EUROPEAN CHEMICALS AGENCY

Helsinki, 27 April 2017

Addressee: [redacted]

Decision number: TPE-D-2114359620-51-01/F
Substance name: Reaction products of 1H-Imidazole-1-ethanol, 4,5-dihydro-, 2-(C7-C17 odd-numbered, C17-unsatd. alkyl) derivs. and sodium hydroxide and chloroacetic acid
EC number: 931-291-0
CAS number: -
Registration number: [redacted]
Submission number: [redacted]
Submission date: 07.10.2016
Registered tonnage band: 1000+T

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA examined your testing proposal(s) and decided as follows.

Your testing proposals are accepted and you are requested to carry out:

1. **In vivo mammalian bone marrow chromosomal aberration test (Annex IX, Section 8.4., column 2; test method: OECD TG 475) in mice or rats, oral route using the registered substance.**
2. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats using the registered substance.**
3. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route using the registered substance.**

While your originally proposed test for **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210)** using the analogue substance Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide (EC No 271-794-6) is rejected, you are requested to perform:

4. **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) using the registered substance.**

You are additionally requested to perform:

5. **Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: Daphnia sp. Acute Immobilisation Test, EU C.2/OECD TG 202) using the registered substance.**

6. **Growth inhibition study on aquatic algae and cyanobacteria (Annex VII, Section 9.1.2.; test method: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, EU C.3 /OECD TG 201) using the registered substance.**

7. **Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: Fish, Acute Toxicity Test, EU C.1 /OECD TG 203) using the registered substance.**

8. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance.**

In order to ensure use of the integrated testing strategy for the environmental requests, the aquatic short-term toxicity testing (no 5-7 above) are to be conducted first before long-term testing (no 4 and 8 above) is commenced, as further explained in Appendix 1, section 'Environmental testing'.

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **4 November 2019**. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Kevin Pollard, Head of Unit, Evaluation E1

[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

The decision of ECHA is based on the examination of the testing proposal(s) submitted by you and scientific information submitted by third parties.

0. Grouping of substances and read-across approach

a. Legal Background on ECHA's assessment of the grouping of substances and read-across hypothesis

ECHA based its decision on the examination of your testing proposal for the registered substance proposed to be performed with the analogue substance Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide (EC No 271-794-6) (thereafter Amphoacetates C12 or the source substance) and the submitted grouping and read-across justification.

The evaluation by ECHA of testing proposals submitted by registrants aims at ensuring that generation of information is tailored to real information needs. To this end, it is necessary to consider whether testing proposed by registrants are appropriate to fulfil the relevant information requirements and to guarantee the identification of health and environmental hazards of substances. In that respect, the REACH Regulation aims at promoting wherever possible the use of alternative means, where equivalent results to the prescribed test are provided on health and environmental hazards.

The first Recital and the first Article of the REACH Regulation establish the "*promotion of alternative methods for assessment of hazards of substances*" as an objective pursued by the Regulation. In accordance with that objective, ECHA considers whether a prediction of the relevant properties of the substance subject to this decision by using the results of the proposed test is sufficiently plausible based on the information currently available.

Article 13(1) of the REACH Regulation requires information on intrinsic properties of substances on human toxicity to be generated whenever possible by means other than vertebrate animal tests, including information from structurally related substances (grouping or read-across), "*provided that the conditions set out in Annex XI are met*".

Annex XI, 1.5 requires a structural similarity among the substances within a group or category such that relevant properties of a substance within the group can be predicted from the data on reference substance(s) within the group by interpolation.

b. Introduction of the grouping approach and read-across hypothesis proposed

According to the information provided in the category justification document attached to the technical dossier, you have built a category of chemicals based on "*similarities in the general chemical process, functional groups and general composition*" and specified that "*the main variable resides in the alkyl chain distribution present in the raw materials*".

You indicated in the category justification document that the "*following substances are currently in the category*":

- Amphoacetates C8-C18, EC No 931-291-0
- Amphoacetates C12-14, EC No 938-645-3
- Amphoacetates C12, EC No 271-794-6

You concluded "*that based on the similar composition and structural similarity of the components present and their expected water solubility, partition coefficient, vapour pressure and surface activity, the substances of the chemical category will be distributed similarly in the environment and in the human body and may have similar (eco)toxicological properties*".

You proposed to conduct further testing with a member of the category as detailed below:

- Fish early-life stage (FELS) toxicity test according to the OECD TG 210 proposed to be performed with the category member Amphoacetates C12. You justified the selection of this species and this source substance since "*Based on the results obtained from the short-term toxicity studies, fish is considered substantially more sensitive than Daphnia and amphoacetates C12 seems to be the most hazardous to fish*".

You indicated in the category justification document that you consider it adequate to read-across the results from these proposed studies with source substances to the other members of the category (target substances) "*because the substances are considered similar based on the physico-chemistry data, their (eco)toxicological properties and their environmental fate and because the main components in the substances are similar (the C12 and C14 derivatives, characterised by an increase in C12 content)*".

c) Information submitted to support the grouping approach and read-across hypothesis

In order to support the grouping approach based on "*similarities in the general chemical process, functional groups and general composition*", you have provided information on each of these aspects in the category justification document.

Specifically, you have included a general overview of the chemistry of the manufacture of alkyl amphoacetates, outlining the main reactions involved in the synthesis of this type of substance.

You have also elaborated on the common structural features among the members of the category consisting in the presence of an amide bond, the presence of a hydroxyl group and an aminoglycinate function. You also presented theoretical structures of constituents of the category members and stressed that the "*precise structure (i.e. positioning of the acetate and hydroxyl groups) and respective percentages are variable and cannot be analytically determined due to the lack of a suitable analytical method for these complex UVCB substances*".

Information on the typical composition of each category member was presented with details of the alkyl chain distribution for each member of the category. You further identified differences in the composition of the category members and associated this variability with the use of starting materials containing a mixture of constituents with different alkyl chain lengths. You also reported that "*All substances in the category contain mono- and diacetate structures and contain a majority of the C12 and C14 derivatives. The ratio of mono and di-acetate constituents can be different due to the relative amount of chloroacetic acid used in the manufacturing process*".

In addition, information outlining similarities in physico-chemical properties of the category members and your assessment of the impact of these similarities on the distribution of the substances in the environmental and physiological compartments was reported.
You concluded on the basis of this information that "*the substances of the chemical category will be distributed similarly in the environment and in the human body and may have similar (eco)toxicological properties*".

In order to support the read-across approach within this category, you have elaborated on similarities in multiple physico-chemical properties among the members of the category such as water solubility, vapour pressure, density, flammability and pyrophoric and explosive properties. You also attributed differences in other properties such as melting point to the relative content of molecules with a similar alkyl chain length affecting their organisation when crystallising and melting. A data matrix presenting a range of physico-chemical properties for the three members of the category was included in the category justification document.

Similarly, you have presented and compared information on environmental fate and eco-toxicological properties of the category members in a data matrix. You concluded that all category members are considered to be readily biodegradable, are not expected to adhere to organic matter and would mainly reach the aquatic compartment. You further elaborated on the outcome of aquatic toxicity data available for the amphoacetates C8-18 and C12 and concluded that "*amphoacetates C8-C18 has a similar toxicity towards fish and Daphnia (L(E)C50's: 2.5 – 18.5 mg/L), while amphoacetates C12 is clearly more toxic towards fish than towards Daphnia (and more toxic towards fish than amphoacetates C8-C18)*". You considered that since "*amphoacetates C12-C14 has also mainly C12 and C14 mono- and diacetates similar to the tested substances, amphoacetates C12-C14 is considered to have a similar toxicity and is readacross to the lowest value in the category*".

You have reported your assessment of a set of available toxicological data for the category members and compiled this data in a matrix. Information on toxicokinetic properties, acute toxicity, skin and eye irritation, skin sensitisation, genotoxicity and repeated dose toxicity was evaluated. On that basis, you considered that "*the assumption that the properties of the members of the category are similar was also verified*".

d) ECHA analysis of the grouping approach in light of the requirements of Annex XI, 1.5

On the basis of the information provided in the category justification document ECHA understands that the grouping approach is based on similarities in the general chemical process, similarities in functional groups and similarities in the general composition of the members of the category.

The category justification document contains information on the alkyl chain distribution, established on the basis of the raw materials used to manufacture these substances, and high level information on the composition of these substances. You indicated that "*An important difference is the use of various types of raw materials, differing mainly by the linear alkyl chains present in the carboxylic acid starting material. UVCB-type substances derived from oleochemicals consist in mixtures of multiple chain lengths at varying amounts. The amount of each chain length depends on the source of fatty acids, which usually originates from natural fats and oils (containing for example the alkyl chain range from C8 to C18) but can also be from synthetic origin*". You also described in that document general structures of the main constituents, and indicate the presence of mono and diacetates in the composition of the difference substances. According to the information included in the category justification document, the "*ratio of mono and di-acetate constituents can be different due to the relative amount of chloroacetic acid used in the manufacturing process*".

The raw materials used and their ratio in the manufacturing process may lead to important variations in the composition of the substances, affecting both the distribution of the alkyl chain length and the ratio of mono- and diacetate for each alkyl derivative. The limited, generic information on the composition of the members of the category provided in the category justification document does not allow ECHA to verify the claimed compositional similarity. Specifically, no information on the typical concentration and on the concentration ranges discriminating the mono- and diacetates for each alkyl derivative included in the composition of the substances is provided. Therefore, ECHA considers that you have not sufficiently characterised the structural and compositional similarity and variability of the substances concerned by the category.

ECHA further points out that the category definition, as described in your category justification document, does not define the applicability domain of this category. You have described similarities in the chemistry and in the physico-chemical properties of the members of the category. You also identified factors causing some variability in the composition of the substances included in the category, such as the use of various types of raw material, differing mainly in the alkyl chain length, and the amount of chloroacetic acid used in the manufacturing process of the substances. Whilst this information presents similarities and possible differences among the three substances presented as members of the category, it does not constitute a set of inclusion and exclusion rules establishing the molecular structure that a substance must have to be part of the category and describing the accepted structural differenes within the category. According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 1, May 2008), Chapter R.6, such criteria should be described in order to identify the range of values within which reliable estimations can be made for the members of the category and to define the borders of the category. In the absence of a clear identification of the applicability domain of the category, ECHA considers that this grouping approach does not fulfil the requirement set in Annex XI, section 1.5 whereby "*Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or category of substances*".

Consequently, for the reasons presented above, ECHA considers that the category approach, as currently documented in your dossier and applied to the proposed testing on long-term fish toxicity of these substances, does not fulfil the requirement defined in Annex XI, 1.5. Nevertheless, the determination that these substances cannot be considered as a category in accordance with Annex XI, 1.5 does not affect the possibility for you to invoke a read-across approach in order to predict the environmental effects of these substances individually on the basis of a one-to-one analogue approach. Irrespective of the unsuitability of the category approach, ECHA also analysed your proposal to predict properties of the registered substance from a test to be performed on the proposed source substance (one-to-one analogue approach).

e) ECHA analysis of the read-across hypothesis for ecotoxicological properties in light of the requirements of Annex XI, 1.5

You have proposed to perform testing on long-term toxicity to fish using Amphoacetates C12, EC No 271-794-6 as source substance and proposed to read-across the results from these studies to the target substance, the registered substance Amphoacetates C8-C18 (EC No 931-291-0).

According to the provisions of Annex XI, section 1.5 of the REACH Regulation, application of the read-across concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach). ECHA understands from the information provided in the category justification document that your hypothesis according to which you consider that you can predict the properties of the substances within this read-across approach is based on your consideration that *"the substances are considered similar based on the physico-chemistry data, their (eco)toxicological properties and their environmental fate and because the main components in the substances are similar (the C12 and C14 derivatives, characterised by an increase in C12 content)"*.

Furthermore ECHA understands that the reasoning for your testing proposal using the source substance Amphoacetates C12 as test material is as follows: *"Due to their wide dispersive uses and their EU volumes, information about the long-term aquatic toxicity of the members of the category is considered to be essential. Based on the results obtained from the short-term toxicity studies, fish is considered substantially more sensitive than Daphnia and amphoacetates C12 seems to be the most hazardous to fish.*

The substance amphoacetates C12 is more than a factor of 50 more sensitive to fish than to Daphnids: 96h-LC50 (zebrafish): 1.6 mg/L vs. 48h-EC50: 89 mg/L. This study is proposed as it is considered as the most sensitive of the fish tests (in accordance with the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b: Endpoint specific guidance, May 2008)."

ECHA observes that there is limited information supporting some elements of this read-across hypothesis in the registration dossier. In addition to issues that were already described under section d) above, ECHA observes that there are also limited information supporting some elements of the ecotoxicological read-across hypothesis in the registration dossier.

- Absence of property-specific hypothesis

ECHA points out that you have not explained in a property-specific read-across justification on how and why the claimed structural similarity, and in this specific case also the claimed compositional similarity, among source and target UVCB substances constitutes a basis to predict the properties of fish early-life stage (FELS) toxicity test.

- Characterisation of the composition of the substances

You refer in your read-across hypothesis to similarities in the main constituents of the substances, with a particular emphasis on the C12 derivatives. As outlined in section d) above, ECHA considers that the limited information on the chemical structures and the compositions of the source and target substances is not sufficient to verify that the main constituents of the substances included in this read-across approach are indeed similar. More specifically you have not explained how the source substance containing mainly (≥[redacted]%) C12 can cover the properties of more complex composition of C8-C18 were C12 is reported to cover [redacted]% of the alkyl chain distribution (based on the information on the raw materials). Without property specific rationale and justification for example based on common mechanisms of action and similarities in chemical (or biochemical) reactivity it not possible for ECHA to understand and verify this read-across approach proposed in your dossier.

According to the general rules for adaptation of the standard testing regime set out in Annexes VII to X, and regarding spesifically Annex XI, 1.5, in all cases, the results should be adequate for the purpose of classification and labelling and/or risk assessment. ECHA considers that in the grouping and read-across context this means that the predicted properties shall not underestimate the hazards, and the adequate and reliable documentation of the applied methods and approach proposed needs to be provided to allow ECHA to verify and accept the proposed adaptation according to Annex XI, 1.5.

- Supporting information for read-across on long-term toxicity environmental endpoints

ECHA considers that it is important to provide supporting information to strengthen the rationale for the read-across. As part of your category justification, you have provided a data matrix containing physico-chemical properties for the category members. Also you have also provided a data matrix representing environmental fate and toxicity studies for the Amphoacetates category. ECHA notices that as all the substances in the category are UVCB and surfactants, the lack of detailed information on the test materials and sample preparation makes it difficult to compare the results reported in these two tables. Given the nature of the substances and regarding the physco-chemical properties as a supporting information for read-across and grouping ECHA was expecting to see ranges of values and explanations for the variation between the results and if this would give rise to different aquatic toxicity effects. Or if similarity of action or reaction can be assumed regardless of the variation observed. Furthermore ECHA notes that there are no acceptable studies available for aquatic toxicity with the registered substance or with Amphoacetates C12.

As all the substances in the category are surface active, it is known that they can form dispersions or emulsions in which the bioavailability is difficult to ascertain, even with careful solution preparation. Moreover, the micelle formation can result in an overestimation of the bioavailable fraction even when solution seem to be formed. This may present significant problems of interpretation. It is recommended in the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b: Endpoint specific guidance, Version 3.0, February 2016, that *"Toxic effect concentrations for dispersions and emulsions should be compared with the dispersibility limit (i.e., the limit at which phase separation takes place) or the critical micelle concentration (CMC) for a substance in water rather than with its water solubility limit. The bioavailable concentration does not change above the CMC, even at higher dosing levels. The highest test concentration should either be 1000 mg active ingredient/litre or the dispersibility limit/CMC, whichever is lower."*

However, ECHA notes that you have not discussed the surface activity as a potential challenge in interpretation of the results obtained from the aquatic toxicity studies or for the selection of the test material for your testing proposal in your category justification document or in the technical dossier.

- Source data for the read-across

The two key studies for your testing proposal justification establishing the sensitivity between Daphnia and fish are on short-term toxicity to fish and aquatic invertebrates tested with the source substance Amphoacetates C12. ECHA notices that you have not provided the robust study summaries in your technical dossier for either of these studies.

As part of you category justification you have provided the following statement:*"The toxicity of amphoacetates C8-C18 and amphoacetates C12 towards fish and algae is similar (96h-LC50's: 1.6 – 13.9 mg/L; 72h-ErC50's: 10 – 30 mg/L). The toxicity of the C12 member towards Daphnia (48h-EC50's: 89 – >100 mg/L) is lower than the toxicity of the C8-C18 member towards Daphnia (48h-EC50's: 2.5 – 18.5 mg/L). From the results can also be derived that amphoacetates C8-C18 has a similar toxicity towards fish and Daphnia (L(E)C50's: 2.5 – 18.5 mg/L), while amphoacetates C12 is clearly more toxic towards fish than towards Daphnia (and more toxic towards fish than amphoacetates C8-C18). The substance amphoacetates C12 is more than a factor of 50 more sensitive to fish than to Daphnids: 96h-LC50 (zebrafish): 1.6 mg/L vs. 48h-EC50: 89 mg/L."*

As you have only provided robust study summaries of aquatic studies tested with the registered substance Amphoacetates C8-C18, and not with Amphoacetates C12 in your technical dossier, ECHA cannot assess or verify the validity of the source studies and the species sensitivity difference based on the statement provided above. Consequently the proposed testing and adaptation strategy for short- and long-term aquatic toxicity of the registered substance fails.

- Consideration of your comments and updated dossier

You have submitted a dossier update on 07 October 2016 (submission number [redacted]). This dossier update includes a document entitled "[redacted]" (dated on 06 October 2016) in IUCLID section 13.

This document contains your views on the points raised by ECHA in the draft decision and describes a proposed step-wise approach to fulfil the data gaps in the dossiers of the members of this category.
Specifically, you indicate that:

- You acknowledge the points raised by ECHA on the limited information on the composition of the members of the category and express their intention to "*undertake more efforts to more adequately specify the substance's composition in order to support the verification of substance similarity. Also, in a tiered approach, new techniques are planned to be explored, e.g. HPLC-NMR, to address the mono-/diacetate ratio questions*".

- You also agree that the borders of the category were not specifically defined. You report that analytical data will be generated to refine the category definition and that based on this new data a decision on whether to pursue in a category approach or to switch to analogue approaches will be made.

- the read-across approach will be revised on the basis of new analytical data. You specify that the read-across approach will be reconsidered based on the RAAF and inform that a tiered testing approach to address toxicological endpoints – specifically sub-chronic repeated dose toxicity and reproductive/developmental toxicity – is being developed and that possibilities to use data on metabolism and toxicokinetics of the analogues to justify the read-across are being explored .

  In the description of their "Step-wise approach to fill the data gaps in the dossiers", you outline the steps already taken and planned to be started:
  - Additional information on the test material used in the available studies has been included in the updated dossiers, with an emphasis on alkyl chain length distribution and/or mono/diacetate ratio.
  - Improve the analytical data sets of analogues, with a particular effort to determine the monoacetate/diacetate ratio.
  - Reconsider the read-across approach and fill the data gaps on toxicological endpoints through *A step-wise approach [...], which will include additional test work and potentially data on metabolism and toxicokinetics of the category members to strengthen the read-across hypothesis*

You consider that this strategy is scientifically valid and respects the principles of animal use reduction and welfare. You also outline that the timeline envisioned by ECHA to have all the information generated within 30 months is very ambitious.

ECHA acknowledges and welcomes your intentions to provide further information on the composition of the members of the category. ECHA observes that the information provided in the upated dossier, i.e. your intentions to generate new analytical data and to revise your read-across approach on that basis and to develop a tiered approach including additional test work and potentially data on metabolism and toxicokinetics information, is informative about your general intentions and plans.

You indicated in your updated dossier, that the revision of your read-across approach will be based on ECHA's read-across assessment framework (RAAF). Whilst no read-across approach for toxicological endpoints is addressed in this decision, ECHA draws your attention to the fact that the RAAF has been developed for assessing read-across approaches for predicting toxicological properties based on mono-constituent substances. The application of grouping and read-across approaches to UVCB substances, such as the substance subject to this decision, requires additional scientific considerations. ECHA will shortly publish on its website a document presenting aspects to be taken into account when evaluating such grouping and read-across approaches. ECHA understands from the information provided in the dossier update that the scientific data constituting the basis for the revised adaptation is not yet available. The information provided in the dossier update does not allow ECHA to conclude on whether the step-wise approach described in very generic terms in your dossier update will be acceptable or plausible to meet the information requirements under consideration. Therefore, in the absence of new scientific information, ECHA considers that, based on the information currently provided in your comments on the draft decision and the udpated dossier, there is no basis currently on which to revise the ECHA's conclusions from the scientific assessment of your adaptation, and and proposed testing of the source substance cannot be considered plausible for the endpoint(s) in consideration of the registered substance.Furthermore, ECHA notes that you have commented on the the timeline given in this decision, but you have not demonstrated its inappropriateness or required (with any justification) an extension. ECHA considers that a deadline of 30 months is a reasonable time period for providing the required information in the form of an updated registration from the date of the adoption of the decision.

f) Conclusion

For the reasons presented above and on the basis of the information provided in your comments on the draft decision and updated registration dossier, ECHA considers that your read-across hypothesis based upon similarities in physico-chemical and ecotoxicological properties is not supported by reliable and comparable evidence and therefore ECHA is not in the position to verify and accept the adaptation proposed. In addition, as explained above you have not provided a property-specific justification for why aquatic toxicity may be predicted for Amphoacetates C8-C18 by using data generated with Amphoacetates C12.

Based on the above considerations ECHA concludes that you have not provided adequate and reliable information to demostrate that the proposed read-across is plausible for the endpoint(s) in consideration.

ECHA therefore concludes that the criteria of Annex XI, Section 1.5. are not met, and consequently the testing proposed on the source substance is not appropriate to fulfil the information requirement(s) of the substance subject to the present decision.

1. In vivo mammalian bone marrow chromosomal aberration test (Annex IX, Section 8.4., column 2)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Mutagenicity" is an information requirement as laid down in Section 8.4. of Annexes VII to X of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant."

The technical dossier contains an *in vitro* study *Genetic toxicity in vitro.493183* performed according to *the OECD test guideline 473 (in vitro mammalian chromosome aberration test)* with the registered substance that show positive results. An increase in the number of polyploid cells was noted with and without the use of a metabolic activation system was observed in this study. The positive results indicate that the substance is inducing chromosomal aberrations under the conditions of the test.

An appropriate *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations is not available for the registered substance. Consequently, there is an information gap. Hence, you have submitted a testing proposal for a Mammalian bone marrow chromosome aberration test to be performed using the registered substance according to the OECD test guideline 475.

ECHA notes that the proposed test is an appropriate test to investigate effects on chromosomal aberrations *in vivo* as described in the ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015), Chapter R.7a, section R.7.7.1. and figure R.7.7-1 if the test substance or its metabolite(s) will reach the target tissue as specified in the respective test method (OECD TG 475).

You proposed testing in rats. You proposed testing by the oral route. According to the test method OECD TG 475, the test shall be performed in mice or rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

In your comments to the draft decision you agreed to conduct the requested study, by stating "*When the decision is final, the test will be initiated with Amphoacetate C8-C18 following ECHA's recommendations.*" In ECHA's understanding, this is the registered substance.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

A third Party has proposed a weight-of evidence approach for ECHA to take into account before further tests on vertebrate animals are required. As part of this approach, the third party provided results by using a read-across from the substance 1-propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-cocoacyl derivs., inner salts (CAS No. 61789-40-0) to the Registrant's read-across substance dodecylamidopropylbetaine (CAS No. 4292-10-8), used by the registrant for an expert statement on toxicokinetics.

ECHA has taken the information provided into account and concludes that it is insufficient for demonstrating that the conditions of Annex XI, Section 1.2 and 1.5 of the REACH Regulation are met. More specifically, while evidence on gene mutation was negative, ECHA could not conclude that the substance has not a particular dangerous property because the in vitro chromosome aberration test with the registered substance showed a dose-dependent increase in the number of polyploid cells with and without metabolic activation. Furthermore, the proposed read-across approach as an element of the weight of evidence justification did not demonstrate that physicochemical properties/human health effects/environmental effects or environmental fate of the registered substance may be predicted from data on the reference substance.

Although ECHA recognises that the information as provided by the third party might be scientifically valid, it does not fulfil Annex XI requirements and is therefore not sufficient to allow ECHA to reject the testing proposal. Nevertheless, ECHA acknowledges that the Registrant may himself supplement under its own responsibility the argumentation and information provided by the third party in order to make use of adaptation possibilities. This would require that the Registrant documents, using several independent sources of information, that there is a sufficient weight of evidence leading to the assumption/conclusion that a substance has or has not particular dangerous properties, according to the criteria laid down in Annex XI of the REACH Regulation.

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed study with the registered substance subject to the present: *In vivo* mammalian bone marrow chromosomal aberration test (test method: OECD TG 475) in mice or rats, oral route.

d) *Notes for your consideration*

According to paragraph 6 of the OECD TG 475 (Mammalian Bone Marrow Chromosomal Aberration Test, updated on 26 Sept 2014) "*If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test*". Additionally, according to paragraph 44 (d) of the OECD TG 475, a negative test result can be considered reliable if "*Bone marrow exposure to the test substance(s) occurred*". Accordingly, if a substance is negative in this test, and if it is not possible to demonstrate that bone marrow exposure to the substance occurred, then it may not be an appropriate test to meet the information requirements under the REACH Regulation and ECHA will consider any remaining uncertainty concerning the mutagenic potential of the substance and whether to request any further information.You are reminded that according to Annex IX, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered". You may consider making a testing proposal to conduct the mammalian spermatogonial chromosome aberration test (OECD TG 483) whenever the results of the somatic *in vivo* genotoxicity tests indicate that chromosomal aberrations occurred.

2. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.)

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

A sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) by the oral route according to EU B.26./OECD TG 408 on the registered substance.

You proposed testing by the oral route. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA agrees that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is a solid marketed or used in aqueous solution and there are no indications for significant inhalation exposure of humans (e.g., spray application). Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.

You did not specify the species to be used for testing. According to the test method EU B.26./OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed study with the registered substance subject to the present decision: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26./OECD TG 408).

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.
A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for a pre-natal developmental toxicity study in rats according to EU B.31./OECD TG 414 by the oral route on the registered substance.

ECHA considers that the proposed study performed with the registered substance is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

You proposed testing with the rat as a first species. According to the test method EU B.31./OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default consideration, ECHA considers testing should be performed with the rat or rabbit as a first species.

You proposed testing by the oral route. ECHA agrees that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in a first species (rats or rabbits), oral route (test method: EU B.31./OECD TG 414).

Notes for your consideration

For the selection of the appropriate species you are advised to consult ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015), Chapter R.7a, section R.7.6.2.3.2.

Environmental testing:

In order to ensure use of the integrated testing strategy, the aquatic short-term toxicity testing on algae, *Daphnia* and fish are to be conducted first to determine the most sensitive species for the aquatic long term toxicity testing.

If, based on the results, either fish or aquatic invertebrates are shown to be substantially more sensitive than the respective other species, according to ECHA Guidance on information requirements and chemical safety assessment (version 3.0, February 2016), Chapter R7b (Section R.7.8.5 including Figure R.7.8-4), a long-term study on the more sensitive species is required, i.e. either on invertebrates or fish. On the contrary, if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. In such a case, according to the integrated testing strategy, the invertebrate study (*Daphnia* preferred) is to be conducted first. If, based on the results of the long-term invertebrate study and the application of a relevant assessment factor no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted. However, if a risk is indicated, then also long-term fish testing may need to be conducted.

4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other tests in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

"Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for testing the analogue substance Amphoacetates C12 (EC No 271-794-6) for long-term toxicity testing on fish according to Fish, early-life stage toxicity test, OECD TG 210 with the following justification: "*This substance (amphoacetates C8-C18) is a member of the amphoacetate category. A fish early-life stage (FELS) toxicity test (OECD 210) will be performed with another member of the chemical category (amphoacetates C12, EC 271-794-6) and this REACH Annex IX study will be read-across to this substance.*".

ECHA has evaluated your proposal to perform the test with the analogue substance Amphoacetates C12 (EC No 271-794-6). As explained above in Appendix 1, section 0 of this decision, your adaptation provided in your comments on the draft decision and updated technical dossier of the information requirement is rejected.

ECHA notes that you have not submitted a testing proposal on a "Long-term toxicity testing on invertebrates", which is a standard information requirement as laid down in Annex IX, Section 9.1.5. of the REACH Regulation. Furthermore, there were no indications in the dossier from the short-term toxicity studies on aquatic species that fish would be substantially more sensitive than aquatic invertebrates or algae.

In your dossier you have aquatic toxicity data available for short-tem toxicity to fish, short-term toxicity to aquatic invertebrates and for the growth inhibition on aquatic algae with the registered substance. However, you have adapted the standard information requirements for the long-term toxicity testing on invertebrates based on short-term aquatic toxicity studies on the analogue substance amphoacetate C12. As explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected. In addition, the aquatic toxicity data available with the registered substance were considered not reliable and valid (see Sections 5 to 8 below) and therefore sensitivity between the aquatic species cannot be established. Consequently, there are information gaps in your dossier on aquatic toxicity. The additional request to conduct the long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.) and the other additional aquatic toxicity studies will be addressed in the Sections 5 to 8 below.

ECHA considers that the proposed test method for long-term toxicity testing on fish is appropriate to fulfil the information requirement of Annex IX, Section 9.1.6 of the REACH regulation.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the proposed test using the registered substance subject to the present decision: Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.; test method: Fish, early-life stage toxicity test, OECD TG 210) while your originally proposed test for a Fish, early-life stage toxicity test, OECD TG 210, with the analogue substance Amphoacetates C12 (EC No 271-794-6) is rejected according to Article 40(3)(d) of the REACH Regulation..

Notes for your consideration related to Appendix 1, sections 4-8

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, February 2016), Chapter R7b, (Section R.7.8.5 including Figure R.7.8-4), if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. In such case, according to the integrated testing strategy, the *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study and the application of a relevant assessment factor, no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted.

As the registered substance is a UVCB and has surface active properties, you should consult OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6 and ECHA Guidance, Chapter R7b, table R. 7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested ecotoxicity tests and for calculation and expression of the result of this test. Furthermore, ECHA notes that if the registered substance is likely to be unstable in the aquatic environment, a decision to test the registered substance relevant constituents of the registered substance and/or its possibly identified degradation product(s) should be based on a consideration of the half-life of the registered substance under test and real-world conditions. It is your responsibilty to design the test in such a way that the effects on aquatic organisms are adequately assessed.

5. to 8. Additional aquatic toxicity tests

5. **Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.),**

6. **Growth inhibition study on aquatic algae and cyanobacteria (Annex VII, Section 9.1.2.),**

7. **Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.),**

8. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5).**

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other tests in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

(i) Information provided by you on short term-toxicity test on aquatic invertebrates, short-term toxicity to fish, growth inhibition study on aquatic algae and cyanobacteria and long-term toxicity test on aquatic invertebrates.

For the standard information requirement of short-term toxicity on aquatic invertebrates you have provided two key studies and six supporting studies, indicating that for all studies below the identity of test material corresponds to the registered substance Amphoacetates C8-C18 (EC No 931-291-0):

1) key study according to OECD TG 202, GLP, [redacted] (1992), with test material name Ampholak XCO-30/Rewoteric AM2CNM (test material form aqueous solution; Lot/Batch No.: 1/86; composition of test material, percentage of components reported: surfactant concentration: 33.7%, separately reported: solid content: 39.5%, water content: 60.5%, NaCl content: 8.5%). Results 48h EC50 2.5 mg/L concentration expressed as solid content (nominal) based on mobility (range: 2.1-3 mg/L);

2) supporting study according to DIN 38412, Teil 11, GLP, [redacted]. (1993), with test material name Dehyton G (test material form aqueous solution; Batch/Lot number: 451211 / 51Z1600011000 / 7015; composition of test material, percentage of components reported: surfactant concentration: 30%, separately reported: solid content: 37%, water content: 63%, NaCl content: 7%). Results 48h EC50 641 mg/L concentration expressed as solid content (nominal) based on mobility (no range reported);

3) supporting study according to EU Method C.2, GLP, [redacted] (1994), with test material name Miranol C2M Conc NP (test material form aqueous solution; Batch/Lot number: LP943; composition of test material reported, percentage of components: surfactant content: 39.3%, solid content: 50.5%, water content: 49.5%, NaCl content: 11.2%). Results 48 h EC50: 12.6 mg/L concentration expressed as solid content (nominal) based on mobility (range: 8.1-16.7 mg/L);

4) supporting study according to EU C.2, GLP, [redacted] (1995), with test material name Miranol Ultra C32 (test material form aqueous solution; Batch/Lot number: LS 0423; composition of test material reported, percentage of components: surfactant content: 31%, solid content: 39.6%, water content: 60.4%, NaCl content: 7.5%). Results 48 h EC50: 100 mg/L concentration expressed as solid content (nominal) based on mobility;

5) supporting study according to OECD TG 202, GLP, [redacted] (1996), with test material name Empigen CDR 60 (test material form aqueous solution; Batch/Lot number: E/2051; composition of test material reported, percentage of components: surfactant content: 32.9%, separately reported: solid content: 42%, water content: 58%, NaCl content: 9%). Results 48 h EC50: 18.5 mg/L concentration expressed as solid content (nominal) based on mobility (range: 16-21 mg/L);

6) key study according to OECD 202, GLP, [redacted] (2001), with test material name Ampholak XCO-30/Rewoteric AM2CNM (test material form aqueous solution; Batch/Lot number 1768; composition of test material reported, percentage of components: surfactant content: 32%, separately reported: solid content: 39%, water content: 61%, NaCl content: 7%). Results 48 h EC50: 17.9 mg/L concentration expressed as solid content (nominal) based on mobility (no range reported);

7) supporting study according to OECD 202, non GLP, [redacted] (2010a), with test material name Euroglyc MD (EC No 931-291-0, test material form aqueous solution; no Batch or Lot number reported; composition of test material reported, percentage of components: surfactant content: 38%, solid content: 50%, water content: 50%, NaCl content: 12%). Results 48 h EC50: 8.2 mg/L concentration expressed as solid content (nominal) based on mobility (range: 4.4-16.2 mg/L);

8) supporting study according to OECD 202, non GLP, [redacted] (2010b), with test material Euroglyc AMS (EC 931-291-0, test material form aqueous solution; no Batch or Lot number reported; composition of test material reported, percentage of components: surfactant content: 32.5%, solid content: 40%, water content: 60%, NaCl content: 7.5%). Results 48 h EC50: 6 mg/L concentration expressed as solid content (nominal) based on mobility (range: 3.1-12.3 mg/L).

For the standard information requirement of short-term toxicity to fish, you have submitted two key studies and six supporting studies, indicating that for all studies below the identity of test material corresponds to the registered substance Amphoacetates C8-C18 (EC No 931-291-0):

1) key study, according to EU C.1, GLP, [redacted] (1995), with test material name Miranol ultra C32 (test material form aqueous solution; Batch/Lot number: LS 0423; composition of test material reported, percentage of components: surfactant content: 31%, solid content: 39.6%, water content: 60.4%, NaCl content: 7.5%). Results: semi-static, freshwater 96 h LC50: 4.2 mg/L concentration expressed as solid content (nominal) based on: mortality of Oncorhynchus mykiss (range: 3-6 mg/L);

2) supporting study, according to OECD 203, GLP, [redacted] (1996), with test material name Miranol C2M Conc NP (test material form aqueous solution; Batch/Lot number: LBULK471; composition of test material reported, percentage of components: surfactant content: 38%, solid content: 49.5%, water content: 50.5%, NaCl content: 11.5%). Results: semi-static, freshwater 96 h LC50: 6.4 mg/L concentration expressed as solid content (nominal) based on: mortality of Oncorhynchus mykiss (range: 4.6-8.9 mg/L).

3) supporting study, according to OECD 203, GLP, [redacted] (1996), with test material name Rewoteric AM 2 C NM (test material form aqueous solution; Batch/Lot number: 89845; composition of test material reported, percentage of components: surfactant content: 39.7%, solid content: 49.5%, water content: 50.5%, NaCl content: 9.8%). Results: semi-static, freshwater 96 h LC50: 13.9 mg/L concentration expressed as solid content (nominal) based on: mortality of Leuciscus idus (no range reported).

4) supporting study, according to OECD 203, GLP, [redacted] (1996), with test material name Empigen CDR 60 (test material form aqueous solution; Batch/Lot number: E/2051; composition of test material reported, percentage of components: surfactant content: 32.9%, separately reported: solid content: 42%, water content: 58%, NaCl content: 9%). Results: semi-static, freshwater 96 h LC50: 5.5 mg/L concentration expressed as solid content (nominal) based on: mortality of Oncorhynchus mykiss (range: 4.2-7.6 mg/L).

5) supporting study, according to EU C.1, GLP, [redacted] (1998), with test material name Dehyton G (test material form aqueous solution; no Batch or Lot number reported; composition of test material reported, percentage of components: surfactant content: 30%, solid content: 37%, water content: 63%, NaCl content: 7%). Results: semi-static, freshwater 96 h LC50: 10 mg/L concentration expressed as solid content (nominal) based on: mortality of Danio rerio (no range reported).

6) supporting study, according to OECD 203, GLP, [redacted] (2001), with test material name SAT 010787 (Dehyton MC) (test material form aqueous solution; Batch/Lot number: 1058987; composition of test material reported, percentage of components: surfactant concentration: 31.72%, separately reported: solid content: 39%, water content: 61%, NaCl content: 7%). Results semi-static, freshwater 96 h LC50: 8.5 mg/L concentration expressed as solid content (nominal) based on mortality of Danio rerio (range: 7.4-9.8 mg/L).

7) supporting study, according to OECD 203, GLP, [redacted] (2002), with test material name Rewoteric AM C (test material form aqueous solution; Batch/Lot number: 16S0016716; composition of test material reported, percentage of components: surfactant content: 31.5%, solid content: 38.5%, water content: 61.5%, NaCl content: 6.9%). Results semi-static, freshwater 96 h LC50 = 8.24 mg/L concentration expressed as solid content (nominal) based on mortality of Danio rerio (range: 6.67 - 10.24 mg/L).

8) supporting study, according to OECD 203, GLP, [redacted] (2002), with test material name AMPHOTENSID GB 2009 (test material form aqueous solution; Batch/Lot number: 43794/01; composition of test material reported, percentage of components: surfactant content: 39.5%, solid content: 50.7%, water content: 49.3%, NaCl content: 9-11.5%). Results: static, freshwater 96 h LC50 = 23 mg/L concentration expressed as solid content (nominal) based on mortality of Danio rerio (no range reported).

For the standard information requirement toxicity to aquatic algae and cyanobacteria you have provided two key studies and two supporting studies, indicating that for all studies below the identity of test material corresponds to the registered substance Amphoacetates C8-C18 (EC No 931-291-0):

1) key study, according to EU C.3, GLP, [redacted] (1995), with test material name Miranol Ultra C32 (test material form aqueous solution; Batch/Lot number: LS 0423.; composition of test material reported, percentage of components: surfactant content 31%, solid content 39.6%, water content 60.4%, NaCl content 7.5%). Results 72h EC50 10 mg/L concentration expressed as solid content (nominal) based on growth rate of Pseudokirchnerella subcapitata (range: 9-12 mg/L).

2) key study, according to OECD 201, GLP, [redacted] (1998), with test material name Rewoteric AM 2 C NM (test material form aqueous solution; Batch/Lot number: 96317; composition of test material reported, percentage of components: surfactant content: 40.5%, solid content: 50.2%, water content: 49.8%, NaCl content: 9%). Results 72h EC50 30 mg/L concentration expressed as solid content (nominal) based on growth rate of Desmodesmus subspicatus (no range reported).

3) supporting study, according to OECD 201, GLP, [redacted] (2001), with test material name Miranol C2M Conc NP (test material form aqueous solution; Batch/Lot number: LB0001; composition of test material reported, percentage of components: surfactant content: 37.5-39.5%, NaCl content: 11-12%, separately reported: solid content: 50%, water content: 50%). Results 72h EC50 28.5 mg/L concentration expressed as solid content (nominal) based on growth rate of Pseudokirchnerella subcapitata (range: 25.5-33.2 mg/L).

4) supporting study, according to OECD 201, GLP, Mead, C. (1996) IUC4#1/Ch.4.3, with test material name Miranol C2M Conc NP (sodium cocoamphodiacetate) (test material form not reported; Batch/Lot number: LBULK471.; composition of test material reported, percentage of components: percentage of components: active substance content 37.96%, solid content 49.5%, NaCl content 11.54%). Results 72h EC50 3.7 mg/L concentration expressed as solid content (nominal) based on growth rate of Desmodesmus subspicatus (no range reported).

For the information requirement of long-term toxicity to aquatic invertebrates you have given the following statement: "*This substance (amphoacetates C8-C18) is a member of the amphoacetate category. A long-term toxicity study with fish (OECD 210) will be performed with another member of the chemical category (amphoacetates C12) and this REACH Annex IX study will be read-across to this substance. Pending the outcome of this study, a long-term toxicity study with Daphnids with any member of the chemical category is waived as Daphnids are not the most sensitive species. The substance amphoacetates C12 is more than a factor of 50 more sensitive to fish than to Daphnids: 96h-LC50 (zebrafish): 1.6 mg/L vs. 48h-EC50: 89 mg/L.*"

(ii) Assessment of the aquatic toxicity studies

In your dossier you have aquatic toxicity data available with the registered substance, all of which show deficiencies. As the substance tested is surface active, it is known that such substances can form dispersions or emulsions in which the bioavailability is difficult to ascertain, even with careful solution preparation. Moreover, the micelle formation can result in an overestimation of the bioavailable fraction even when solution seems to be formed. This may present significant problems of interpretation. However, ECHA notes that you have not discussed the surface activity as a potential challenge in interpretation of any of the results obtained from the aquatic toxicity studies.
According to OECD 201 OECD 202 and OECD 203, results can be reported as nominal concentrations if there is evidence that the concentration of the test substance has been satisfactorily maintained within ± 20% of the nominal or measured initial concentration throughout the test. This is also one of the validity criteria of OECD 203. Most of the aquatic toxicity studies provided were conducted without analytical monitoring. Only 4 out of 20 aquatic toxicity studies had analytical monitoring reported as TOC or DOC measurements. However, there is no evidence that DOC and TOC measurements are suitable for the analytical monitoring of surface active substances.

This is acknowledged by you when reporting the results of the analytical monitoring in the algae toxicity study 2 referred above: "*A high DOC value was measured in the control at t=72h, therefore it is not ingenious to give any correct recovery values. This may be due to metabolites formed by algae during growth.*" In addition, in the fish toxicity study 6 referred above "*Three test concentrations were analytically monitored by TOC.*", but the TOC concentration was measured only at t=0h and at t=24h and not for the whole test duration of 96h. At some of the concentrations, the TOC varied more than ± 20% (e.g. at 25 mg/L, TOC is 2.49 mg/L at t=0h and 8.03 mg/L at t=24h, see table "Determined TOC-concentrations"). Due to the absence of measured concentrations of the test material during the tests, especially when the registered material is known to be surface active, it is impossible to verify the reliability of any the test results reported.

There are additional reasons not to consider the studies valid. The OECD TG 202 on short-term toxicity to aquatic invertebrates requires the use of "*young daphnids, aged less than 24 hours at the start of the test*". If the test organisms used are >24 h old, their sensitivity might be lower. In Daphnia study 6 referred above, the age of the Daphnids was not reported, but a justification was provided that the age was "*expected to be < 24 hours as it is mentioned that neonates are used.*" However, in other studies you have neither reported the age of the Daphnids nor demonstrated that the age was less than 24 hours at the start of the test to be able to demonstrate that the test results are comparable. In the Daphnia study 2 referred above according to OECD TG 202, you indicated that "*the age of the Daphnids is not provided; it is expected that this is >>24 hours*" and considered this study "*reliable as the validity criteria can be verified and are fulfilled (and the age of the Daphnids is not known/sensitivity can not be checked)*". This is in contradiction with the fact that you did not consider the result of this study for the calculation of the PNEC in the Chemical Safety Assessment (CSA) since it was "*less reliable*" than Daphnia studies 1, 3, 5-8 referred above. In four tests (Daphnia studies 3, 4, 7 and 8), the age of the Daphnids was not provided and thus it cannot be verified that they are aged less than 24 hours at the start of the test, as requested by OECD TG 202.

Another reason for which some tests cannot be considered valid is related to the variation of pH in the controls. According to OECD TG 201 and 202, the pH of the control media should not increase by more than 1.5 units during the test. In the algae studies 2 and 4 referred above, the variation of the pH in the control medium during the test was higher than 2 units. In algae study 2 the pH of the control media increased from 7.98 to 10.23, and this study cannot be considered reliable due to a variation of pH of more than 1.5 units. This is also acknowledged by you in the algae study 4 report, where you indicated the fact that the "[...] *pH at the end of the test = pH initial +/- 2.1 unit (not in conformity with OECD method (+/- 1 unit) and EEC method C3 (+/- 1.5 unit))*" as one of the reasons not to consider this study valid and did not use it in the CSA. In additon, in four tests with aquatic invertebrates (Daphnia studies 3, 5, 6, 7) the pH value in the controls was not provided thus it is not possible to verify if the variation was within 1.5 units.
Overall, based on all the deficiencies reported above, ECHA considers that the validity criteria of aquatic toxicity testing cannot be considered fulfilled and the reporting is not adequate, so the aquatic toxicity test results submitted cannot be considered to be reliable. Consequently, the information provided for the registered substance in the technical dossier does not meet the information requirements.

You have sought to adapt the information requirement of long-term toxicity to aquatic invertebrates by indicating that Daphnids are not the most sensitive species based on the short-term aquatic toxicity test results of an analogue UVCB test material Amphoacetate C12 (EC No 271-794-6). As the category and read-across proposed in your dossier is not meeting the general rules for adaptation under Annex XI section 1.5 (as explained in the Section 0. of this decision), there is a information gap in you dossier also in the long-term toxicity to aquatic invertebrates, which is a standard information requirement as laid down in Annex IX, Section 9.1.5. of the REACH Regulation.

In the absence of reliable information on toxicity to algae, Daphnia and fish, it cannot be concluded if fish or invertebrates or algae/aquatic plants are shown to be substantially more sensitive.

(iii) Consideration of your comments and updated dossier

You submitted a dossier update on 07 October 2016 (submission number [redacted]). In your update you have added a document entitled "[redacted]" (date on 06 October 2016) in IUCLID section 13. In this document related to aquatic toxicity testing you state: *"New short-term data with appropriate analytics will be added to the current data-set. The new data will be used to re-evaluate the current data set and determine potential data gaps. Also, it is expected that the new data will allow for a conclusion regarding the question of which organism is the most sensitive species. Based on the outcome, and taking into account the ECHA integrated test strategy, the most relevant species to perform long-term tests with will be determined. Based on the current data-set, it is expected that the relevant follow-up will be the daphnia reproduction toxicity test. Furthermore, the most relevant analogue, or analogues to perform long-term tests with will be determined."*

ECHA acknowledges your strategy for generating the new data and your aim to follow the ECHA's integrated testing strategy as described in this decision.

(iv) Conclusion

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the following additional tests using the registered substance subject to the present decision as listed above:

5. Short-term toxicity testing on aquatic invertebrates (test method: Acute immobilisation on Daphnia, OECD TG 202 / EU C.2)
6. Growth inhibition study on aquatic algae and cyanobacteria (test method: Algal inhibition test, OECD TG 201 / EU C.3)
7. Short-term toxicity testing on fish (test method: Acute toxicity test to Fish, OECD TG 203 / EU C. 1)
8. Long-term toxicity testing on aquatic invertebrates (test method: Daphnia magna Reproduction Test (EU C. 20 / OECD TG 211)

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal(s) for examination pursuant to Article 40(1) on 4 December 2014.

ECHA held a third party consultation for the testing proposal(s) from 16 August 2011 until 30 September 2011. This public consultation called for information on the substance subject to this decision on the endpoints for which you had submitted testing proposals in your dossier with the submission number QT894423-00, i.e. genetic toxicity, sub-chronic toxicity, pre-natal developmental toxicity and long-term toxicity testing on fish. ECHA received information from third parties (see Appendix 1). Notwithstanding later updates of your registration dossier. ECHA considers that the information obtained in the initial public consultation still applies.

You were initially notified that the draft decision does not take into account any updates after 8 August 2016. However, following your request and justification provided (including the complexity of the category involving additional two substances), ECHA exceptionally granted you an additonal two months for the update. Your update of 7 October 2016 with submission number NF643527-34 was subsequently taken into account when processing this decision.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the Enforcement Authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new test(s) is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new test(s) must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.