbered), C18 unsaturated alkyl amino)propyl]propane-1,3- diamine (CAS RN 1219458-11-3)

j) Tetramine OV: Oleyl(vegetable oil) tripropylene tetramine (CAS RN 67228-83-5)

8 Branched polyamines

k) Triamine Y12: Dodecyl dipropylene triamine, branched (CAS RN 2372-82-9)

l) Triamine YT: Tallow dipropylene triamine, branched (CAS RN 85632-63-9) also referred to as N-(3-aminopropyl)-N-N-(C16-18 evennumbered, 18 unsaturated)-alkylpropane-1,3-diamine (CAS RN 1219826-66-0)

9 You justify the grouping of the substances as:

- "*Structurally, the linear di-, tri- and tetramines are very similar: a linear alkyl chain and a primary amine at the end, with 1, 2 or 3 secondary amines in between. Consequently, they share the same chemical reactivity and their physico-chemical properties are very similar from which a comparable toxicological profile can be expected.* [...] *the reactivity profile as indicated by QSAR Toolbox (v.3.4) is identical for all structures* [...]. *Only the triamine-Y structures have an additional alert for 'DNA binding by OECD', which is based on tert. amine structure with potential P450 metabolism to a reactive iminium. However, none of the fatty amine derived substances with tertiary amine structures we have tested showed genotoxicity hazards, and besides, none of the other genotoxicity alerts in the Toolbox were triggered. Also the metabolism/transformation predictions showed are comparable for all polyamine structures*".
- "*The variability of the alkyl chain length* […] *is suspected to influence aspects related to bioavailability, but not aspects of chemical reactivity, metabolism/transformations, and specific mechanisms of toxicity e.g. sensitization and genotoxicity. For these reasons, many of the toxicological studies can best be performed on the substance with the shortest chain length within the sub-category, as this is considered to result to the lowest NOAEL or most likely able to show specific effects where for ecotoxicology and fate studies can best be focussed on the extremes of the category*".
- "*For the physco-chemical properties and related toxicological profile there are clear trends that can be observed over all structures that is related to the length of the alkyl chain, and the number of DP (diamino propane) groups*".
- "*ADME studies indicate slow absorption and likely these substances are not easily metabolized. However, if there is metabolism, the pattern can be expected to be similar for all category members, as is also indicated by metabolism simulators" and "Metabolism profile is not expected to be principally different, and metabolites shows the same variation in alkyl chain lengths. This is supported by the QSAR (OECD) Toolbox (v.3.4) rat liver S9 metabolism and skin metabolism simulators, which show the same metabolism profiles* […]. *Only for the Oleyl chain, some additional metabolic targets are presented related to the available unsaturated bond. However, from common physiological knowledge of fatty acid metabolism, it is known that this is of no concern in practice*".
- "*All category members are produced following the same production processes [and] the products show similar purity and impurity profiles. The conversion of the primary amines into a diamine is not fully complete. The same applies for the subsequent steps to triamine and tetramine. The composition descriptions of these products therefore also include a fraction of remaining primary alkyl amines and polyamines from earlier steps*".

10 You define the applicability domain as: You define the applicability domain as: "*substances that contain multiple (2 or more) 1,3-diamine propane (DP) groups linked to a fatty amine. These can be linearly linked based on two DP and fatty amine (triamine structure: alkyl dipropylenetriamine) or 3 DP with a fatty amine (tetraamine structure: alkyl tripropylenetetraamine), or in a branched or Y-amine form of two DP that are both linked to the nitrogen of a fatty amine (The annotation 'branched' does not refer to the alkyl chains). The alkyl chain for the structures under consideration, can vary in length from relatively short (C8) to longer (C18). Also the level of unsaturation of the fatty acids can be a factor to be considered for category members" and "tetramines also contain for a large part triamines and some diamines, and the triamines can contain a considerable amount of diamines and some tetramines*".

0.1.2. Predictions for fate properties

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

Diamine C12/14	C12/14 propylene diamine (CAS RN 90640-43-0)
Diamine C2	N-(Coco alkyl)trimethylenediamine (CAS RN 61791-63-7)
Diamine O	Oleyl propylene diamine (CAS RN 7173-62-8
Diamine T	Tallow propylene diamine (CAS RN 61791-55-7) also referred to as N-C16-18-alkyl-(evennumbered, C18 unsaturated) propane-1,3-diamine (CAS RN 1219010-04-4)

12 You provide the following reasoning for the prediction of fate properties:

- "*Although micro-organisms capable of degrading surfactants are immensely diverse, the central metabolism (b-oxidation and TCA cycle) is remarkably similar*. […] *This unity is the key to justification of the use of read-across of biodegradability test results*".
- "*[…] it is unlikely that the biodegradability of these surfactants differs significantly with varying alkyl chain lengths*".
- "*The adequate ready biodegradability test result obtained and the scientific evidence that consortia of hydrophilic moiety and alkyl-utilizing micro-organisms through a joint biodegradation pathway degrade all triamines, alkyl lead to the conclusion that all triamines, alkyl are readily biodegradable*".

13 ECHA understands 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.

14 We have identified the following issues which are common to the predictions of toxicological and fate properties, or specific only to the predictions of toxicological properties:

0.1.2.1. Inadequate or unreliable source studies.

15 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.

16 Specific reasons why the studies on the source substance does not meet these criteria are explained further below under the applicable information requirement section 2. Therefore, no reliable predictions can be made for this information requirement.

0.1.3. Conclusion on the read-across approach

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

Reasons related to the information under Annex VII of REACH

1. Growth inhibition study aquatic plants

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

1.1. Information provided

19 You have provided the following information on the Substance:

(i) a toxicity study to aquatic algae and cyanobacteria (2012) according to OECD TG 201

20 You have also adapted this information requirement by using Annex XI, Section 1.5. (Grouping of substances and read-across approach) based on experimental data from the following substances:

(ii) a toxicity study to aquatic algae and cyanobacteria (2019) according to OECD TG 201 with the analogue substance oleic acid, compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (CAS RN 40027-38-1).

21 ECHA understands that this analogue Substance does not fall into the category definition described in Section 0.1 and that this information is provided as an analogue read-across. ECHA has therefore addressed this adaptation separately under this section.

22 You provide the following reasoning for the prediction of this information requirement: "*a GLP study according to the TG OECD 201 with a similar substance is available to assess the inhibition of the growth of the freshwater green alga Pseudokirchneriella subcapitata in a standard medium*".

23 ECHA assumes 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.

1.2. Assessment of the information provided

1.2.1. Information on the Substance

1.2.1.1. Insufficient information provided to confirm whether the test material used in study (i) is representative of the Substance

24 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. Information to confirm the test material identity includes purity, composition, carbon chain length, saturation, branching, depending on the type of substance.

25 The study (i) has been conducted with the N-[(9Z)-octadec-9-en-1-yl]propane-1,3-diaminium di[(9Z)-octadec-9-enoate (CAS 34140-91-5 / EC 251-846-4). You claimed that the test material was representative of the boundary composition of the Substance. However, you did not provide any information on composition (including carbon chain length, saturation) to support your claim.

26 In the absence of detailed 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.

1.2.1.2. The provided study (i) does not meet the specifications of the test guideline

27 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:

28 Technical specifications impacting the sensitivity/reliability of the test

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

29 Characterisation of exposure

b) the test media prepared specifically for analysis of exposure concentrations during the test is treated identically to those used for testing (*i.e.* inoculated with algae and incubated under identical conditions);
c) for volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24 hour intervals is required;

30 Additional requirements applicable to difficult to test substances

d) 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.

31 In study (i) described as growth inhibition study on aquatic plants/algae:

32 Technical specifications impacting the sensitivity/reliability of the test

a) the test medium is described as Water of the river Innerste. You state 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*";

33 Characterisation of exposure

b) the test media prepared specifically for analysis of exposure concentrations was not inoculated with algae;
c) the Substance has low water solubility (c.a. 0.005 mg/L), adsorptive properties (Kd up to 38000 L/kg based on read-across) and ionisable properties (pKa for the first amine of > 9), and therefore the substance is considered to be highly adsorptive. You have observed significant loss from the test medium at t=72h and no additional sampling for analysis at 24 h interval was conducted;

34 Additional requirements applicable to difficult to test substances

d) as explained above the substance is considered to be highly adsorptive. You report that the test was conducted with natural freshwater with a TOC content of 3.66 mg/L.

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

- you have not used one of the two alternative growth medium and the TOC content of the test medium was above the mandatory value of 2 mg/L which is not adequate to investigate the intrinsic hazards of the Substance. ECHA notes that information on intrinsic properties of a substance must 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 Guidance on Application of CLP Criteria, Section 1.1.3., specifies 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. Therefore, the bulk approach which aims at mimicking exposure under "more environmentally realistic" conditions must not be used for classification and labelling. 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 under 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.

- you have not demonstrated that exposure was satisfactorily maintained and that effect concentrations can be expressed based on nominal concentrations as (i) not all required test concentrations were analytically monitored, (ii) the samples were not inoculated with algae, and (iii) the sampling frequency was not adequate.

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

37 In your comments on the draft decision, you "*acknowledge* [the] *deficiencies* [identified above] *and thus downgrade the Klimisch score of this study to 3 (not reliable) and the study adequacy to "supporting study" instead of key study*".

1.2.2. Assessment of your read-across adaptation

38 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.

39 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).

40 We have identified the following issue with the prediction of growth inhibition on aquatic plants:

1.2.2.1. Absence of read-across documentation

41 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).

42 You have provided a robust study summary for a study conducted with another substance 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.

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

1.2.2.2. Insufficient information provided to confirm identity of the test material used in study (ii)

44 To comply with this information requirement, the test material in a study must be representative for the substance to be tested; Article 10 and Recital 19 of REACH; Guidance on IRs and CSA, Section R.4.1). The Test Methods Regulation (EU) 440/2008, as amended by Regulation (EU) 2016/266, requires that "if the test method is used for the testing of a [...] UVCB [...] sufficient information on its composition should be made available, as far as possible, e.g. by the chemical identity of its constituents, their quantitative occurrence, and relevant properties of the constituents". Such information includes purity, composition, carbon chain length, saturation, branching, depending on the type of UVCB substance.

45 The study (ii) has been conducted with the UVCB Oleic acid, compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (CAS 40027-38-1). You did not provide any information on composition (including carbon chain length, saturation).

46 In the absence of detailed information on the UVCB 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 that was intended to be tested.

47 In your comments on the draft decision, you explain that "[f]*or study (ii), missing details were identified in terms of confirming that the composition of the analogue substance CAS no. 40027-38-1 is representative of the registered substance*". You have updated your dossier with this information and state that "[w]*ith the proposed changes, the Registrant selects the 2019 study conducted with substance CAS 40027-38-1) as the sole key study*".

48 ECHA acknowledges that the additional information provided addresses the issue identified above. However, while the test material used in study (ii) shows that the relative amounts of the main constituents (C18:1 diamine and Oleic Acid) are consistent with the definition of the Substance, you acknowledged that there are discrepancies between the composition of this test material and the boundary composition of the Substance, in particular the different distribution of fatty acids and alkyl diamines (C6 – C24). Therefore, the test material cannot be regarded as representative of the Substance.

1.2.2.3. Conclusion

49 As explained above, you have not established that relevant properties of the Substance can be predicted from data on the source substance(s). Therefore, your read-across approach under Annex XI, Section 1.5. is rejected and the information requirement is not fulfilled.

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

1.3. Study design and test specifications

51 The Substance is difficult to test due to the low water solubility (c.a. 0.005 mg/L), adsorptive properties (Kd up to 38000 L/kg based on read-across) and ionisable properties (pKa for the first amine of > 9). OECD TG 202 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. 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 202. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

52 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).

53 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.

2. Ready biodegradability

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

2.1. Information provided

55 You have provided the following information on the Substance:

(i) a ready biodegradability study (2004) according to OECD TG 301F

56 You have also adapted this information requirement by using Annex XI, Section 1.5. (Grouping of substances and read-across approach) based on experimental data from the following substances:

(ii) a ready biodegradability study (2008) according to OECD TG 301D with the source substance Oleyl propylene diamine (CAS RN 7173-62-8)

(iii) a ready biodegradability study (1992) according to OECD TG 301F with the source substance Oleyl propylene diamine (CAS RN 7173-62-8)

(iv) a ready biodegradability study (1993, report no. [redacted]) according to OECD TG 301D with the source substance Oleyl propylene diamine (CAS RN 7173-62-8)

(v) a ready biodegradability study (2004) according to OECD TG 301D with the source substance N-(Coco alkyl)trimethylenediamine (CAS RN 61791-63-7)

(vi) a ready biodegradability study (1990) according to OECD TG 301D with the source substance Tallow propylene diamine (CAS RN 61791-55-7) also referred to as N-C16-18-alkyl-(evennumbered, C18 unsaturated) propane-1,3-diamine (CAS RN 1219010-04-4)

(vii) a ready biodegradability study (1993, report no. [redacted]) a ready biodegradability study (1993) according to OECD TG 301D with the source substance Oleyl propylene diamine (CAS RN 7173-62-8)

(viii) a ready biodegradability study (2004) according to OECD TG 301D with the source substance C12/14 propylene diamine (CAS RN 90640-43-0)

57 Finally, you have adapted this information requirement by using Annex XI, Section 1.3. (Qualitative or Quantitative Structure-Activity Relationships, (Q)SARs). To support the adaptation, you have provided the following information:

(ix) a prediction from Biowin v4.10 (2022)

2.2. Assessment of the information provided

2.2.1. Information on the Substance

2.2.1.1. Insufficient information provided to confirm whether the test material used in study (i) is representative of the Substance

58 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. Information to confirm the test material identity includes purity, composition, carbon chain length, saturation, branching, depending on the type of substance.

59 The study (i) has been conducted with the N-[(9Z)-octadec-9-en-1-yl]propane-1,3-diaminium di[(9Z)-octadec-9-enoate (CAS 34140-91-5 / EC 251-846-4). You claimed that the test material was representative of the boundary composition of the Substance. However, you did not provide any information on composition (including carbon chain length, saturation) to support your claim.

60 In the absence of detailed 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.

2.2.1.2. Ready biodegradation tests are normally intended for pure substances (studies (i))

61 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.

62 You have provided a study conducted with a test material claimed to be representative of the Substance. In section 1.2 of your IUCLID, you specify that the alkyl diamine and fatty acid C-chain length varies from C6 to C24, the main fraction (> 60%) corresponding to C18:1 (the sum of other C-chain length amounting for less than 15%. You also explain that the Substance may contain 0-10% primary amines and secondary amides of various C-chain length. Finally the Substance includes 0-5% free fatty acid.

63 The test materials used in study (i) is a complex substance which contain constituents with significant structural differences described above. Therefore, the provided studies do not provide unequivocal conclusion that all constituents can safely be regarded as readily biodegradable.

2.2.1.3. The provided study on the Substance (study (i)) does not meet the specifications of the test guideline

64 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 301F, the following requirements must be met:

65 Reporting of the methodology and results

a) the source of the inoculum, its concentration in the test and any pre-conditioning treatment are reported;
b) the inoculum concentration in the test vessel is reported as cells/L in the test vessel and as volume of added inoculum:
   - for the OECD TG 301F, the concentration of the inoculum is set to reach a bacterial cell density of 10^7 to 10^8 cells/L. The suspended solid concentration is below 30 mg/L and the volume of added effluent is < 100 mL/L;
c) the calculation of the ThOD is described and justified;
d) 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).

66 Your registration dossier provides a study conducted according to OECD TG 301F (study (i)) showing the following:

67 Reporting of the methodology and results

a) you have not specified whether the inoculum was adapted to the test material;
b) you have not reported inoculum density in cells/mL;
c) you state that "*ThOD of* [the test material] *calculated using the raw formula is 2.969 mg O2/mg*". However, you do not described how this raw formula was used considering the complex composition of the tested substance;
d) you have not reported whether a correction for nitrification was applied on the theoretical oxygen demand.

68 Based on the above, the reporting of the study is not sufficient to fully assess its reliability. More specifically,

- as you have not specified whether the inoculum was adapted to the test material, it is not possible to verify whether this study qualifies for a ready biodegradability test;
- 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 301F.
  In your comments on the draft decision, you consider "that reporting the inoculum density in cells/mL is not a requirement in accordance with the OECD Test Guideline 301 F". You further state that "*Table 2, "Test Conditions", provides an approximate range of cell densities for each variant of the OECD 301 methods, but these elements are not under the mandatory data to be reported. Under paragraph 27, "Test report", the Test Guideline lists a series of aspects that are mandatory in test reports. With regards to the inoculum, the Test Guideline lists as mandatory: "inoculum: nature and sampling site(s), concentration and any preconditioning treatment*". You consider that "*each of those pieces of information is reported, as required*" and that "*this specific aspect should not be listed under the criteria to invalidate a study*".
  ECHA points out that table 2 of OECD TG 301 is entitled "test conditions" and therefore should be seen as the conditions under which the various test methods described in the test guideline must be conducted. The limit values for the inoculum density in mg/L (e.g., for sludge or soil) or mL/L (e.g., for surface water or effluent) are set to ensure that the introduction of exogeneous organic matter in the test system is within an acceptable range. Such parameter does not provide a direct estimate of bacterial biomass (as the density of bacteria in, for e.g., a sludge sample

or a secondary effluent may vary by orders of magnitude). Accordingly, Appendix R.7.9-1 of ECHA Guidance on IRs and CSA specifies inoculum conditions as cell density (cells/mL) present in a relevant media (e.g. surface waters, unchlorinated sewage treatment works, activated sludge). In your comments, you consider, based on paragraph 27 of OECD TG 301, that inoculum density in cells/mL should not be seen as a mandatory reporting requirement. However, ECHA notes that paragraph 27 does not specify that inoculum density should be expressed in mg/L only (as no unit is specified). It should then be understood that adequate information must be provided to document that inoculum density was within the requirements specified in table 2 (i.e. which include bacterial cell density).

- you have not specified how 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). Therefore, the interpretation of the study results cannot be assessed.

69 Therefore, study (i) does not meet the requirements of the corresponding test guideline.

2.2.2. Assessment of your read-across adaptation

2.2.2.1. Read-across adaptation rejected

70 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(s) addressed below.

2.2.2.2. Insufficient information provided to confirm test material identity (studies (ii) to (viii))

71 To comply with this information requirement, the test material in a study must be representative for the substance to be tested; Article 10 and Recital 19 of REACH; Guidance on IRs and CSA, Section R.4.1). The Test Methods Regulation (EU) 440/2008, as amended by Regulation (EU) 2016/266, requires that "if the test method is used for the testing of a [...] UVCB [...] sufficient information on its composition should be made available, as far as possible, e.g. by the chemical identity of its constituents, their quantitative occurrence, and relevant properties of the constituents". Such information includes purity, composition, carbon chain length, saturation, branching, depending on the type of UVCB substance.

72 The studies (ii) to (viii) were conducted with UVCB substances. Your provide the following information on the purity/composition of the corresponding test materials:

- Study (ii): you state "*total diamine* [redacted]*%,* [redacted]*% primary amines;* [redacted]*% water*"
- Study (iii): you state that purity was [redacted]%
- Study (iv): you state that purity > 85%
- Study (v): you state "*Diamine content:* [redacted]*%*"
- Study (vi): you state "*Analytical purity: >92% (MW = 319)*"
- Study (vii): you state "*diamine* [redacted]*%*"
- Study (viii): you state that purity was [redacted]%

73 You have provided no information on composition for any of the studies (ii) to (viii).

74 In the absence of detailed information on the UVCB 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 substances that were intended to be tested.

2.2.2.3. Inadequate or unreliable study on the source substance (studies (ii) to (viii))

75 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 study that must normally be performed for a particular information requirement, in this case the OECD TG 301. Therefore, for a study according to OECD TG 301D or F, the following specifications must be met:

76 Technical specifications impacting the sensitivity/reliability of the test

a) test solutions are prepared using an appropriate nutrient medium, which includes ammonium chloride;
b) for the OECD TG 301D, the concentration of the test material is 100 mg /L, corresponding to 50 to 100 mg ThOD/L;

77 Reporting of the methodology and results

c) 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);
   - for OECD TG 301F, the concentration of the inoculum is set to reach a bacterial cell density of 10^7 to 10^8 cells/L. The suspended solid concentration is below 30 mg/L and the volume of added effluent is < 100 mL/L;
d) the results of measurements at each sampling point in each replicate is reported in a tabular form;
e) the calculation of the ThOD is described and justified;
f) 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).

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

79 Technical specifications impacting the sensitivity/reliability of the test

a) for studies (ii), (vi) and (vii), you report that "*Ammonium chloride was omitted from medium to prevent nitrification*". For study (iv), (v) and (viii), you have not described what test medium was used.
b) for study (iii), the test material concentration equivalent to 339 mg/L chemical oxygen demand which is over three times above the maximum value allowed by the OECD TG 301F.

80 Reporting of the methodology and results

c) for study (ii), you report that "*The closed bottles were filled with river water and medium at a ratio of 1:1"*. For study (v), you state that "[t]*he test medium is inoculated with 7.8 ml/L of filtered sludge*". For study (vi) to (viii), you have not specified the volume of inoculum added to the test bottles. You have not reported inoculum density in cells/mL in studies (ii) to (viii).
d) you have not reported the results of measurements at each sampling point in each replicate in studies (ii), (iii) and (v) to (viii).
e) you have not reported the ThOD (studies (iii), (vi) and (viii)) nor described and justified the ThOD calculation (taking into account the fact that the substance is a UVCB) (studies (ii) to (viii));
f) you have not reported whether a correction for nitrification was applied on the

theoretical oxygen demand in studies (ii) to (viii).

81 Based on the above,

a) there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically:

- in studies (ii), (vi) and (vii), 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. For study (iv), (v) and (viii), the adequacy of the test medium cannot be independently assessed.
- the information you provided on study (ii) indicates that the volume of added inoculum was 100 times above the maximum value specifies in OECD TG 301D. The volume of added inoculum was too high also in study (v);

b) 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 in studies (ii) to (viii), it is not possible to verify if the inoculum density was low enough to be consistent with the specifications of OECD TG *301D or F.*
- as you have not provided an adequate reporting of the study results for studies (ii), (iii) and (v) to (viii), it is not possible to verify if validity criteria consistent with the specifications of OECD TG 301D or F were met;
- you have not specified how 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) for any of the above studies.

82 Therefore, studies (ii) to (viii) do not meet the requirements of OECD TG 301D.

83 Based on the above, your read-across adaptation is rejected.

2.2.3. Assessment of your QSAR adaptation

2.2.3.1. Lack of/Inadequate justification of the representativeness of the structure(s)

84 Under Guidance on IRs and CSA R.6.1.7.3. a prediction is adequate for the purpose of classification and labelling and/or risk assessment if the following cumulative condition(s) is/are met:

- representative structure(s) for the assessment are selected.

85 Your registration dossier provides the following information:

- In Section 1.1 of your technical dossier, you define the Substance as a mono-constituent substance
- In Section 1.2, you indicate the following constituents in the composition of your Substance:
  - oleic acid, compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (2:1): >=60<=100 % (w/w)
  - reaction product of 1 mole of amine with 1 mole of fatty acid: >=0<10 % (w/w). You state that "*this family approach covered all chain repartition*

Cx, under 10% each and the sum of the family is clearly under 10%".
    - Diamine fatty acid salt (other chain length than C18:1): >=0<15 % (w/w). You state that "this family approach covered all chain repartition Cx, under 10% each"
    - amidification of oleic acid,compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (2:1) with fatty or oleic acids: >=0<15 % (w/w). You state that "this family approach covered all chain repartition Cx, under 10% each"
    - Fatty acid that you consider to be an undefined group of substances: >=0<5 % (w/w)

  You also provide information on C-chain length distribution of the alkyl diamine and of the fatty acid indicating that the C-chain length may range from C6 to C24.
- For the assessment, you provided predictions for the following structures: Fatty acid C18:1.

86 You have considered Fatty acid C18:1 as representative structure. You justify the selection of representative structure as follows: "*it represents the main constituent of the fatty acid part of the registered salt*".

87 However, ECHA disagrees with your selection of the representative structure, because the Substance has a complex composition that includes mono- and diamine fatty acid salts, amidification products and free fatty acids (including constituents with a wide range of C-chain length within each fraction). The selection of a single fatty acid (Fatty acid C18:1) constituent cannot be regarded as being representative of the Substance as a whole.

88 Therefore, you have not demonstrated that the prediction is adequate for the purpose of classification and labelling and/or risk assessment and your adaptation is rejected.

2.2.3.2. (Q)SAR results only are not sufficient to fulfil the information requirement under Annex VII, Section 9.2.1.1.

89 Guidance on IRs and CSA, Section R.7.9.5.1. specifies that (Q)SARs for predicting ready biodegradation are not yet sufficiently accurate to predict rapid degradation. However, when no useful information on degradability is available (either experimentally derived or estimated), (Q)SAR predictions can be used as supporting evidence of that the substance is not rapidly degradable.

90 For the reasons explained above, you dossier does not include any reliable experimental data on ready biodegradability for the Substance. You have used your QSAR predictions to conclude that the Substance is readily biodegradable. As explained above, (Q)SARs predictions alone is not adequate to conclude on the persistence of the Substance. Therefore, this information does not fulfil the information requirement and your adaptation is rejected.

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

92 In your comments on the draft decision, you agree to perform the requested study.

2.3. Study design and test specification

93 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.

94 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.

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).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).
All guidance documents are 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.

The deadline of the decision is set based on standard practices 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 currently longer lead times in contract research organisations.

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

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

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

You have provided comments during the decision-making phase which were found to address the incompliance identified in the draft decision and you included this information in an update of your registration dossier (submission date: 14 October 2022). Therefore the original requests for

- In vitro gene mutation study in bacteria,
- In vitro cytogenicity study in mammalian cells, and
- Sub-chronic toxicity study (90-day)

were removed.

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

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)

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

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,
- The reported composition must also include other parameters relevant for the property to be tested, in this case the relative abundance of monoamine, diamine and triamine, the distribution of C-chain length, the degree of unsaturation within each of fractions and the relative abundance of branched versus linear polyamines.

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

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[3].

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.

[3] https://echa.europa.eu/manuals