~~Confidential~~

Helsinki, 15 March 2023

Addressees
Registrant(s) of DoAc_C16-18_C18UNSAT_JS as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
19/10/2021

Registered substance subject to this decision ("the Substance")
Substance name: Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, diacetates
EC/List number: 800-153-0

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

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

3. 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

4. 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)

5. 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)

6. 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

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

8. 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 addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

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

How to comply with your information requirements

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

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

Appeal

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

Failure to comply

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

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

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

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

Appendix 1: Reasons for the request(s)

Contents

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.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)
- Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)
- Ready biodegradability (Annex VII, Section 9.2.1.1.)
- Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2)

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

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

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

0.1.1. Scope of the grouping of substances (category)

5 You provide a read-across justification document entitled "[redacted]*.pdf*" (October 2020) in IUCLID Section 13.2. Under the above endpoints, you also attached a category justification document entitled "[redacted]*.pdf*". ECHA understands that you have extended the previously formed category of polyamines to include polyamine acetates.

6 In your comments on the draft decision, you "*stress that ECHA must take both documents "[redacted].pdf" and "[redacted]" as a combined documented approach to the read-across of Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, diacetates to the broader category of polyamines*".

7 ECHA confirms that the assessment of your read-across adaptations (and the issue listed below) are based on an assessment of both documents.

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

9 Linear polyamines

- Diamine C12/14: C12/14 propylene diamine (CAS RN 90640-43-0)
- C12-alkyl diamine : N-dodecylpropane-1,3-diamine (CAS RN 5538-95-4)
- Diamine C: Coco propylene diamine (CAS RN 91771-18-5) also referred to as Amines, N-C12-18-alkyltrimethylenedi- (CAS RN 68155-37-3)
- 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 HT: Hydrogenated tallow propylene diamine (CAS RN 68603-64-5) also referred to as Amines, N-C16-18-alkyl (evennumbered) propane-1,3-diamine (CAS RN 133779-11-0)
- Diamine O: Oleyl propylene diamine (CAS RN 7173-62-8)
- N-Oleyl-1,3-diaminopropane diacetate (CAS RN 7173-67-3)
- 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)
- 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)

10 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), i.e. the Substance

11 You justify the grouping of the substances as:

- "*Structurally, the linear di-, tri- and tetramines are very similar: a linear alkyl chain and a primary amine at the end, with 1, 2 or 3 secondary amines in between. Consequently, they share the same chemical reactivity and their physico-chemical properties are very similar from which a comparable toxicological profile can be expected*. [...] *the reactivity profile as indicated by QSAR Toolbox (v.3.4) is identical for all structures* [...]. *Only the triamine-Y structures have an additional alert for 'DNA binding by OECD', which is based on tert. amine structure with potential P450 metabolism to a reactive iminium. However, none of the fatty amine derived substances with tertiary amine structures we have tested showed genotoxicity hazards, and besides, none of the other genotoxicity alerts in the Toolbox were triggered. Also the metabolism/transformation predictions showed are comparable for all polyamine structures*".
- "*The variability of the alkyl chain length* […] *is suspected to influence aspects related to bioavailability, but not aspects of chemical reactivity, metabolism/transformations, and specific mechanisms of toxicity e.g. sensitization and genotoxicity. For these reasons, many of the toxicological studies can best be performed on the substance with the shortest chain length within the sub-category, as this is considered to result to the lowest NOAEL or most likely able to show*

specific effects where for ecotoxicology and fate studies can best be focussed on the extremes of the category".

- "*For the physco-chemical properties and related toxicological profile there are clear trends that can be observed over all structures that is related to the length of the alkyl chain, and the number of DP (diamino propane) groups*".
- "*ADME studies indicate slow absorption and likely these substances are not easily metabolized. However, if there is metabolism, the pattern can be expected to be similar for all category members, as is also indicated by metabolism simulators" and "Metabolism profile is not expected to be principally different, and metabolites shows the same variation in alkyl chain lengths. This is supported by the QSAR (OECD) Toolbox (v.3.4) rat liver S9 metabolism and skin metabolism simulators, which show the same metabolism profiles* […]. *Only for the Oleyl chain, some additional metabolic targets are presented related to the available unsaturated bond. However, from common physiological knowledge of fatty acid metabolism, it is known that this is of no concern in practice*".
- "*All category members are produced following the same production processes [and] the products show similar purity and impurity profiles. The conversion of the primary amines into a diamine is not fully complete. The same applies for the subsequent steps to triamine and tetramine. The composition descriptions of these products therefore also include a fraction of remaining primary alkyl amines and polyamines from earlier steps*".

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

13 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

14 You provide a read-across justification document in the Section 'Linked category' in IUCLID.

15 <u>Toxicological properties</u>

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

Diamine C12/14	C12/14 propylene diamine (CAS RN 90640-43-0)
C12-alkyl diamine	N-dodecylpropate-1,3-diamine (CAS RN 5538-95-4)
Diamine C:	Coco propylene diamine (CAS RN 91771-18-5) also referred to as Amines, N-C12-18-alkyltrimethylenedi- (CAS RN 68155-37-3)
Diamine HT	Hydrogenated tallow propylene diamine (CAS RN 68603-64-5) also referred to as Amines, N-C16-18-alkyl (evennumbered) propane-1,3-diamine (CAS RN 133779-11-0)

Diamine O	Oleyl propylene diamine (CAS RN 7173-62-8)
N-Oleyl-1,3-diaminopropane diacetate (CAS RN 7173-67-3)	
Triamine Y12	Dodecyl dipropylene triamine, branched (CAS RN 2372-82-9)

17 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."*
- *"This mechanism of action is the basis for their general toxicity profile, characterized by local effects (often corrosive to skin) […]"*
- *"Diamine acetates salts (DoAc) correspond to a simple mixing of the corresponding alkyl-1,3-diamino propane (diamines) with acetic acid."*
- *"the toxicity of the DoAc is attributed to the hydrated protonated amine ion whereas the negative counter acetate ion does not contribute to toxic effects."*
- *"the (eco)toxicological data of the diamines can be used for the corresponding salts"*

18 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.

19 <u>Ecotoxicological properties</u>

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

21 You provide the following reasoning for the prediction of ecotoxicological properties:

- *"The tests reveal a comparable toxicity, independent of the alkyl chain length"*.

- "*the toxicity of the DoAc is not determined by the presence of acetates and that these substances have the same ecotoxicty profile*".

22 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.

23 Fate properties

24 You predict the fate 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)

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

- "*Although micro-organisms capable of degrading surfactants are immensely diverse, the central metabolism (b-oxidation and TCA cycle) is remarkably similar*. […] *This unity is the key to justification of the use of read-across of biodegradability test results*".
- "[…] *it is unlikely that the biodegradability of these surfactants differs significantly with varying alkyl chain lengths*".
- "*The adequate ready biodegradability test result obtained and the scientific evidence that consortia of hydrophilic moiety and alkyl-utilizing micro-organisms through a joint biodegradation pathway degrade all triamines, alkyl led to the conclusion that all triamines, alkyl are readily biodegradable*".
- "*The same conclusion was obtained for all the tested substances, diamines and DoAc: they are readily biodegradable confirming that the presence of acetates has no influence on the degradability of these surfactants*".

26 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.

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

0.1.2.1. Insufficient data density

28 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".

29 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.

30 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.

31 You have provided information on:

- one category member for *in vitro* gene mutation in mammalian cells (Diamine O)
- one category member (*i.e.,* Triamine Y12) for screening for reproductive/ developmental toxicity
- two category members (*i.e.*, Diamine O and Diamine HT or Diamine O and Diamine T) for Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.), Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.) and Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2)
- two category members (*i.e.*, or Diamine O and Diamine T) for Ready biodegradability

32 Information for one or few category members is not sufficient to establish a trend across the category consisting of 12 substances (and their acetate salts). In the absence of data for substances for the upper and lower borders of the category as well as between the borders, it cannot be confirmed 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 and properties are likely to follow a regular pattern.

33 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*".

34 ECHA notes that, in paragraph 31 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.

35 In your comments on the draft decision, you refer to ECHA Guidance on IRs and CSA, Section R.6.2.4.1., Step 1.

36 However, the quote you have extracted from ECHA Guidance on IRs and CSA refers to the development of the category hypothesis and definition and the identification of individual members of the category. The quote refers to the selection of category members which are based on the reasoning behind the category definition, for e.g. structural similarity and/or common functional group(s) and/or common mode of action and/or similar metabolic pathways etc. The "*pragmatic criteria*" refers to means of identifying category members which are "*empirical and non-systematic*". The guidance document suggests referring to the grouping approaches from empirical OECD HPV and EU ESR programmes but specifies that a category "*may also contain substances that are not produced in high volumes* [or] *substances that are not necessarily commercially available*". It is then stated that "[t]*he formation of a category has in many cases also been dependant on which chemicals are manufactured by the consortium of companies sponsoring the category. However, it should be noted that a category may also contain substances that are produced by a number of different companies. It is therefore important for industries wishing to use this approach to consider the formation of a consortium (e.g. based on an Industry sector group) in order to obtain appropriate support and information*".

37 ECHA notes that the quote in your comments on the draft decision does not refer to assessing data density within a given category. ECHA agrees that full data coverage for the category members is not expected. However, ECHA maintains that for larger categories

sufficient data density must be provided to identify potential breaks in trends which could affect the reliability of interpolation. For the information requirements listed above under paragraph 31, independent of the assessment of the reliability of the supporting information, you have provided insufficient information to demonstrate such trend within the category as a whole.

0.1.2.2. Inadequate or unreliable source studies.

38 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.

39 Specific reasons why the studies on the source substance does not meet these criteria are explained further below under the applicable information requirement sections 1 to 5, 8 and 9. Therefore, no reliable predictions can be made for these information requirements.

40 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*".

41 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 nor your comments on the draft decision provides such documentation.

42 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"".

43 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

44 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.).

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

46 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).

47 You have provided the following 28-d or 90-d repeated dose toxicity studies, and reproductive and developmental toxicity studies in your registration dossier:

- a short-term repeated dose toxicity study according to OECD TG 407 with source substance Diamine O (2010).
- a sub-chronic repeated dose toxicity study according to OECD TG 408 with source substance Diamine C12/14 (2010)
- a 2-generation reproductive toxicity study according to OECD TG 416 with source substance Triamine Y12 (1995) and a pre-natal developmental toxicity study according to OECD TG 414 with Diamine O.

48 In the available sub-chronic 90 days study on Diamine C12/14 (at the lower border of the category), a NOAEL of 0.4 mg/kg bw/d was derived. In comparison to the NOAEL observed in 28 days study on Diamine O (at the higher border of the sub-category for diamines), a NOAEL of 1.25 mg/kg bw/d was derived. The NOAEL from 28-d study is ca. 3-fold higher compared to the NOAEL from 90-d study, but the exposure time is accordingly 3 times shorter. Therefore, the available information, including other short-term 14 d studies, on repeated dose toxicity does not provide sufficient support for your argument "*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*". No supporting information is provided either in the available reproductive and developmental toxicity studies or in vitro genotoxicity studies. Therefore, you have not provided sufficient experimental evidence for the worst-case prediction. Apart from these studies on the source substances, 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.

49 In the absence of such information, you have not established that the source substances constitute 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.

50 In your comments on the draft decision, you state that "[i]*t is understandable ECHA's assumption that the hypothesis may not directly correlate when applied to direct extrapolation of acute to sub-chronic repeat-dose studies for the presented information*". You consider that "[w]*orking under the assumption that smaller chain alkyls are indeed more hazardous is the conservative approach for this read-across as this trend has been demonstrated in a similar category of Primary Fatty Amines*". You therefore conclude that "*that the hypothesis for this category approach is valid as a weak underlying trend may not be observable through the use of extrapolation on the data presented*".

51 ECHA takes note of your intention to submit additional supporting information to substantiate your worst-case hypothesis.

0.1.3. Conclusion on the read-across approach

52 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. Short-term toxicity testing on aquatic invertebrates

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

1.1. Information provided

54 You have adapted this information requirement by using Column 2 of Annex VII, Section 9.1.2. To support the adaptation, you have provided following information:

(i) a study on long-term toxicity to aquatic invertebrates (2008) according to OECD TG 211 on the analogue substance Oleyl propylene diamine (CAS RN 7173-62-8)

(ii) a study on long-term toxicity to aquatic invertebrates (2008) according to OECD TG 211 on 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)

1.2. Assessment of the information provided

55 Under Column 2 of Annex VII, Section 9.1.2, the study may be omitted if reliable information on long-term toxicity to aquatic invertebrates is available. We have identified the following issue with the information you submitted:

1.2.1. Read-across adaptation rejected

56 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.

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

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

58 Technical specifications impacting the sensitivity/reliability of the test

a) the test is conducted with a fully defined test medium. Any deviation (e.g. use of undefined additives) must be specified and clearly described (including its impact on the test medium composition);

59 Additional requirements applicable to difficult to test substances

b) 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.

60 Reporting of the methodology and results

c) adequate information on the method and results of the analytical determination of

exposure concentrations are provided.

61 You have provided studies described as long-term toxicity studies on daphnids according to OECD TG 211:

62 Technical specifications impacting the sensitivity/reliability of the test

a) For studies (i) and (ii), you described the test medium as "*Natural river water, river grane, located in the low mountain range "Harz", D-38685 Langelsheim, Herzog-Julius-Hütte, Im Granetal; additionally 80 % of the components of the culture medium acc. to* [redacted] *(1990) were added to enable a sufficient total water hardness of more than 140 mg CaCO3/L and a sufficient growth and reproduction of the daphnids*". You justify the deviation by stating "*Standard guideline studies are inappropriate to test substances with such properties and the current EU Technical Guidance and RIP Documents do not provide sufficient guidance concerning bioavailability and exposure assessment for cationic surface-active substances like the diamines as these were written with normal hydrophobic chemicals in mind, failing to take into account the lack of bioavailability that occurs in the environment with these substances. The aquatic ecotoxicity tests with diamines were therefore performed in river water*" and you refer to the bulk approach from [redacted] (2001).

63 Additional requirements applicable to difficult to test substances

b) studies (i) and (ii) were conducted under semi-static conditions with a renewal rate of test solutions (frequency) of 3 times per week. You report that measured test concentrations were below 80% of nominal concentrations.

64 Reporting of the methodology and results

c) for studies (i) and (ii), you provide mean measured test item concentrations. However, you have not provided individuals measurements and you have not specified whether (1) measurements were conducted on both fresh and old test solution and (2) the sampling frequency. Also, you report that no pre-treatment was conducted prior to using the extraction solvent and you have not justified that this approach is adequate to determine truly dissolved concentrations.

65 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically,
    - you have not used a fully defined test medium as required by the test guideline. Instead you used natural water and you refer to the bulk approach (ECETOC, 2001). The reported DOC concentration of the natural water was below 2 mg/L. However, it is unknown how the component of the river water may have impacted the bioavailability of the test material used in studies (i) and (ii). 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 on the draft decision, you provided information on water quality parameters for the natural water used as dilution water and for the additional nutrients added to allow sufficient growth and reproduction of the test animals. However, you did not provide any evidence to demonstrate that the use of natural water did not influence the bioavailability of the test materials used in these studies.

You also state that "The Bulk approach has been accepted by the Technical Meetings (TM's) for the EU risk assessments of e.g. DODMAC and primary alkyl amines category (COM070_410_412_429_430_env)". ECHA notes that the references you are referring to do not address (or support) the use of the bulk approach for the purpose of classification and labelling or the PBT assessment. Therefore, ECHA maintains the above assessment.
Finally you explain that "performing aquatic studies under standard conditions with this kind of highly adsorbing substances with a low water solubility will lead to the described difficulties in analytical recovery and homogeneous distribution of the substances in the standard medium. Hereby, it will not be possible to determine the true intrinsic toxicity. [you] are of the opinion that based on this conservative approach (by using a mitigation factor of 10) for C&L on the bulk approach test results, it is not expected that the newly generated results from non-bulk approach tests will significantly change the classification".

ECHA takes note of 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 use of natural water from a specific sampling site may mitigate 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 test design for studies (i) and (ii) 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.

- the reporting of study (i) and (ii) is not sufficient to conduct an independent assessment of their reliability. More specifically, in the absence of adequate reporting of the results of the analytical monitoring of exposure concentrations in studies (i) and (ii), it is not possible to verify that exposure was satisfactorily maintained under the conditions of these tests. Also, you failed to justify that the analytical method allows determining truly dissolved concentrations.

  In your comments on the draft decision, you state that "*measured concentrations are included in the report and this information will be added to the IUCLID 6 RSS*

of the registration dossier. For old media, replicates without test organisms were prepared and stored under test conditions. These results show that the test substance is correctly added to the test and is stable under test conditions. For cationic surfactants the analytical recoveries in samples where the algae are removed, very low ranging in general from < LOQ – 15%. This is caused by the strong sorption of cationic surfactants to algae. Binding of these cationic surfactants to algae has shown to be largely irreversible as very low recoveries were obtained when trying to find the best procedure to recover the substance from algae. Daphnids are thus during a long-term daphnia test mainly exposed via ingestion of the algae and to a much lesser extend via the dissolved fraction". You provided measured concentration for studies (i) and (ii) as an attachment to your comments.

ECHA notes that the values determined at the end of the test cannot be regarded as reflecting truly dissolved concentrations under the conditions of the test as replicates without test organisms (and algae) were used. Your claim that test organisms are mainly exposed via ingestion is not a valid justification for this deviation unless you can demonstrate that the substance exert similar toxicity through both the aqueous and feed exposure route. ECHA notes you have not provided such justification.

66 As a result, the submitted studies on long-term toxicity on aquatic invertebrates do not provide an adequate and reliable coverage of the key parameter(s) of the corresponding OECD TG 211.

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

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

1.3. Study design and test specifications

68 The Substance is difficult to test due to the surface activity (surface tension of 43 mN/m at 1 g/L), adsorptive properties (Log Koc> 4 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.

69 In your comments on the draft decision, you state that "quantification of the truly dissolved concentration in a test where algae are present is very unreliable because it requires separation of the algae from the aqueous phase via filtration or centrifugation and during each step of the procedure to remove the algae a fraction of the already low amount of substance in the test is lost".

70 ECHA takes note of the technical challenges in conducting adequate analytical monitoring of exposure for cationic surfactants such as the Substance. In case it is not possible to determine reliable measurements of dissolved concentrations, you should provide supporting evidence to show that reasonable efforts to attempt obtaining reliable results were made.

71 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).

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

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

2. Growth inhibition study aquatic plants

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

2.1. Information provided

74 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 long-term toxicity to aquatic invertebrates (2008) according to OECD TG 201 on the analogue substance Oleyl propylene diamine (CAS RN 7173-62-8)

(ii) a study on long-term toxicity to aquatic invertebrates (2008) according to OECD TG 201 on 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)

2.2. Assessment of the information provided

2.2.1. Read-across adaptation rejected

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

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

76 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 201. If the

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

77 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;

78 Reporting of the methodology and results

b) adequate information on the method and results of the analytical determination of exposure concentrations are provided.

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

80 Technical specifications impacting the sensitivity/reliability of the test

a) For studies (i) and (ii), you described the test medium as "*Natural river water, river grane, located in the low mountain range "Harz", D-38685 Langelsheim, Herzog-Julius-Hütte, Im Granetal; additionally 50 % of the components of the dilution water acc. to the guideline were added to enable a sufficient growth of algae*"

81 Reporting of the methodology and results

b) for studies (i) and (ii), you provide mean recovery values in fresh and old media. However, you have not provided individuals measurements and you have not specified whether the sampling frequency. Also, you report that no pre-treatment was conducted prior to using the extraction solvent and you have not justified that this approach is adequate to determine truly dissolved concentrations.

82 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the results of studies (i) and (ii). More specifically, you have not used one of the two alternative growth medium and you justify this deviation by referring to the "bulk approach" (ECETOC, 2001). As already explained under section 2.2.2., the bulk approach is not adequate for the purpose of classification and labeling and the PBT assessment.
- the reporting of study (i) and (ii) is not sufficient to conduct an independent assessment of their reliability. More specifically, in the absence of adequate reporting of the results of the analytical monitoring of exposure concentrations in studies (i) and (ii), it is not possible to verify that exposure was satisfactorily maintained under the conditions of these tests. Also, you failed to justify that the analytical method allows determining truly dissolved concentrations.

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

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

85 In your comments on the draft decision, you state that "*Similar as for the short-term toxicity to aquatic invertebrates, the registrants suggest improving the robust study summaries with the missing data (e.g. adding the measured concentrations of fresh and aged samples, justification why the analytical method allows determining the truly dissolved concentration) for the algae study. Furthermore, the read-across justification to the substance (Z)-N-9-octadecenylpropane-1,3-diamine (CAS 7173-62-8) will be improved to justify the high structural similarity and similar physicochemical, toxicological and ecotoxicological properties. Furthermore, the information on the composition of the test substances, e.g. chemical identity of its constituents, their quantitative occurrence, and relevant properties of the constituents will be updated*".

86 ECHA notes that you have not provided any of the above information as part of your comments on the draft decision. Therefore, ECHA is not in a position to assess the validity of this additional information.

87 You also reiterate your comments on the adequacy of the bulk approach. For the reasons already explained under Request 2, ECHA disagrees with your conclusions.

2.3. Study design and test specifications

88 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.

3. Ready biodegradability

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

3.1. Information provided

90 You have provided the following information on the Substance:

(i) a ready biodegradability study (1990) according to OECD TG 301D.

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

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

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

3.2. Assessment of information provided

3.2.1. Assessment of the information provided on the Substance

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

92 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.

93 The study above has been conducted with Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, diacetates (CAS RN 68911-78-4), which you consider equivalent to the Substance. You claim that "Based on the qualitative and quantitative information on the

composition, the sample used are representative of the boundary composition shared and agreed by each registrant". However, you provide no information on composition to support your claim.

94 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.

3.2.1.2. Ready biodegradation tests are normally intended for pure substances (study (i))

95 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.

96 You have provided a study (i) conducted on a test material claimed to be representative of the Substance as a whole. In Section 1.1. of your dossier you describe the Substance as UVCB. In Section 1.2, you describe the substance as a mixture of monoamine and diamine ranging from C12 to C18. The alkyl chain can be saturated or mono-unsaturated.

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

98 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*".

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

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

100 Reporting of the methodology and results

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

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

102 Reporting of the methodology and results

a) you have not reported inoculum concentration in the test vessel in cells/L nor the volume of added inoculum;
b) you have not reported the results of measurements at each sampling point in each replicate;
c) you have not reported the ThOD nor described and justified the ThOD calculation (taking into account the fact that the substance is a UVCB);
d) you have not reported whether a correction for nitrification was applied on the theoretical oxygen demand.

103 Based on the above,

- the reporting of the study is not sufficient to fully assess its reliability. More specifically,
  - as you have not reported inoculum concentration in the test vessel in cells/L, it is not possible to verify if the inoculum density was low enough to be consistent with the specifications of OECD TG 301D.
    In your comments on the draft decision you confirm that "*the inoculum was indeed not quantified for this study as this was not specifically required according to the guideline*". You consider that the level of oxygen depletion in the inoculum blank and the residual concentration of oxygen in the test bottles at the end of the test as sufficient to conclude on the validity of the study. Finally, you state that "[t]*he secondary activated sludge from the RWZI Nieuwgraaf is already used for more than 30 years and the historical viable bacteria count (after preconditioning for 7 days and dilution to 2 mg DW/L) shows that the bacterial density expressed as colony forming units (CFU/L), determined by a standard dilution plate count method based on ISO 6222 (1999) guideline was always < 1*10^6 CFU/L*". You state that "[i]*n the potential new test, the short comings as identified by ECHA will be taken into account bacterial density (cells/L) of the inoculum of the test will be measured*".
    ECHA notes that Table 2 of OECD TG 301 is entitled "test conditions" and therefore should be seen as the conditions under which the various test methods described in the test guideline must be conducted. The limit values for the inoculum density in mg/L (e.g., for sludge or soil) or mL/L (e.g., for surface water or effluent) are set to ensure that the introduction of exogeneous organic matter in the test system is within an acceptable range. Such parameter does not provide a direct estimate of bacterial biomass (as the density of bacteria in, for e.g., a sludge sample or a secondary effluent may vary by orders of magnitude). Accordingly, Appendix R.7.9-1 of ECHA Guidance on IRs and CSA specifies inoculum conditions as cell density (cells/mL) present in a relevant media (e.g. surface waters, unchlorinated sewage treatment works, activated sludge). ECHA further notes that you provided no information in support of your claim that the inoculum density using the activated sludge obtained from the selected sampling site always leads to adequate cell density in the test vessels and therefore it remains not possible to verify that inoculum density was appropriate.
  - as you have not provided an adequate reporting of the study results, it is not possible to verify if validity criteria consistent with the specifications of OECD TG 301D were met.
    In your comments on the draft decision, you provided the results of measurements at each sampling point in each replicate. However, for the reasons explained above, adequate reporting of inoculum density is still missing.
  - you have not specified how ThOD was estimated and, as the test material is a

nitrogen-containing substance, that the calculated ThOD takes into account oxygen consumption through nitrification (or alternatively supporting information that nitrification did not occur).
In your comments on the draft decision, you state that "[a] *ThODNH3 of 2.51 g O2/g test substance was calculated for Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, diacetates*". However, you have not described how this value was obtained considering the UVCB nature of the Substance. You also state that "[t]*he elemental composition of this substance will be used to calculate the ThODNO3. The ThODNO3 will then be used to calculate the biodegradation percentages with nitrification*".

Therefore, the requirements of OECD TG 301 are not met.

3.2.2. Assessment of your read-across adaptation

3.2.2.1. Read-across adaptation rejected

104 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.

3.2.2.2. Insufficient information provided to confirm test material identity (studies (ii) and (iii))

105 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.

106 The studies (ii) and (iii) have been conducted with the UVCB substances listed above. On the test materials used in these studies you only report information on purity. However, you provide no information on composition including information on C-chain length distribution and on the relative abundance of unsaturated constituents.

107 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.

3.2.2.3. Ready biodegradation tests are normally intended for pure substances

108 The test material used in studies (ii) and (iii) correspond to complex UVCBs. Therefore, the issue described under section 4.2.1.2. equally applies to this study.

3.2.2.4. Inadequate or unreliable study on the source substance (studies (ii) and (iii))

109 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:

110 Technical specifications impacting the sensitivity/reliability of the test

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

111 Reporting of the methodology and results

b) the inoculum concentration in the test vessel is reported as cells/L in the test vessel and as volume of added inoculum (for OECD TG 301D, the concentration of the inoculum is set to reach a bacterial cell density of 10^4 to 10^6 cells/L in the test vessel. The concentration of added inoculum is ≤ 5 mL);
c) the results of measurements at each sampling point in each replicate is reported in a tabular form;
d) the calculation of the ThOD is described and justified;
e) for nitrogen-containing test materials, correction for nitrification is applied on the theoretical oxygen demand (*i.e.* $ThOD_{NO3}$) unless it can be demonstrated that nitrification did not occur (*e.g.* by monitoring changes in concentrations in nitrite and nitrate).

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

113 Technical specifications impacting the sensitivity/reliability of the test

a) for studies (ii) and (iii), you report that "*Ammonium chloride was omitted from medium to prevent nitrification*".

114 Reporting of the methodology and results

b) for study (ii), you have not specified the volume of inoculum added to the test bottles. For study (iii), you report that "*The closed bottles were filled with river water and medium at a ratio of 1:1"*. You have not reported inoculum density in cells/mL in studies (ii) and (iii).
c) you have not reported the results of measurements at each sampling point in each replicate in studies (ii) and (iii).
d) you have not reported the ThOD (study (ii)) nor described and justified the ThOD calculation (taking into account the fact that the substance is a UVCB) (studies (ii) and (iii));
e) you have not reported whether a correction for nitrification was applied on the theoretical oxygen demand in studies (ii) and (iii).

115 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically:
    - in studies (ii) and (iii), 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 (iii), 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 (iii) indicates that the volume of added inoculum was 100 times above the maximum value specifies in OECD TG 301D;

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

116 Therefore, studies (ii) and (iii) do not meet the requirements of OECD TG 301D.

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

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

3.3. Study design and test specification

119 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.

120 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.

121 In your comments on the draft decision, you state that "*based on the shared biodegradation pathway and the broad substrate specificity of microorganisms degrading polypropyl diamine (acetates) 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 consider that "[o]*bserved differences in the ready biodegradability tests can be explained by biocidal effects and/or limited bioavailability*". You propose to "*perform ready biodegradation screening tests with the substance as a whole and with a realistic worst-case constituent (having a longer alkyl chain length) in case ready biodegradability is observed in the test with the substance as a whole*".

122 ECHA considers that if, after obtaining a positive result in a ready biodegradability study on the whole 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 Substance 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.

Reasons related to the information under Annex VIII of REACH

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

123 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.

4.1. Information provided

124 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 substance:

(i) *in vitro* mammalian chromosome aberration test (2008) with an analogue substance (Z)-N-9-octadecenylpropane-1,3-diamine/Diamine O (EC 230-528-9, CAS RN 7173-62-8).

(ii) *in vitro* mammalian chromosome aberration test (2003) with an analogue substance N-dodecylpropane-1,3-diamine (EC No 226-902-6, CAS RN 5538-95-4).

4.2. Assessment of the information provided in your dossier

4.2.1. Read-across adaptation rejected

125 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. Inadequate or unreliable study (ii) on the source substance

126 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) at least 300 well-spread metaphases are scored per concentration;

b) the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures is reported;

127 In study (ii) described as an in vitro mammalian chromosome aberration test:

a) 50 - 200 metaphases (i.e., less than 300 metaphases) were scored per concentration.

In your comments on the draft decision, you refer to a full study report, but you have provided a full study report only for study (i). In your comments, you consider that examination of 200 metaphases could be sufficient. Currently, the robust study summary in the registration dossier for study (ii) indicates that 25 – 100 metaphases per experimental group and cell culture were examined, and duplicate cultures were used. It seems that even less than 200 metaphases have been examined for some tested concentrations. Therefore, the issue identified under a) above remain.

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

In your comments on the draft decision, you refer to a full study report, but you have provided a full study report only for study (i). Therefore, the issue identified under b) above remain.

128 The information provided does not cover the specifications(s) required by the OECD TG 473.

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

129 In your comments on the draft decision, you state that the "[a]*nalogues presented 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 (especially diamines)*". On this basis we understand that you intended to invoke a weight-of evidence adaptation under section 1.2 of Annex XI of REACH.

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

130 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.

131 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. 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 patten across all category members"*

4.4.1. Lack of documentation justifying the weight of evidence adaptation

132 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.

133 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.

134 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.

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

4.5. Specification of the study design

136 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.

5. In vitro gene mutation study in mammalian cells

137 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.

5.1. Triggering of the information requirement

138 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.

139 The information for the in vitro cytogenicity study in mammalian cells provided in the dossier is rejected for the reasons provided in request 4.

140 The result of the request 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.

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

5.2. Information provided

142 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 substance:

(i) *in vitro* mammalian cell gene mutation test (2010) with an analogue substance (Z)-N-9-octadecenylpropane-1,3-diamine/Diamine O (EC 230-528-9, CAS RN 7173-62-8).

5.3. Assessment of the information provided

5.3.1. Read-across adaptation rejected

143 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.

144 In your comments on the draft decision, you provided the same consideration as already detailed under Request 4 with reference to your intention to use the data from your dossier as part of weight of evidence and the same claim that existing information is sufficient to demonstrate a trend of absence of effects throughout the category.

145 ECHA's reply equally applies to this information requirement.

146 Annex XI, Section 1.2. further states that there may be sufficient weight of evidence from several independent sources of information based on which a conclusion on the information requirement can be drawn. ECHA notes that you have provided only provided one source of information for this information requirement. Therefore, the available information does not qualify for a weight of evidence adaptation.

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

5.4. Specification of the study design

148 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.

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

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

6.1. Information provided

150 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 (2010) with an analogue substance Amines, N-C12-14-alkyltrimethylenedi-/Diamine C12/14 (EC No 292-562-0, CAS RN 90640-43-0).

(ii) a short-term 28 days repeated dose toxicity study via oral route in rats (2010) with an analogue substance (Z)-N-9-octadecenylpropane-1,3-diamine/Diamine O (EC No 230-528-9, CAS RN 7173-62-8).

(iii) a short-term 14 days repeated dose toxicity study via oral route in rats (2009) with an analogue substance (Z)-N-9-octadecenylpropane-1,3-diamine/Diamine O (EC No 230-528-9, CAS RN 7173-62-8).

(iv) a short-term 14 days repeated dose toxicity study via oral route in rats (2009) with an analogue substance N-Oleyl-1,3-diaminopropane diacetate (EC No 230-532-0, CAS RN 7173-67-3).

6.2. Assessment of the information provided

6.2.1. Read-across adaptation rejected

151 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.

6.2.2. Inadequate or unreliable studies (i) to (iv) on the source substance

152 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) full histopathology, including incidence and severity, is performed as specified in paragraphs 47-49 of the test guideline.

153 The study (i) is described as a sub-chronic repeated dose toxicity study in rat. However, the following specifications are not according to the requirements of OECD TG 407:

d) the following functional observations were not assessed: sensory activity, grip strength and motor activity.

In your comments on the draft decision, you provided a full study report for study (i). This information addresses the reporting issue identified above. You will have to submit this information in an updated registration dossier by the deadline set in the decision.

154 The study (ii) is described as a short-term 28 days repeated dose toxicity study in rat. However, the following specifications are not according to the requirements of OECD TG 407:

d) the following functional observations were not assessed: sensory activity and grip strength.

In your comments on the draft decision, you provided a full study report for study (ii). This information addresses the reporting issue identified above. You will have to submit this information in an updated registration dossier by the deadline set in the decision.

155 The studies (iii) and (iv) are described as a short-term 14 days repeated dose toxicity study in rat. However, the following specifications are not according to the requirements of OECD TG 407:

a) only two dose levels were described in study (iii);

b) only 3 males and 3 females were included in each test and control group in studies (iii) and (iv);

c) the exposure duration was limited to 14 days in studies (iii) and (iv);

d) the following functional aspects were not assessed in studies (iii) and (iv): sensory activity, grip strength and motor activity;

e) histopathological examination was not performed in studies (iii) and (iv).

156 The information provided in studies (i) to (iv) does not cover the specification(s) required by the OECD TG 407.

157 Based on the above, the studies (i) to (iv) do not provide an adequate and reliable coverage of the key parameter(s) addressed by the OECD TG 407 and these studies are not an adequate basis for your read-across predictions.

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

158 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.

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

159 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.

160 However, you only rely on information from analogues substances and the read-across is rejected for the reasons specified under Section 0.1.

6.4.1. Lack of documentation justifying the weight of evidence adaptation

161 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.

162 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.

163 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.

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

6.5. Specification of the study design

165 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.).

166 For information on the study design see request for OECD TG 422 below (section 8).

7. Screening for reproductive/developmental toxicity

167 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.

7.1. 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 substance:

(i) a two-generation reproduction toxicity study via oral route in rats (1995) with an analogue substance N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine/Triamine Y12 (CAS RN 2372-82-9).

7.2. Assessment of the information provided

7.2.1. 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.

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

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 substance.

171 For study (i), you indicated "*Analytical purity:* [redacted]*% aqueous solution*". However, you have not provided any information on composition of the test material.

172 In the absence of detailed information on the test material, the identity of the test material and its impurities cannot be assessed, and you have not demonstrated that the test material is representative for the substance intended to be tested.

173 In your comments on the draft decision, you state that "*the test material identity for the studies in the registration dossier will be reviewed by the respective study owners in the polyamines consortium. This will improve the adequacy and reliability of the data*". You have attached a full study report for study (i) which includes a certificate of analysis for the test material.

174 The certificate of analysis provided for study (ii) does not provide any additional information on composition. Therefore, the above deficiency remains.

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

175 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.63/OECD TG 421 or EU B.64/OECD TG 422. As specified under Annex X, Section 8.7.3., two-generation reproductive toxicity studies (B.35, OECD TG 416) that were initiated before 13 March 2015 shall be considered appropriate to address the information requirement for an extended one-generation reproductive toxicity study and, by extension, the information requirement for a screening reproductive and developmental toxicity study. For a study according to OECD TG 416, the following specifications must be met:

a) histopathology of reproductive organs and tissues is performed, and the presence or absence, incidence and severity of abnormalities is evaluated.

176 In study (i) described as a two-generation reproduction toxicity study:

a) data on histopathology findings, including incidence and severity of abnormalities, are not reported.

177 In your comments on the draft decision, you provided a full study report for study (i), but it does not provide any data on histopathological findings.

178 The information provided does not provide an adequate and reliable coverage of the specifications required by the OECD TG 416.

179 Therefore, the study (i) is not an adequate basis for your read-across predictions.

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

7.3. Specification of the study design

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

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

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

8. Short-term toxicity testing on fish

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

8.1. Information provided

185 You have provided the following information on the Substance:

(i) a study on short-term toxicity to fish (1990) according to OECD TG 203.

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

(ii) a study on short-term toxicity to fish (1990) according to OECD TG 203 with the analogue substance Tallow propylene diamine (CAS RN 61791-55-7) also referred to as N-C16-18-alkyl-(evennumbered, C18 unsaturated) propane-1,3-diamine (CAS RN 1219010-04-4)

(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)

8.2. Assessment of information provided

8.2.1. Assessment of the information provided on the Substance

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

187 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.

188 The study above has been conducted with Amines, N-(C16-18 and C18-unsatd. alkyl)trimethylenedi-, diacetates (CAS RN 68911-78-4), which you consider equivalent to

the Substance. You claim that "*Based on the qualitative and quantitative information on the composition, the sample used are representative of the boundary composition shared and agreed by each registrant*". However, you provide no information on composition to support your claim.

189 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.

8.2.1.2. Inadequate or unreliable study on the Substance

190 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:

191 Validity criteria

a) the analytical measurement of test concentrations is conducted.

192 In study (i) described as a short-term toxicity study on fish:

193 Validity criteria

a) no analytical measurement of test concentrations was conducted.

194 Based on the above, the validity criteria of OECD TG 203 are not met. 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.

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

8.2.2. Assessment of your read-across adaptation

8.2.2.1. Read-across adaptation rejected

196 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.

8.2.2.2. Insufficient information provided to confirm test material identity

197 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.

198 The studies (ii) and (iii) have been conducted with the UVCB substances listed above. On the test materials used in these studies you only report information on purity or study (ii). You provide no information on composition including information on C-chain length distribution and on the relative abundance of unsaturated constituents in either study (ii) or (iii).

199 In the absence of detailed information on the UVCB test materials, the identity of the test materials and their impurities cannot be assessed, and you have not demonstrated that the test materials are representative for the substances intended to be tested.

8.2.2.3. Inadequate or unreliable studies on the source substances

200 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:

201 Validity criteria

a) the analytical measurement of test concentrations is conducted.

202 In studies (ii) and (iii) described as a short-term toxicity studies on fish:

203 Validity criteria

a) no analytical measurement of test concentrations was conducted in stuydies (ii) and (iii)

204 Based on the above, the validity criteria of OECD TG 203 are not met. In particular, in the absence of analytical monitoring of exposure during these tests, you have not demonstrated that exposure to the test material was satisfactorily maintained and that effect values can reliably be based on nominal concentrations.

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

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

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

8.1. Study design and test specifications

208 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 and removed the request for In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method OECD TG 471, 2020) but did not amend the other 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: Addressees of this decision and their corresponding information requirements

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

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

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

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

Appendix 4: Conducting and reporting new tests for REACH purposes

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

1.1. Test methods, GLP requirements and reporting

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

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

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

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

1.2. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

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,

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

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,

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 C-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.