~~Confidential~~

Helsinki, 15 March 2023

Addressee
Registrant of Triamine_C18_unsat as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
26/10/2021

Registered substance subject to this decision ("the Substance")
Substance name: (Z)-N-(3-aminopropyl)-N'-9-octadecenylpropane-1,3-diamine
EC/List number: 249-276-6

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***24 March 2025.***

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

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

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

2. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202)

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

4. 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

5. 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)

6. 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)

7. Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.) to be combined with the Screening for reproductive/developmental toxicity requested below

8. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.64/OECD TG 422) by oral route, in rats. Due to reasons explained in Section 8. of Appendix 1, the test sample must be chosen to minimise gastrointestinal irritation and to allow investigation of intrinsic properties at adequate dose levels. This could be achieved by testing a neutral salt of the Substance.

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

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 addressee of the decision and its corresponding information requirements based on registered tonnage band are listed in Appendix 3.

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)

Contents

0. Reasons common to several requests 4

Reasons related to the information under Annex VII of REACH 11

1. In vitro gene mutation study in bacteria 11
2. Short-term toxicity testing on aquatic invertebrates 12
3. Growth inhibition study aquatic plants 18
4. Ready biodegradability 21

Reasons related to the information under Annex VIII of REACH 28

5. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study 28
6. In vitro gene mutation study in mammalian cells 30
7. Short-term repeated dose toxicity (28 days) 33
8. Screening for reproductive/developmental toxicity 35
9. Short-term toxicity testing on fish 37

References 40

0. Reasons common to several requests

0.1. Read-across adaptation rejected

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

- 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.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
- Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)
- 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 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. Scope of the grouping of substances (category)

5 You provide two read-across justification documents in the IUCLID Section 'Linked category' (entitled "[redacted]*.pdf*") and in IUCLID Section 13.2 (entitled "[redacted]*.pdf*"), respectively. ECHA understands that the justification provided under Section 13.2 (2016) replaces the earlier read-across justification document (2010) provided under section 'Linked category' and has assessed your read-across on this basis.

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

7 Linear polyamines

- Diamine C12/14: C12/14 propylene diamine (CAS RN 90640-43-0)
- Diamine O: Oleyl propylene diamine (CAS RN 7173-62-8)
- Triamine C: Coco dipropylene triamine (CAS RN 91771-18-5)
- 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)

- Triamine OV: Oleyl (vegetable oil) dipropylene triamine (CAS RN 28872-01-7), i.e. the Substance
- 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)
- Tetramine OV: Oleyl(vegetable oil) tripropylene tetramine (CAS RN 67228-83-5)

8 Branched polyamines

- Triamine Y12: Dodecyl dipropylene triamine, branched (CAS RN 2372-82-9)
- 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 variability of the alkyl chain length* […] *is suspected to influence aspects related to bioavailability, but not aspects of chemical reactivity, route of metabolism, 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*".
- "*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 1 or more repeating 1,3-diamino propane (DP) groups linked to a fatty amine. These can be linearly linked based on one DP and fatty amine (diamine), two DP and fatty amine (triamine structure: alkyl dipropylene triamine) or 3 DP with a fatty amine (tetramine structure: alkyl tripropylene tetramine), 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' in this case does not refer to the alkyl chain). 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*".

11 ECHA understands that this is the applicability domain of the grouping and your predictions are assessed on this basis.

0.1.2. Predictions for (eco)toxicological and fate properties

12 You provide a read-across justification document in IUCLID Section 13.2.

13 Toxicological properties

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

Triamine T	N-(3-aminopropyl)-N'-C16-18 (evennumbered), C18 unsaturated alkyl – propane-1,3-diamine (CAS RN 1219458-14-6)
Triamine C	Coco dipropylene triamine (CAS RN 91771-18-5)

15 You provide the following reasoning for the prediction of toxicological properties:

- "*Due to the identical position of the functional amine groups and the identical CH2 groups adjacent to the diamine group, no difference in chemical reactivity can be expected for this functional group.*"
- "*The variation of the length of the alkyl chains will result to some trends in their properties within each sub-group, consequently resulting in a possible trend in level of bioavailability, absorption and toxicokinetics.*"
- "*[…] 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 […]*"
- "*Metabolism profile is not expected to be principally different, and metabolites shows the same variation in alkyl chain lengths.*"
- "*Cytotoxicity at the local site of contact through disruption of cell membrane is considered the most prominent mechanism of action for toxic effects.*"

16 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance based on a worst-case approach.

17 Ecotoxicological properties

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

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)
Diamine S	Hydrogenated tallow propylene diamine (CAS RN 68603-64-5)
Triamine Y12	Dodecyl dipropylene triamine, branched (CAS RN 2372-82-9)
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).

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

19 You provide the following reasoning for the prediction of ecotoxicological properties: "*The tests reveal a comparable toxicity, independent of the alkyl chain length*".

20 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.

21 Fate properties

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

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

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

- "*All polyamines are found to be readily biodegradable*".

24 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.

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

0.1.2.1. Insufficient data density

26 Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances".

27 According to the Guidance on IRs and CSA, Section R.6.2.1.5., one of the factors in determining the robustness of a category is the density and distribution of the available data across the category. To identify a regular pattern and/or to derive reliable prediction of the properties of the members of the category, adequate and reliable information covering the range of structural variations identified among the category members needs to be available.

28 Furthermore, in larger categories there may be breaks in trends which could affect the reliability of interpolation (Guidance on IRs and CSA, Section R.6.2.2.2.). To confirm that there are no such breakpoints, adequate and reliable information needs to cover also substances within a range of homologous series.

29 You have provided information on:

- a single category member (*i.e.*, Triamine T) for screening for reproductive/developmental toxicity.
- Two category members (*i.e.*, Triamine T and Triamine C) for *in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study, *in vitro* gene mutation study mammalian cells, and short-term repeated dose toxicity.
- two category members (*i.e.*, Triamine C and Tetramine T) for Ready biodegradability

30 Information for one or two category members is not sufficient to establish a trend across the category consisting of 9 substances. Such limited information does not allow confirming

that there is no breakpoint in toxicity trend within the given range of chain length and that (i) the relative abundance of mono, di, tri and tetramine, (ii) the presence of amines in a branched or Y-amine form and (iii) the presence of unsaturation of the alkyl chain will not impact the predictions. Therefore, the information provided is not sufficient to conclude that (eco)toxicological properties are likely to follow a regular pattern.

31 In your comments on the draft decision, you state that "*data density and distribution should be evaluated on an endpoint specific basis IR &CSA R.6.2.1. Endpoints for which there is possibly sufficient data density (i.e. in vitro gene mutagenicity assay in bacteria) have been dismissed by ECHA under an entire category read-across rejection approach for all endpoints*".

32 ECHA notes that, in paragraph 29 above, the endpoints to which this issue applies are specified. Therefore, data density is not stated as a basis to reject your read-across adaptations for all endpoints listed under paragraph 1.

0.1.2.2. Inadequate or unreliable source studies.

33 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.

34 Specific reasons why the studies on the source substance does not meet these criteria are explained further below under the applicable information requirement sections 2, 4 to 7 and 9. Therefore, no reliable predictions can be made for these information requirements.

35 In your comments on the draft decision, you consider that "*common endpoints such as repeat dose studies should be assess in a WoE when read-across is utilize*".

36 ECHA notes that your registration currently does not provide any reference to weight of evidence in relation to the information requirement on repeated dose toxicity. In addition, Annex XI, Section 1.2. requires that adequate and reliable documentation is provided to describe a 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. ECHA notes that neither your dossier or your comments on the draft decision provides such documentation.

37 Finally, you state that "*in light of any new information presented in the following comments we ask ECHA to reconsider the validity of the read-across and studies requested in adherence to section R.6.2.2.1f, "In cases where there are convincing arguments for a read-across approach, the need to generate new data with tests on vertebrates should require a strong and convincing argument, whether to remove an unwanted classification or confirm a non-classification""*.

38 ECHA agrees that when a valid read-across adaptation is provided, including among others adequate and reliable studies on the source substance(s), normally no further information on the Substance is needed. However, for the reasons described throughout this decision, your read-across adaptations do not meet the requirements of Annex XI, Section 1.5.

0.1.2.3. Missing supporting information to substantiate worst-case consideration for toxicological properties

39 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.).

40 Supporting information must include information to confirm your claimed worst-case prediction.

41 As indicated above, your read-across hypothesis for the prediction of toxicological properties is based on the assumption that the source substance constitutes a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and the source substance(s) is necessary to confirm a conservative prediction of the properties of the Substance from the data on the source substance(s). Such information can be obtained, for example, from bridging studies of comparable design and duration for the source substance(s).

42 For the source substances Triamine T and Triamine C, you provide in vitro chromosome aberration and gene mutation tests in mammalian cells used in the prediction for in vitro genotoxicity in the registration dossier. For Triamine T, you provide sub-chronic toxicity study used in the prediction for repeated dose toxicity, and for Triamine C, you provide a 10 days short-term repeated dose toxicity study and a combined repeated dose toxicity study with reproduction/developmental toxicity screening test used in the prediction of repeated dose and reproductive toxicity in the registration dossier. Apart from these studies, your read-across justification or the registration dossier does not include any robust study summaries or descriptions of data for the Substance that would confirm a conservative prediction of the properties of the Substance.

43 In the absence of such information, you have not established that the source substance constitutes a worst-case for the prediction of the property under consideration of the Substance. Therefore you have not provided sufficient supporting information to scientifically justify the read-across.

44 In your comments on the draft decision, you further explain that the read across document "[redacted].pdf" refers to additional studies for the endpoints listed above. However, you acknowledge that "*ECHA was not provided robust study summaries on every category member's endpoints that were relevant*". You then state that "*the dossier was completed with only the relevant robust study summaries that were used in the final read-across to endpoints that were structurally similar and presented worst-case scenarios based off identifiable trends in endpoint data in relation to structure*". You then state that "*Triamine T and Triamine C are structurally very similar to the target chemical substantially limiting possibility of breakpoints in toxicity. Furthermore, Triamine T and Triamine C have an average shortened alkyl chain length which are considered worst-case as there is a significant amount of evidence that attribute shortening alkyl-chain length with increased levels of toxicity in human health studies in both alkyl monoamines and polyamines*". Finally, you state that "*ECHA's dismissal of the sub-category data on triamines in favor of greater data for the entire category will likely not lead to any further conclusions that have not already been established in this dossier*".

45 ECHA notes that your comments on the draft decision provide contradictory statement with regard the read-across approach submitted in your registration dossier. On the one hand, you refer to studies available on other category members from you read-across justification document as relevant information to support trend identification within the category as a whole. On the other hand, you refer to a sub-category of triamines, where according to you, *Triamine T and Triamine C* should be regarded as worst cases. ECHA further notes that your category justification document (including the category, definition, category

hypothesis and applicability domain) does specify in an unambiguous manner that you intend to rely on a sub-category of triamines. If this is you intention, you should revise your read-across justification document accordingly. Also ECHA emphasizes the absence of adequate supporting information for the claimed worst case considerations (as specified below under section 0.1.2.3.).

46 In your comments on the draft decisions, you also state that "*the worst-case approach is substantiated on the basis that decreased alkyl chain length and decreasing number of linear amines as independent variables results in decreased NOAELs for repeat dose studies*". You have provided references to the results of acute studies on Tetramine T, Tetramine O, Diamine O and Diamine C12/14 and sub-chronic studies on Triamine T and Diamine C12/14.

47 ECHA takes note of the provided information in your comments. However, you have not provided any study results on the Substance to confirm a conservative prediction of the properties of the Substance.

0.1.3. Conclusion on the read-across approach

48 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. In vitro gene mutation study in bacteria

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

1.1. Information provided

50 You have provided:

(i) an *in vitro* gene mutation study in bacteria (2009) with the Substance.

1.2. Assessment of the information provided

1.2.1. Insufficient information provided to confirm whether the test material is representative of the Substance

51 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). 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.

52 The study (i) has been conducted with N-(3-aminopropyl)-N'-octadec-9-en-1-ylpropane-1,3-diamine (EC No 249-276-6, CAS RN 28872-01-7). You have not provided any information on purity and composition (including carbon chain length, saturation, branching when relevant).

53 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.

54 In your comments on the draft decision, you have provided the certificate of analysis of the test material which shows that its composition was consistent with the identity of the Substance. The information provided as part of your comments addresses the missing information identified above. 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.

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

55 To fulfil the information requirement, a study must comply with the OECD TG 471 (Article 13(3) of REACH). Therefore, the following specifications must be met:

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

56 In study (i) described as an in vitro gene mutation study on bacteria:

a) you have not reported that the positive control substances 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, in your comments, you have provided p-values calculated for positive control when compared to solvent controls using a two-sample t-test assuming equal variance. You state that "*all positive controls were found to have a p-value < 0.01*".

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 should be outside the distribution of the historical negative control data. You have used a parametric test (t-test) which is formally not applicable to small sample size (i.e., only three observations per condition in study (i)) as it cannot be verified whether observations follow a normal distribution and show homoscedasticity (as required by such test). However, while the statistical analysis is erroneous, the information you provided is indicative that positive controls induced a clear positive response when compared to concurrent solvent controls. The information provided as part of your comments addresses the missing information identified above. 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.

b) 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 provided the missing information listed and you stated your intention to submit this information in an update of your registration dossier. You will have to submit this information in an updated registration dossier by the deadline set in the decision

57 Therefore, as the information provided in your comments on the draft decision has not yet been provided in your registration dossier, the information requirement is currently not fulfilled.

2. Short-term toxicity testing on aquatic invertebrates

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

2.1. Information provided

59 You have 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:

(i) a study on short-term toxicity to aquatic invertebrates (2002) according to OECD

TG 202 with the analogue substance Dodecyl dipropylene triamine, branched (CAS RN 2372-82-9).

(ii) a study on short-term toxicity to aquatic invertebrates (1997) according to EPA OTS 797.100 with the analogue substance Oleyl(vegetable oil) tripropylene tetramine (CAS RN 67228-83-5)

(iii) a study on short-term toxicity to aquatic invertebrates (1992) according to OECD TG 202 with the analogue substance 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).

(iv) a study on short-term toxicity to aquatic invertebrates (1999) according to OECD TG 202 with the analogue substance Oleyl propylene diamine (CAS RN 7173-62-8)

(v) a study on short-term toxicity to aquatic invertebrates (1992) according to OECD TG 202 with the analogue substance Oleyl propylene diamine (CAS RN 7173-62-8)

60 You have also provided the following information on the Substance:

(vi) a study on long-term toxicity to aquatic invertebrates (2010) according to OECD TG 211.

61 Under Section 6.1.3. of IUCLID, you state that "*Most of the short-term toxicity daphnia tests were conducted in a period when no reliable specific method of analyses was available. The concentrations were therefore not analytically verified, the reliability of the results is limited because of the poor solubility of the test substances and partial sorption onto the walls of test vessels*" and "*For oleyl triamine a long term test result is available with specific chemical analysis which is used for the hazard assessment because the results can be used for the bulk approach where the sorption to glassware was minimal and solution stability much better*". While you have not explicitly claimed such adaptation, ECHA has also assessed study (ii) under Column 2 of Annex VII, Section 9.1.1, second indent.

2.2. Assessment of the information provided

2.2.1. Read-across adaptation rejected

62 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.

2.2.1.1. Insufficient information provided to confirm test material identity (studies (i) to (v))

63 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.

64 The study (i) to (v) have been conducted with the UVCB substances listed above. For studies (i) and (ii), you claimed that the test material was representative of the boundary composition of the respective registered substances. However, you did not provide any information on purity and composition (including carbon chain length, saturation, branching when relevant) to support your claim. For study (iii), you only state that the "active content" was [redacted]%. For study (iv), you did not provide any information on purity and composition. Finally, for study (v), you only report that the "active content" was [redacted]%.

65 In your comments on the draft decision, you state that "*Test material identity for several of the indicated studies will be reviewed by the respective study owners in the polyamines consortium*". You did not provide new information as part of your comments on the draft decision and therefore ECHA is not in a position to conduct an independent assessment. As a result, the deficiency remains.

66 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.

2.2.1.2. Inadequate or unreliable studies on the source substances (studies (i) to (v))

67 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 202. If the analogue substance is difficult to test, the requirements of OECD GD 23 must be followed. Therefore, the following specifications must be met:

68 Characterisation of exposure

a) analytical monitoring must be conducted. A reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range must be available;

69 Reporting of the methodology and results

b) adequate information on the analytical method (including performance parameters of the method) and on the results of the analytical determination of exposure concentrations is provided.

70 In study (i) to (v) are described as short-term toxicity studies on daphnids:

71 Characterisation of exposure

a) no analytical monitoring was conducted for studies (i) to (iii) and (v). You state that "*Most of the short-term toxicity daphnia tests were conducted in a period when no reliable specific method of analyses was available. The concentrations were therefore not analytically verified, the reliability of the results is limited because of the poor solubility of the test substances and partial sorption onto the walls of test vessels*";

72 Reporting of the methodology and results

b) information on the results of the analytical determination of exposure concentrations is not provided for study (iv).

73 In your comments on the draft decision, you state that "[d]*etailed information on specificity, recovery efficiency, precision, limits of determination and working range is available in the study report on the analytical method used for the quantification of the exposure concentrations in the test*". You further acknowledge that "the working range of the

analytical method was unfortunately not low enough in 2002 to verify all test concentrations". You did not provide any information on the analytical method as part of your comments on the draft decision and therefore ECHA is not in a position to conduct an independent assessment. As a result, the deficiency remains.

74 Based on the above, there are critical methodological deficiencies resulting in the rejection of these studies. More specifically, you have not demonstrated that exposure was satisfactorily maintained in any of these studies and that effect concentrations can be expressed based on nominal concentrations. Therefore, this study does not provide a reliable coverage of the key parameters addressed in the OECD TG 202.

2.2.2. Assessment of your adaptation under Column 2 of Annex VII, Section 9.1.1, second indent

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

75 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). 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.

76 The study (vi) has been conducted with a test material considered by you as representative to the Substance. You provide the following information on the test material: "*Primary fatty amine:* [redacted] *(area %), Di amine:* [redacted] *(area %), Tri amine:* [redacted] *(area %), Tetra amine:* [redacted] *(area %)*". You have not provided information on the distribution of the carbon chain length, on the presence of unsaturated constituents and, if relevant, on branching.

77 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.

78 In your comments on the draft decision, you explain that the study was conducted with a test material from the same batch as the one used to conduct the algae study (see section 3.1). You have provided the certificate of analysis of the test material which shows that its composition was consistent with the identity of the Substance. The information provided as part of your comments addresses the missing information identified above. 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.

2.2.2.2. The provided long-term study (vi) is not reliable.

79 To fulfil the information requirement for long-term toxicity on aquatic invertebrates, a study must comply with the OECD TG 211 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:

80 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;

81 Characterisation of exposure

b) if the concentrations of the test material in a semi-static test are not expected to remain within ± 20 % of the nominal concentration, then all test concentrations must be determined when freshly prepared and at the time of renewal on one occasion during each week of the test;

82 Additional requirements applicable to difficult to test substances

c) a continuous flow through exposure system is used if exposure concentrations cannot be maintained within 80-120% of nominal in a semi-static exposure system with a renewal frequency of 24 hours.

83 In study (ii) described as a long-term toxicity study on daphnids according to OECD TG 211:

84 Technical specifications impacting the sensitivity/reliability of the test

a) you specify that natural river water from the river Leine was used as test medium. You report that the TOC concentration was [redacted] mg/L;

85 Characterisation of exposure

b) you report measured concentrations that are below ± 20 % of the nominal concentration in both fresh and old media throughout the test at [redacted], [redacted] and [redacted] µg/L. However, you have not provided the results of the analytical monitoring for the test at [redacted] µg/L and you have provided a single measurement at [redacted] µg/L;

86 Additional requirements applicable to difficult to test substances

c) test was conducted under semi-static conditions with a renewal rate of test solutions (frequency) of 3 times per week. Measured test concentrations were:

- [redacted] µg/L: recovery in fresh media ranged from 52 to 87% and in old media from 0% (i.e. measured value was below LOQ) to 44%
- [redacted] µg/L (measured only in one occasion): recovery in fresh media was 9% and in old media recovery was 0% (i.e. measured value was below LOQ);
- [redacted] µg/L: recovery in fresh media ranged from 0% (i.e. measured value was below LOQ) to 167% and in old media recovery was always 0% (i.e. measured value was below LOQ).

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

- 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. You justify the use of natural water with high TOC by referring to the "bulk approach" (ECETOC, 2001). However, 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.

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.

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*".

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.

- the monitoring of exposure concentrations did not cover all required concentrations and, for some concentrations did not have an appropriate frequency over the exposure phase.
- the test design for the study was not adequate to maximize the exposure to the test material. The reported results on the analytical monitoring of exposure shows that concentrations were not maintained below ± 20 % of the nominal concentration. However, you have not attempted to increase the frequency of test medium renewal to 24 hours or used a flow-through test set-up as required by the OECD GD 23.

88 Therefore, the study submitted in long-term toxicity study present in your registration dossier does not provide an adequate and reliable coverage of the key parameter(s) of the corresponding OECD TG 211.

89 Therefore, the requirements of Column 2 of Annex VII, Section 9.1.1, second indent are not met.

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

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

2.3. Study design and test specifications

92 The Substance is difficult to test due to the low water solubility (CMC of 73 mg/L), surface activity (surface tension of 29 mN/m at 1 g/L), adsorptive properties (Kd up to 220000 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.

93 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).

94 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.

95 In your comments on the draft decision, you state that "[i]n *the absence of any algae in the test solutions and test concentrations in the 10 – 100 µg/L range, it is expected that relatively reliable results can be obtained for the verification of the exposure concentrations*". ECHA takes note that you consider the analytical monitoring of exposure to the Substance as technically feasible.

96 In the comments to the draft decision, you also state that "the substance is extremely sorbing to any surface to which it is in contact. Establishing constant exposure concentrations in the test solutions without using solvent will be very difficult or impossible to achieve. The use of solvents will make the substance more bioavailable and due to that unrealistically more toxic. The use of solvents should therefore be restricted to situations where no other acceptable method of test solution preparation is available. For this reason, there is a clear preference for a semi-static test setup with a renewal frequency of 24h". ECHA acknowledges the technical challenges in conducting adequate analytical monitoring of exposure for cationic surfactants such as the Substance. When conducting the new study, 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.

3. Growth inhibition study aquatic plants

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

3.1. Information provided

98 You have provided a toxicity study to aquatic algae and cyanobacteria (2009) according to OECD TG 201 with the Substance.

3.2. Assessment of the information provided

3.2.1. Insufficient information provided to confirm whether the test material is representative of the Substance

99 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). 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.

100 The study above has been conducted with N-(3-aminopropyl)-N'-octadec-9-en-1-ylpropane-1,3-diamine (CAS RN 28872-01-7), which you consider representative of the Substance. You provide the following information on the test material: "*Primary fatty amine:* [redacted] *(area %), Di amine:* [redacted] *(area %), Tri amine:* [redacted] *(area %), Tetra amine:* [redacted] *(area %)*". You have not provided information on the distribution of the carbon chain length, on the presence of unsaturated constituents and, if relevant, on branching.

101 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.

102 In your comments on the draft decision, you have provided the certificate of analysis of the test material which shows that its composition was consistent with the identity of the Substance. The information provided as part of your comments addresses the missing information identified above. 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.

3.2.2. The provided study does not meet the specifications of the test guideline

103 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:

104 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;

105 Characterisation of exposure

b) the concentrations of the test material are measured at least at the beginning and end of the test:

- at the highest, and
- at the lowest test concentration, and
- at a concentration around the expected EC_{50}.

c) for volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24 hour intervals is required;

106 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.

107 In the provided study described as a toxicity study to aquatic algae and cyanobacteria according to OECD TG 201:

108 Technical specifications impacting the sensitivity/reliability of the test

a) the test medium is described as Water from the river Innerste. You have provided the following justification for not using one of the two alternative growth medium of OECD TG 201: "*The aquatic ecotoxicity tests with polyamines were therefore performed in river water to allow a PECaquatic, bulk/PNECaquatic, bulk approach and is considered to be conservative but more environmentally realistic than the standard method";*

109 Characterisation of exposure

b) the concentration of the test material was only verified at beginning and end of the test, at the second lowest and the second highest test concentration ([redacted] and [redacted] mg/L);

c) as explained under request 2, 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;

110 Additional requirements applicable to difficult to test substances

d) as already explained under request 2, the substance is considered to be highly adsorptive. You report that the test was conducted with natural freshwater with a TOC content of [redacted] mg/L.

111 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. You justify this deviation by referring to the "bulk approach" (ECETOC, 2001). As already explained under section 2.2.2.2., the bulk approach is not adequate for the purpose of classification and labeling and the PBT assessment.
  In the comments to the draft decision, you provided the same comments on the bulk approach as those detailed under Request 2. ECHA's reply equally applies to this information requirement.
- 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.
  In the comments on the draft decision, you refer to the use of the bulk approach to justify this deviation by the use of the "bulk approach". However, for the reasons already stated above and under Request 2, tests conducted using this approach do not meet the information requirement.

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

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

3.3. Study design and test specifications

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

4. Ready biodegradability

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

4.1. Information provided

116 You have 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:

(i) a ready biodegradability study (2009) according to OECD TG 301D with the analogue Substance Coco dipropylene triamine (CAS RN 91771-18-5)

(ii) a ready biodegradability study (1992) according to OECD TG 301B 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)

117 Finally you have also attached the following publicly available literature references:

(iii) Raymond, D. & Alexander M. (1977). Bacterial Metabolism of quaternary ammonium compounds. Appl. Environ. Microbiol., 33 , 1037-1041

(iv) CG van Ginkel (1995). Biodegradation of cationic surfactants; Biodegradation of surfactants. Eds M.R. Porter and R. Karsa In Blackie Academic & Professional pp 183 203.

(v) CG van Ginkel (1996). Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms. Biodegradation 7; 151-164.

(vi) CG van Ginkel (2007). Ultimate biodegradation of ingredients of cleaning agents. Handbook of Cleaning Agents/Decontamination of Surfaces, Eds. I Johansson and P Somasundaran; Elsevier, Amsterdam the Netherlands Vol. 2, pp 655-694.

(vii) [redacted] (2009). [redacted]

(viii) Rothkopf, G.S. and R Bartha (1984). Structure-Biodegradability correlation among xenobiotic industrial amines. JAOCS 61 977-980.

(ix) PJ Large (1992). Enzymes and pathways of polyamine breakdown in micro-organisms. FEMS Microbiol Rev 88 249-262.

(x) Kluyver AJ and Donker HJL (1926). Die einheit in der BiochemieChemie der Zelle und Gewebe 13: 134-190

118 The information listed under (iii) to (vi) and (viii) has unclear relevance as it relates to the biodegradation of substances which are outside the scope of your category approach. The information listed under (iv) to (vi) correspond to review articles which cannot be subject to an independent assessment. Study (vii) is a summary of a preliminary study to study (i) above investigating the impact of test conditions (in particular, adaptation, nature of the inoculum, addition of various sorbent) on mineralisation under the conditions of the OECD TG 301D. The source of information (ix) to (x) are review articles of degradation pathways and does not provide information on ready biodegradability.

119 None of the information listed under (iii) to (x) provide relevant information to meet this information requirement, therefore this information is not assessed further.

120 In your comments to the draft decision, you specify that you provided this information in a "*weight of evidence argumentation based on Expert Judgment* […] *which considers the biodegradation potential of all Alkyl polypropylenepolyamines*".

121 However, your dossier does not contain any weight of evidence adaptation and your comment referring merely to an "*expert judgement for the weight of evidence argumentation*". In the absence of an explicit reference to such adaptation and of a clear justification explaining why the sources of information together provide a conclusion on the information requirement, ECHA understands that you did not intended fulfil the information requirement with a weight of evidence adaptation under Section 1.2 of Annex XI.

4.2. Assessment of information provided

4.2.1. Read-across adaptation rejected

122 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.

4.2.2. Insufficient information provided to confirm test material identity (studies (i) and (ii))

123 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.

124 The studies (i) and (ii) have been conducted with the UVCB substances listed above. For the test material used in study (i), you report that it contains "*coco dipropylene triamine ([redacted]%), coco propylene diamine ([redacted]%), coco propylene tetramine ([redacted]%), coco amine ([redacted]%)*". You did not provide any information on carbon chain length, and if relevant, saturation and branching. For study (ii) you have not provided any information on purity or composition (you state that no data about purity is available).

125 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 intended to be tested for studies (i) and (ii).

4.2.3. Ready biodegradation tests are normally intended for pure substances (studies (i) and (ii))

126 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.

127 You have provided studies conducted on UVCBs which you describe as mixtures of monoamine, diamine and branched triamines with various Carbon chain length. The test material in study (ii) also includes constituents with alkyl chain substituents that can be saturated or mono-unsaturated.

128 The test materials used in studies (i) and (ii) are complex substances 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.

129 In your comments on the draft decision, you agree that *"Ready biodegradation screening tests have a low distinguishing power and* [you] *therefore agree with ECHA on the remark that a single ready biodegradability test result of the substance as a whole does not allow to conclude on ready biodegradability of all constituents*".

4.2.4. Inadequate or unreliable studies (i) and (ii) on the source substances

130 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, the following specifications must be met:

131 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 a study according to OECD TG 301B, the concentration of the test material is in the range of 10-20 mg DOC (or TOC)/L.

132 Reporting of the methodology and results

c) the source of the inoculum, its concentration in the test and any pre-conditioning treatment are reported;
d) 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 301B, 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;
e) for OECD TG 301D, 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);
g) the results of measurements at each sampling point in each replicate is reported in

a tabular form.

133 Your registration dossier provides studies claimed to be conducted according to OECD TG 301B and D showing the following:

134 Technical specifications impacting the sensitivity/reliability of the test

a) for studies (i), 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*";
b) for study (ii), the test material concentration corresponded to 27.7 mg DOC/L;

135 Reporting of the methodology and results

c) for study (ii), you have not specified whether the inoculum was adapted to the test material and whether the inoculum came from a STP treating predominantly domestic effluents;
d) for study (i), you have not specified the volume of inoculum added to the test bottles and you have not reported inoculum density in cells/mL. for study (ii), you specify that the inoculum concentration was 1.25 x 10^8 cells/L
e) for studies (i), you provide a generic description of the equation used to derive the $ThOD_{NH3}$. However, you do not describe how this equation was used considering the UVCB nature of the tested substance.
f) you have not reported whether a correction for nitrification was applied on the theoretical oxygen demand for study (i).
g) the results of measurements at each sampling point in each replicate is not reported in a tabular form for study (ii).

136 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically,
    - in study (i), 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.

      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*".

      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.

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).

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 study (i), the respiration in the inoculum blank after 28 days was already close to the cut-off value (i.e. 1 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.

- the information you provided on study (ii) indicates that the test material concentration was above the maximum values specified in OECD TG 301B;

- the reporting of the studies 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 (i), it is not possible to verify if the inoculum density was low enough to be consistent with the specifications of OECD TG 301D.

    In your comments on the draft decision, you specify regarding study (i) that "[t]*he Heveadorp sampling site is in use for many years and the historical viable bacteria count* […] *was always <1 * 10^6 CFU/L"*. However, you provided no information in support of your claim and therefore it remains not possible to verify that inoculum density was appropriate for study (ii) and (iii).

    You state that "[f]*or new tests* [you] *will provide the bacterial density (cells/L) of the inoculum of the test*".
  - you have not specified how ThOD was estimated for study (i) 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 for study (i), "*the ThODNH3 and ThODNO3 of 2.79 and 3.39 mg/mg were both calculated […] using an elemental composition of C18.6 H39.7 N2.8 which was calculated from the information included in the CoA of triamine C"*. You also provide the calculation of the % degradation after applying a correction for nitrification. You explain that you will provide this information in an update of the registration dossier.
    ECHA considers this information addresses the deficiency identified above.

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.

- For study (ii), the source of the inoculum is unclear. Further, in the absence of the results of measurements at each sampling point in each replicate, the validity criteria of the test guideline and the interpretation of the results cannot be assessed.

137 Therefore, studies (i) and (ii) do not meet the requirements of the corresponding test guideline.

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

4.3. Study design and test specification

139 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. The Substance is a UVCB that includes amines, diamines, triamines and tetramines with varying Carbon chain length. Some constituent are also unsaturated.

140 The Substance is a complex substance and contains constituents with significant structural differences described above.

141 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.

142 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.

143 In your comments on the draft decision, you state that "*based on the shared biodegradation pathway and the broad substrate specificity of microorganisms degrading these linear polypropylene amines with respect to the alkyl chain length, it is unlikely that biodegradability (the potential for biodegradation) of alkylamines differs significantly with varying chain lengths*". You however acknowledge that "[d]*egradation observed in ready biodegradability tests with these alkyl polypropylene amines were however not the same*" and that "[o]*bserved differences in the ready biodegradability tests can be explained by biocidal effects and/or limited bioavailability*". To demonstrate these differences between

the constituents you propose to perform ready biodegradation screening tests with Tetramine T as a whole and in case ready biodegradability is observed for the substance as a whole also a test will be performed with one realistic worst-case constituent (having a long alkyl chain length). You intend to provide sufficient justification for the reasonable worst-case selection of testing a constituent with a low bioavailability.

144 ECHA considers that if, after obtaining a positive result in a ready biodegradability study on the selected analogue substance, you can demonstrate through further testing that its worst-case constituent meets the criteria for ready biodegradability, it will we reasonable to conclude that the selected analogue can regarded as readily biodegradable. However, ECHA notes that this strategy requires to provide adequate supporting information to justify the selection of the worst-case constituent.

145 Further, as this strategy relies on data which is yet to be generated for the proposed category member, no conclusion on the compliance of the proposed adaptation can be made. You remain responsible for complying with this decision by the set deadline.

Reasons related to the information under Annex VIII of REACH

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

146 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.

5.1. Information provided

147 You have 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:

(i) *in vitro* mammalian chromosome aberration test (2009) with an analogue substance N-(3-aminopropyl)-N'-C16-18 (evennumbered), C18 unsaturated alkyl -propane-1,3-diamine/Tallow dipropylene triamine (List No 628-863-4, CAS RN 1219458-14-6).

(ii) *in vitro* mammalian chromosome aberration test (2009) with an analogue substance N-(3-aminopropyl)-N-(C12-18 evennumbered) alkyl-propane-1,3-diamine/Coco dipropylene triamine (CAS RN 91771-18-5).

5.2. Assessment of the information provided

5.2.1. 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. In addition, ECHA identified endpoint specific issue(s) addressed below.

5.2.2. Insufficient information provided to confirm test material identity (studies (i) and (ii))

149 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.

150 The studies (i) and (ii) have been conducted with the UVCB substances listed above. For these studies, you stated only that the test material was "technical grade", but you did not provide any other information on purity and composition (including carbon chain length, saturation, branching when relevant).

151 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 intended to be tested.

152 In your comments on the draft decision, you have provided the certificate of analysis of the test materials used in studies (i) and (ii). The information provided as part of your

comments addresses the missing information identified above. 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.

5.2.3. Inadequate or unreliable studies (i) and (ii) on the source substance

153 As explained in Section 0.1., 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 473. Therefore, the following specifications must be met:

a) the maximum concentration tested induces 55+5% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration corresponds to 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest;
b) data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures is reported.

154 In studies (i) and (ii) described as an in vitro mammalian chromosome aberration tests:

a) the maximum tested concentration is based on cytotoxicity but it is not reported whether it induced 55±5% of cytotoxicity compared to the negative control as it is only stated "*appropriate toxicity was reached*";

   In your comments to the draft decision you provide full study reports for studies (i) and (ii). These report states that "*based on the results of the dose range finding test an appropriate range of dose levels was chosen for the cytogenetic assays considering the highest dose level had an inhibition of the mitotic index of 50% or greater whereas the mitotic index of the lowest dose level was approximately the same as the mitotic index of the solvent control*". You provide results showing full cell lysis occurred at 33 µg/mL and above in both studies. This information addresses the issue identified above.

b) the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures were not reported.

155 In your comments on the draft decision, you have provided the information listed under points a) and b) for studies (i) and (ii). The information provided as part of your comments addresses the missing information identified above. 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.

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

156 In your comments on the draft decision, you state that the "[a]*nalogues presented in the dossier and the read-across document for this endpoint not only can be used for additional strength of evidence for the endpoint, but also act as bridging studies for the category establishing a reliably predictable trend that has basis in the similar structural and physicochemical properties of the category.* […] *There is sufficient data density to establish a trend that results in a negative patten across all category members (including other branched triamines*)". On this basis we understand that you intended to invoke a weight-of evidence adaptation under section 1.2 of Annex XI of REACH.

5.4. Assessment of the weight of evidence provided in your comments on the draft decision

157 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.

158 However, you only rely on information from analogues substances and the read-across is rejected for the reasons specified under Section 0.1. More specifically, as already explained above, your registration dossier includes information on two analogue substances. You have provided no additional study on other analogues to cover the proposed category. While your read-across justification document refers to studies on other analogues, ECHA is not in a position to assess the reliability of this information. Therefore, you have not provided adequate information to justify the claimed trend of "*negative patte*[r]*n across all category members"*

5.4.1. Lack of documentation justifying the weight of evidence adaptation

159 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.

160 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.

161 Therefore, while you claim you intend to use the information currently in your registration dossier as a weight of evidence, the requirements of Annex XI, Section 1.2 are currently not met. On this basis, the information requirement is not fulfilled.

5.5. Specification of the study design

162 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.

6. In vitro gene mutation study in mammalian cells

163 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.

6.1. Triggering of the information requirement

164 Your dossier contains data 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.

165 The information for the in vitro gene mutation study in bacteria and for the in vitro cytogenicity study in mammalian cells provided in the dossier are rejected for the reasons provided in requests 1 and 5.

166 The result of the requests for an in vitro gene mutation study in bacteria, and for an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study 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.

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

6.2. Information provided

168 You have 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:

(i) *in vitro* mammalian cell gene mutation test (2009) with an analogue substance N-(3-aminopropyl)-N'-C16-18 (evennumbered), C18 unsaturated alkyl -propane-1,3-diamine/Tallow dipropylene triamine (List No 628-863-4, CAS RN 1219458-14-6).

(ii) *in vitro* mammalian cell gene mutation test (2009) with an analogue substance N-(3-aminopropyl)-N-(C12-18 evennumbered) alkyl-propane-1,3-diamine/Coco dipropylene triamine (CAS RN 91771-18-5).

6.3. Assessment of the information provided

6.3.1. Read-across adaptation rejected

169 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.

6.3.2. Insufficient information provided to confirm test material identity (studies (i) and (ii))

170 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.

171 The studies (i) and (ii) have been conducted with the UVCB substances listed above. For these studies, you stated only that the test material was "technical grade", but you did not provide any other information on purity and composition (including carbon chain length, saturation, branching when relevant).

172 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.

173 In your comments on the draft decision, you have provided the certificate of analysis of the test materials used in studies (i) and (ii). The information provided as part of your comments addresses the missing information identified above. 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.

6.3.3. Inadequate or unreliable studies (i) and (ii) on the source substance

174 As explained in Section 0.1., 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 476 or 490. Therefore, the following specifications must be met:

a) the maximum concentration tested induces 80-90% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration corresponds to 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest;
b) the concurrent positive controls induce responses that are compatible with those generated in the historical positive control database
c) the concurrent positive controls produce a statistically significant increase compared with the concurrent negative control;
d) data on the cytotoxicity and the mutation frequency for the treated and control cultures are reported.

175 In study (i) described as an in vitro gene mutation study in mammalian cells:

b) you have not reported whether the concurrent positive controls induced responses that are compatible with those generated in the historical positive control database
c) you have not reported whether the concurrent positive controls produced a statistically significant increase compared with the concurrent negative control;
d) data on the cytotoxicity is only reported for the highest tested concentration and the mutation frequency for the treated and control cultures are not reported.

176 In your comments on the draft decision, you provided full a study report for study (i). This additional information addresses the reporting deficiencies listed under point b) to d) and confirm the validity of the study. 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.

177 In study (ii) described as an in vitro gene mutation study in mammalian cells:

a) the maximum concentration tested concentration did not induce 80-90% of cytotoxicity compared to the negative control, or the precipitation of the tested substance, and it was less than 10 mM, 2 mg/mL or 2 µL/mL in the presence of metabolic activation.

   In your comments to the draft decision you provide a full study report for study (ii). In this report, it is stated that "*In the absence of S9-mix, the relative suspension growth was 4% at the test substance concentration of 3 µg/ml compared to the relative suspension growth of the solvent control. No cell survival was observed at test substance concentrations of 10 µg/ml and above. In the presence of S9-mix, the relative suspension growth was 85% at the test substance concentration of 10 µg/ml compared to the relative suspension growth of the solvent control. No cell survival was observed at test substance concentrations of 33 µg/ml and above*". You provide results showing cell survival in the dose range-finding experiments. This information addresses the issue identified above.

d) data on the cytotoxicity is only reported for the highest tested concentration and the mutation frequency for the treated and control cultures are not reported.

178 In your comments on the draft decision, you provided full a study report for study (ii). This additional information addresses the reporting deficiencies listed under point a) and d) and confirm the validity of the study. 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.

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

180 In your comments on the draft decision, you provided the same considerations as already detailed under Request 5 with reference to your intention to use data from your dossier part of a weight of evidence and the same claim that existing information is sufficient to demonstrate a trend of absence of effects throughout the category.

181 ECHA's replies provided under Request 5 equally apply to this information requirement.

6.4. Specification of the study design

182 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.

7. Short-term repeated dose toxicity (28 days)

183 A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII, Section 8.6.1.

7.1. Information provided

184 You have 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:

(i) a sub-chronic 90 days repeated dose toxicity study via oral route in rats (2013) with an analogue substance N-(3-aminopropyl)-N'-C16-18 (evennumbered), C18 unsaturated alkyl -propane-1,3-diamine/Tallow dipropylene triamine (List No 628-863-4, CAS RN 1219458-14-6).

(ii) a combined repeated dose toxicity study with reproduction/developmental toxicity screening test (2010) with an analogue substance N-(3-aminopropyl)-N-(C12-18 evennumbered) alkyl-propane-1,3-diamine/Coco dipropylene triamine (CAS RN 91771-18-5).

(iii) a range finding and maximum tolerated dose study via oral route in rats (2010) with an analogue substance N-(3-aminopropyl)-N-(C12-18 evennumbered) alkyl-propane-1,3-diamine/Coco dipropylene triamine (CAS RN 91771-18-5).

7.2. Assessment of the information provided

7.2.1. Read-across adaptation rejected

185 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.

7.2.2. Insufficient information provided to confirm test material identity (studies (i) to (iii))

186 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.

187 The studies (i) to (iii) have been conducted with the UVCB substances listed above. For these studies, you stated only that the test material was "technical grade", but you did not provide any other information on purity and composition (including carbon chain length, saturation, branching when relevant).

188 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.

189 In your comments on the draft decision, you have provided the certificate of analysis of the test materials used in studies (i) and (ii). The information provided as part of your comments addresses the missing information identified above. 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.

7.2.3. Inadequate or unreliable study (iii) on the source substance

190 As explained in Section 0.1., the study to be read across must have an adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3), in this case EU B.7/OECD TG 407. Therefore, the following specifications must be met:

a) testing is performed with at least three dose levels (unless conducted at the limit dose) and with concurrent controls;
b) at least 5 male and 5 female animals are used for each concentration and control group;
c) dosing of the test substance is performed daily for a minimum of 28 days;
d) functional observations (i.e., sensory activity, grip strength and motor activity) are made during the fourth exposure week;
e) terminal organ weights are measured;
f) full histopathology, including incidence and severity, is performed as specified in paragraphs 47-49 of the test guideline;

191 The study (iii) is described as short-term repeated dose toxicity study. However, the following specifications are not according to the requirements of OECD TG 407:

a) only two dose levels and no concurrent controls were described;
b) only 3 males and 3 females were included in each test group;
c) the exposure duration was limited to 10 days;
d) the following functional aspects were not assessed: sensory activity, grip strength and motor activity;
e) terminal organ weights were only recorded for the following organs: adrenal glands, heart, kidneys, liver, lung, spleen, thymus;
f) histopathological examination was not performed.

192 The information provided does not cover the specification(s) required by the OECD TG 407.

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

193 In your comments on the draft decision, you state that "*repeat dose studies should be assess in a WoE when read-across is utilize*" and ECHA understands that you intended to invoke a weight of evidence adaptation under section 1.2 of Annex XI.

7.4. Assessment of the weight of evidence provided in your comments on the draft decision

194 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.

195 However, you only rely on information from analogues substances and the read-across is rejected for the reasons specified under Section 0.1. More specifically, as already explained above, your registration dossier includes information on two analogue substances. You have provided no additional study on other analogues to cover the proposed category. While your read-across justification document refers to studies on other analogues, ECHA is not in a position to assess the reliability of this information.

7.4.1. Lack of documentation justifying the weight of evidence adaptation

196 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.

197 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.

198 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.

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

7.5. Specification of the study design

200 When there is no information available neither for the 28-day repeated dose toxicity (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the same time fulfil the information requirement of REACH Annex VIII, Section 8.6.1 and that of REACH Annex VIII, Section 8.7.1. (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

201 For information on the study design see request for OECD TG 422 below.

8. Screening for reproductive/developmental toxicity

202 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.

8.1. Information provided

203 You have 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:

(i) a combined repeated dose toxicity study with reproduction/developmental toxicity screening test (2010) with an analogue substance N-(3-aminopropyl)-N-(C12-18 evennumbered) alkyl-propane-1,3-diamine/Coco dipropylene triamine (CAS RN 91771-18-5).

8.2. Assessment of the information provided

8.2.1. Read-across adaptation rejected

204 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.

8.2.2. Insufficient information provided to confirm test material identity (study (i))

205 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.

206 The study (i) has been conducted with the UVCB substance listed above. For this study, you stated only that the test material was "technical grade", but you did not provide any other information on purity and composition (including carbon chain length, saturation, branching when relevant).

207 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.

208 In your comments on the draft decision, you have provided the certificate of analysis of the test material used in study (i). The information provided as part of your comments addresses the missing information identified above. 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.On this basis, the information requirement is not fulfilled.

8.3. Specification of the study design

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

210 The Substance is a corrosive liquid and you apply a self-classification as Skin Corr. 1C (H314). Corrosive or highly irritating substances must be tested preferably via the oral route. Therefore, the study must be conducted with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.). However, testing at concentration/dose levels causing corrosivity must be avoided. Testing of neutral salts of alkaline or acidic substances is therefore more appropriate as it allows the investigation of intrinsic properties at adequate dose levels.

211 The test sample must be chosen to minimise gastrointestinal irritation and to allow investigation of intrinsic properties at adequate dose levels. This could be achieved by testing a neutralised salt of the Substance.

212 If the Screening for reproductive/developmental toxicity study submitted in response of this decision does not deliver reliable results because of gastrointestinal irritation, further testing may be considered necessary in order to investigate the intrinsic properties at adequate dose levels. Therefore, if the Member State competent authorities consider that a concern must be clarified in that respect, they may decide to require further testing under Substance Evaluation.

213 In your comments on the draft decision, you "*disagree with ECHA's stance that neutralized salts of test material must be used for identification of intrinsic properties at adequate dose levels*" for the following reasons:

- you are not aware of any area of application where the Substance is used in neutralized form. Hence, studies conducted with neutralized test material do not reflect realistic exposure scenarios relevant for the registered substance.
- You state that REACH does not require to assess substances that results from chemical reactions that occurs when pH neutralizers are used and you refer to the ECHA Guidance for Annex V on Exemptions from the obligations to register.
- You state that "*testing neutralized test material means to assess the toxicity of corresponding salt, which practically means testing on different substances than the test material which would be subject separate registrations in most if not all cases. In the current draft decision ECHA points out that testing on neutralized material would be a way to avoid the corrosive effects. However, the corrosivity involved in inducing local effects belongs in part to the intrinsic property of the test materials molecular structure*". In addition, you refer to 28-day studies from Oleic acid, compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (2:1) and (Z)-N-9-octadecenylpropane-1,3-diamine which indicates that testing with a salt of alkyl-polyamines will not produce different pattern of results or conclusions.

214 ECHA takes note of your comments but stresses that the draft decision only state testing a neutralized salt as an option to mitigate corrosivity and subsequent gastrointestinal irritation. You remain responsible to select an appropriate test material to conduct the test.

215 ECHA further notes that your reference to the ECHA Guidance for Annex V on Exemptions from the obligations to register is irrelevant. The guidance refers to registration obligations (i.e., in this case, the exemption of registration of substances generated as a result of the chemical reaction with a pH neutraliser functions as intended, provided it is not itself manufactured, imported or placed on the market) and not to the characterisation of intrinsic properties of substances subject to registration.

9. Short-term toxicity testing on fish

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

9.1. Information provided

217 You have 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:

(i) a study on short-term toxicity to fish (2002) according to OECD TG 203 with the analogue substance Dodecyl dipropylene triamine, branched (CAS RN 2372-82-9).

(ii) a study on short-term toxicity to fish (1997) according to OECD TG 203 with the analogue substance Oleyl(vegetable oil) tripropylene tetramine (CAS RN 67228-83-5)

(iii) a study on short-term toxicity to fish (1990) according to OECD TG 203 with the analogue substance 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)

(iv) a study on short-term toxicity to fish (1985) according to EU Method C.1 with the analogue substance Oleyl propylene diamine (CAS RN 7173-62-8)

(v) a study on short-term toxicity to fish (1997) according to OECD TG 203 with the analogue substance Oleyl propylene diamine (CAS RN 7173-62-8)

(vi) a study on short-term toxicity to fish (1990) according to OECD TG 203 with the analogue substance 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)

9.2. Assessment of the information provided

9.2.1. Read-across adaptation rejected

218 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.

9.2.2. Insufficient information provided to confirm test material identity (studies (i) to (vi))

219 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.

220 The study (i) to (vi) have been conducted with the UVCB substances listed above. For studies (i) and (ii), you claimed that the test material was representative of the boundary composition of the respective registered substances. However, you did not provide any information on purity and composition (including carbon chain length, saturation, branching when relevant) to support your claim. For study (iii) and (v), you did not provide any information on purity and composition. For study (iv) and (vi), you only state that the purity was about 100%.

221 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.

9.2.3. Inadequate or unreliable studies on the source substances (studies (i) to (vi))

222 To fulfil the information requirement, a study must comply with OECD TG 203 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:

223 Validity criteria

a) the analytical measurement of test concentrations is conducted.

224 Characterisation of exposure

b) the concentrations of the test material are measured at least at the highest and lowest test concentration, at the beginning and end of the test.

225 In studies (i) to (vi) described as a short-term toxicity study on fish:

226 Validity criteria

a) no analytical monitoring was conducted for studies (i) to (iv) and (vi). You state that "*Most of the short-term toxicity daphnia tests were conducted in a period when no reliable specific method of analyses was available. The concentrations were therefore not analytically verified, the reliability of the results is limited because of the poor solubility of the test substances and partial sorption onto the walls of test vessels*";

227 Characterisation of exposure

b) for study (v), you state that the concentrations of the test material were measured only at the beginning of the test. Furthermore, the results of these measurements are not reported.

228 Based on the above,

- the validity criteria of OECD TG 203 are not met for studies (i) to (iv) and (vi). In particular, in the absence of analytical monitoring of exposure during the test, you have not demonstrated that exposure to the test material was satisfactorily maintained and that effect values can reliably be based on nominal concentrations.
- You have not demonstrated that exposure was satisfactorily maintained in study (v) as the monitoring of exposure did not have an appropriate frequency over the exposure phase.

229 Therefore, the requirements of OECD TG 203 are not met.

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

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

9.3. Study design and test specifications

232 OECD TG 203 specifies that, for difficult to test substances, OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design and test specifications' under Request 2.

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.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix 3: Addressee 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]

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

Selection of the Test material(s)

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

a) the boundary composition(s) of the Substance,
b) 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

c) 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 Carbon chain length, the degree of unsaturation within each of fractions and the relative abundance of branched versus linear polyamines.

With that detailed information, ECHA can confirm whether the Test Material is relevant for the Substance.

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.