EUROPEAN CHEMICALS AGENCY

Helsinki, 27 April 2017

Addressee: [redacted]

Decision number: TPE-D-2114359615-42-01/F
Substance name: Acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide
EC number: 271-794-6
CAS number: 68608-66-2
Registration number: [redacted]
Submission number: [redacted]
Submission date: 7 October 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 proposal is accepted and you are requested to carry out:

1. **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.**

While your originally proposed test for a Sub-chronic toxicity study (90-day), oral route (EU B.26./OECD TG 408) and a Pre-natal developmental toxicity study (EU B.31./OECD TG 414) using the analogue substance 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 No 931-291-0) are rejected, you are requested to perform:

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 (rats or rabbits), [oral/inhalation] route using the registered substance.**

You are additionally requested to perform:

4. **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.**
5. **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.**

6. **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.**
7. **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) using the registered substance.**

In order to ensure use of the integrated testing strategy for the environmental requests, the aquatic short-term toxicity testing (no 4-6 above) are to be conducted first before long-term testing (no 1 and 7 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 **6 May 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.

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 proposals for the registered substance proposed to be performed with the registered substance and with the analogue substance 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 (thereafter Amphoacetates C8-C18 - EC No 931-291-0) 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 members of the category as detailed below:

- Sub-chronic toxicity study (90-day oral) according to the OECD TG 408 in rats proposed to be performed with the category member Amphoacetates C8-18. You justified the selection of this source substance "*because this substance is the mostly used substance of the category and because of its most complex composition. This substance contains also the smaller and higher alkyl chain derivatives and unsaturated C18 alkyl chain derivative, instead of only the C12 and C14 derivatives*".

- Pre-natal developmental toxicity study according to the OECD TG 414 in rats proposed to be performed with the category member Amphoacetates C8-18. You justified the selection of this source substance "*because of its most complex composition. This substance contains also the smaller and higher alkyl chain derivatives and unsaturated C18 alkyl chain derivative, instead of only the C12 and C14 derivatives. It is expected that this substance will show the highest absorption and (therefore) highest toxicity of the category*".

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 sub-chronic toxicity (90-day) and pre-natal developmental toxicity, 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 human health 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 in light of the requirements of Annex XI, 1.5

You have proposed to perform testing for sub-chronic toxicity (90-day) and pre-natal developmental toxicity using the substance Amphoacetates C8-C18 (EC No 931-291-0) as source substance and proposed to read-across the results from these studies to the target substances Amphoacetates C12-14 and Amphoacetates C12.

According to the provisions of Annex XI, section 1.5 of the REACH Regulation, application of the group 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)"*.

ECHA observes that there is limited information supporting some elements of this 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 hypothesis and justification on how and why the claimed structural similarity, and in this specific case also the claimed compositional similarity, among source and target substances constitute a basis to predict the properties sub-chronic toxicity (90-day) and pre-natal developmental toxicity.

- 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 and C14 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 provided in your documentation of this read-across approach does allow to verify that the main constituents of the substances included in this read-across approach are indeed similar.

- Assessment of the impact of the identified structural and compositional differences among the substances

You have identified qualitative and quantitative differences in the composition of source and target substances and associated them with the use of starting materials containing a mixture of constituents with different alkyl chain lengths. You indicated that "*Because most often from a natural origin, the C8-18 alkyl distribution is variable, and can only be given as a range of chain lengths, with the main constituents being C12 and C14. Fractionation can increase the content in specific chain lengths (for example, to > 90% C12-alkyl)*" and specified that "*In the three substances discussed in the present document, the alkyl chain distribution is centred on the C12 alkyl, i.e. it represents the majority of the alkyl chain present in the raw materials used, with a pattern of increasing C12-alkyl content*".

ECHA understands from this information that there are variations in the chain length distribution of the alkyl derivatives and/or the proportion of the different alkyl derivatives in the composition of the substances. You have selected the substance Amphoacetates C8-18 to perform the proposed studies for sub-chronic toxicity and pre-natal developmental toxicity and to read-across this data to Amphoacetates C12-14 and Amphoacetates C12. You indicated in the category justification document that this source substance had been selected for testing "*because of its most complex composition. This substance contains also the smaller and higher alkyl chain derivatives and unsaturated C18 alkyl chain derivative, instead of only the C12 and C14 derivatives. It is expected that this substance will show the highest absorption and (therefore) highest toxicity of the category*".

  - Differences in the distribution of alkyl derivatives

Based on the information provided in your category justification document, the substance Amphoacetates C8-18 has the widest alkyl chain distribution, ranging from C8 to C18 saturated and unsaturated derivatives, whereas the alkyl chain derivatives for the substances Amphoacetates C12-14 and Amphoacetates C12 are mainly in the range of C12 and C14. No unsaturated alkyl derivatives are reported in the composition of Amphoacetates C12-14 and Amphoacetates C12. It is not clear why "*the most complex composition*" qualifies this substance for the selection as source substance for target substances with less complex compositions.

ECHA observes that you have not elaborated on the potential impact of the presence of up to [redacted]% of unsaturated alkyl derivatives in the composition of the proposed test material on the prediction of properties of Amphoacetates C12-14 and Amphoacetates C12. Similarly, ECHA observes that you have not provided any adequate explanation on the impact of the broader alkyl chain distribution in the proposed source substance on the prediction of the properties of Amphoacetates C12-14 and Amphoacetates C12.

In this respect, ECHA further notes that whilst you indicated that among the substances considered in this read-across approach *"the alkyl chain distribution is centred on the C12 alkyl, i.e. it represents the majority of the alkyl chain present in the raw materials used, with a pattern of increasing C12-alkyl content"*, the substance that you have selected as source substance has the lowest content in C12 alkyl derivatives. Based on the compositional information reported in the documentation of your approach, the C12 proportion of the alkyl chain distribution of Amphoacetates C8-18 may vary from [redacted]% to [redacted]%. This proportion is [redacted]% to [redacted]% for the Amphoacetates C12-14 and exceeds [redacted]% for the Amphoacetates C12. Taken together this information suggests that Amphoacetates C8-18 may not constitute the member of the proposed category with the closest composition to Amphoacetate C12. In the absence of further justification addressing the differences in the composition among these substances, the adequacy of Amphoacetates C8-18 as a source substance to predict properties of Amphoacetates with a significantly higher content in C12 alkyl derivatives such as Amphoacetates C12-14 and Amphoacetates C12 is questioned.

- Toxicokinetic properties

You have not provided a scientific argument to support your assumption that the Amphoacetate C8-C18 will show the highest absorption. ECHA assumes that you consider that shorter chain lengths alkyl derivatives in the composition might have a higher absorption. In this case, ECHA points out that the assessment of the impact of the common and varying structural features (e.g. amide bonds, mono-acetates, diacetates, hydroxyl groups) on the absorption of the constituents has not been included in your documentation. ECHA also notes that in your assessment of the toxicological properties of the substances reported in section 5 of your category justification document you considered that "*Oral, dermal and inhalation absorption rates of 100%, 10% and 100% were estimated, respectively*" for Amphoacetates C8-18 and that "*As amphoacetates C12-C14 and amphoacetates C12 contain surfactant constituents structurally similar to the assessed substance and all have similar physico-chemical properties,amphoacetates C12-C14 and amphoacetates C12 are considered to have similar oral, dermal and inhalation absorption rates of 100%, 10% and 100%, respectively*". This conclusion appears to be inconsistent with your argument for selection of Amphoacetates C8-18 as source substance for further testing based on a "*highest absorption*". The source and target substances in this read-across approach are UVCBs. The large number of constituents associated with this type of substance and the structural differences among these constituents limit the relevance of a conclusion on the overall absorption of the substance without discriminating the relative absorption of the different constituents in the context of a read-across approach. ECHA furthermore understands from the information provided that you expect a relationship between increasing absorption and toxicity of the constituents. This assumption is not substantiated with evidence supporting that such a relationship exists for the constituents of the source substance.

On the basis of the compositional information currently reported, it appears that the range of constituents to which the test organism is exposed to after administration of Amphoacetates C8-18 is likely to be different from that after administration of Amphoacetates C12-14 or Amphoacetates C12. ECHA notes that you have not established that the strutural differences such as the alkyl chain length, mono or diacetate structures do not lead to differences in the systemic absorption of these constituents. ECHA further highlights that no or very limited information on the metabolism, distribution, and excretion of the different constituents and their breakdown products has been reported in your category justification document.
In the absence of information on these aspects, it remains unclear which constituents of these substances are systemically available. Consequently, a plausible mechanistic explanation cannot be presented on why and how test results from the source substance can be used to predict systemic properties for the target substances. ECHA considers that you have not established that the properties of these substances relating to systemic toxicity are likely to be similar or follow a regular pattern. Therefore, ECHA considers that you have not provided an adequate scientific basis according to which the properties of Amphoacetates C12-14 and Amphoacetates C12 for the endpoints sub-chronic toxicity and pre-natal developmental toxicity may be predicted from data generated using Amphoacetates C8-18.

- Variations in the ratio of mono- vs diacetates

You have indicated in your category justification document 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*" and provided generic theoretical structures of these mono and diacetates. In your assessment of the variability and differences among the substances, you considered that "*The ratio of the (potential) structures contained in the surfactant part of the substance is not expected to play a significant role with regard to the (eco)toxicological properties of the substances, because the structures all have the same functional groups, i.e. one or two aminoglycinate (-NH-CH2-COONa) functions (i.e. terminal acetate) and hydroxyl, linked to a fatty chain by an amide bond*". As outlined in section d) above, 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. ECHA stresses that the presence of qualitatively similar functional groups such as hydroxyl groups, acetates or alkyl chains in the structure of the constitutents of the source and target substances does not in itself establish that these constituents have similar toxicological properties. On the basis of the information provided ECHA considers that you have not provided scientific information to establish that the possible variations in the number and position of the different functional groups and variations in the alkyl chain length do not impact the toxicological properties of the constituents, and in turn the toxicological properties of the source and target substances.

Furthermore, according the provisions of Annex XI, section 1.5 of the REACH Regulation the toxicological properties of substances included in read-across approaches should be "*likely to be similar or follow a regular pattern as a result of structural similarity*". Based on the information provided in your registration dossier, ECHA considers that there are indications that the toxicological properties of the substances included in this read-across approach differ, as outlined below.

ECHA understands that one of the elements contributing to your consideration that the substances included in this read-across approach are considered similar is their toxicological profile. Whilst the available data on the substances included in this read-across approach and reported in the data matrix provided in the category justification document may suggest similarities in the properties of these substances for properties such as acute toxicity, skin irritation and skin sensitisation, ECHA observes differences in the properties for the endpoints eye irritation, i.e. different classification categories for the substances, and in vitro cytogenicity between the Amphoacetates C8-18 and Amphoacetates C12. Such differences may be indicative of different reactivity in biological systems.

In addition, ECHA stresses that:

- only one data point addressing the property repeated-dose toxicity is available: a 28-day repeated-dose toxicity study performed with Amphoacetates C8-18;
- no information related to the property pre-natal developmental toxicity is available for any of the substances included in this read-across.

Therefore , ECHA considers that the information currently available on the toxicological properties of these substances does not constitute evidence supporting a claim of similarity of these substances. In addition, ECHA is of the opinion that it cannot be established from this data set that the properties of substances can be predicted from other substances in this read-across approach for the endpoints repeated-dose toxicity and pre-natal developmental toxicity.

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 07 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. However the information provided in your comments and in the updated dossier does not include new scientific arguments and evidence for ECHA to assess..

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). 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, there is no basis on which to revise the ECHA's conclusions from the scientific assessment of your adaptation, 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 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 24 months is a reasonable time period for providing the required information in this decision in the form of an updated registration from the date of the adoption of the decision.

ECHA has taken this dossier update into account in this testing proposal examination. However no new information on the grouping and read-across approach and on the testing proposals for toxicological endpoints under consideration in this testing proposal examination has been included in the dossier update. Therefore, in the absence of endpoint specific comments on the draft decision, and in the absence of new scientific information, ECHA considers that there is no basis in the technical dossier on which to revise the conclusions from the scientific assessment of your adaptation, as currently documented.

e) Conclusion

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.

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

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.

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) in rats by the oral route according to EU B.26./OECD TG 408 with the analogue substance Amphoacetates C8-C18 (EC No 931-291-0).

ECHA has evaluated your proposal to perform the test with the analogue substance Amphoacetates C8-C18 (EC No 931-291-0). As explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers 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 registered 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.

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)(c) of the REACH Regulation, you are requested to carry out the additional 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) while your originally proposed test for a Sub-chronic toxicity study (90-day) with the analogue substance Amphoacetates C8-C18 (EC No 931-291-0) is rejected according to Article 40(3)(d) of the REACH Regulation.

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

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.

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 with the analogue substance Amphoacetates C8-C18 (EC No 931-291-0).

ECHA has evaluated your proposal to perform the test with the analogue substance Amphoacetates C8-C18 (EC No 931-291-0). As explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

ECHA considers that the proposed test method is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

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.

ECHA considers 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 a solid marketed or used in aqueous solution, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the additional 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) while your originally proposed test for a Pre-natal developmental toxicity study with the analogue substance Amphoacetates C8-C18 (EC No 931-291-0) is rejected according to Article 40(3)(d) of the REACH Regulation.

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.

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

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

"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 registered substance 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 C12) is a member of the amphoacetate category. 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 with the members of the category, fish is considered substantially more sensitive than Daphnia and the category member 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), page 25).".*

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

However, 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 as explained below in Sections 4 to 7.

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, the aquatic toxicity studies available with the registered substance were considered not reliable and valid (see Sections 4 to 7 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 additional aquatic toxicity studies will be addressed in the Sections 4 to 7 below.

Therefore, pursuant to Article 40(3)(a)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).

Notes for your consideration related to Appendix 1, sections 3-7

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 on information requirements and chemical safety assessment (version 3.0, February 2016), 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.

4. to 7. Additional aquatic toxicity tests

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

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

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

7. **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 one key study and one supporting study with the registered substance Amphoacetates C12 (EC No 271-794-6):

1) Key study, according to Bestimmung der akuten Daphnientoxizität im Daphnientest nach DIN 38412, Teil 11 ([redacted],1993) and GLP. Physical state of test material aqueous solution, name of test material Dehyton W, Lot/batch No.: 4/1/51Z1600012000/66283, composition of test material, percentage of components: solid content ± 35% (33-36%), water content ± 65% (64-67%), surfactant content 18-23%, NaCl content 6.5-8.5% and Sodium glycolate 4-9%. Other information you have reported regarding the test material and test solutions: "the CAS number in the report is correct; the chemical description and purity information is incorrect (separately confirmed). The nominal concentrations are expressed based on an incorrect purity of the substance.

These concentrations are here corrected: Concentrations: 325, 1250 and 7500 mg/L (aqueous solution), at t=0, t=24 and t=48h." 48h EC50 was determined to be 89 mg/L (expressed as solid content).

2) Supporting study accroding to Inhibition of the mobility of Daphnia magna method: NF EN ISO 6341 ([redacted]., 2001) and GLP. The test material physical state: aqueous solution, name of the test material Miranol Ultra L32, Lot/batch No.: LA6628, Composition of test material, percentage of components: solid content 38.1%, water content 61.9%, separately reported NaCl content: 6.35% and surfactant content 30.4%. Limit dose at 100 mg/L (expressed as solid content) = 262.4 mg/L (aqueous solution).

For the standard information requirement of short-term toxicity to fish, you have submitted the following key study with the registered substance Amphoacetates C12 (EC No 271-794-6):

1) Key study, according to ISO 7346-1 (Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 2: Semi-static method) and GLP, [redacted]. (1993), with test material name E Dehyton W (test material form aqueous solution; Batch/Lot No. 66283 / 51 Z 1600012000; composition of test material reported, percentage of components: solid content ± 35% (33-36%), water content 65% (64-67%), surfactant content 18-23%, NaCl content: 6.5-8.5%, Sodium glycolate 4-9%). Other information you have reported related to testing material composition: the CAS number in the report is correct; the chemical description and purity information is incorrect (separately confirmed)). Results: semi-static, freshwater 96 h LC50 1.6 mg/L concentration expressed as solid content (nominal) based on mortality (range not given).

For the standard information requirement toxicity to aquatic algae and cyanobacteria you have provided two key studies and two supporting studies with the registered substance Amphoacetates C12 (EC No EC 271-794-6):

1) Key study, according to OECD 201, Toxicity to Scenedesmus subspicatus in a 72-hour algal growth screening test [redacted]. (2008) non GLP. Test material name Miranol Ultra L-32. Test material form aqueous solution; Lot/Batch No.: W17D081651; composition of test material, percentage of components, solid content 38%, water content 62%, separately reported NaCl content: 7.6% (max.), surfactant content: 30-32%). Result 72h EC50 14.8 mg/L concentration expressed as solid content (nominal) based on growth rate (range not given).

For the information requirement of long-term toxicity to aquatic invertebrates you have given the following adaptation: *"This substance (amphoacetates C12) is a member of the amphoacetate category. A long-term toxicity study with fish (OECD 210) will be performed with 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 studies available with the registered substance Amphoacetates C12, all of which show deficiencies. In particular, most of these studies were conducted completely without analytical monitoring or with no accurate monitoring. Reported absence of measured concentrations of the test material during the tests makes it impossible to verify the reliability of the test results reported, especially when the registered substance is known to be surface active and "*can form dispersions or emulsions in which the bioavailablity is difficult to ascertain, even with careful solution preparation.*", see also ECHA Guidance on information requirements and chemical safety assessment (version 3.0, February 2016), Chapter R7b, Table R.7.8-3 where it is stated that "*Micelle formation can result in an overestimation of the bioavailable fraction even when "solutions" are apparently formed. This presents significant problems of interpretation.*"

ECHA has listed here below some of the examples of the deficiencies and deviations from the test guidelines you have reported in your aquatic toxicity studies:

For short-term toxicity on aquatic invertebrates key study 1) referred above you have reported as follows :"*In the report the results are expressed based on an incorrect purity of the substance (40% instead of 35%). In this summary the results are corrected.*" Also you state that "*Study conducted equivalent to OECD 202 and GLP. Missing information on the age of the Daphnids. No accurate monitoring of the test concentrations and as there is no data reported about any results obtained with a reference substance, the sensitivity can not be checked. Also, missing information about number of animals at each test concentration and for the controls. Still regarded as a reliable study as the validity criteria can be verified and are fulfilled (and there is no definite data about the age of the Dahnids/about the sensitivity).*" In the confidential details on test material you report that:" *the CAS number in the report is correct; the chemical description and purity information is incorrect (separately confirmed)*"

For the supporting short-term toxicity on aquatic invertebrates study 2) you state "*Study conducted in accordance with OECD 202 and GLP, but no analytical monitoring. All the validity criteria can be verified and are fulfilled.*"

For the short-term toxicity to fish you report that "*Study conducted similar to OECD 203, but the reports lacks important study design details, especially about the used fish (length and weight). Also no accurate monitoring of the test concentrations. All the validity criteria can be verified and are fulfilled.*" In the confidential details on test material you report that:" *the CAS number in the report is correct; the chemical description and purity information is incorrect (separately confirmed)*"

You have reported the following deficiencies with the growth inhibition study on aquatic algae which have direct impact on the reliability and the validity of the study results: "*Study conducted in accordance with OECD 201, although the algal biomass was only determined at t=72h and not at t=24h and t=48h (major deviation). Therefore one of the validity criteria can not be verified (the other 2 are fulfilled).*

In addition, no analytical monitoring, no reference control data and the test concentrations were not following the prescribed rules as at least 5 concentrations, arranged in a geometric series with a factor not exceeding 3.2, should have been selected."

Overall, based on all the deficiencies reported above, ECHA considers that the studies available for the aquatic toxicity cannot be considered as adequate, realiable and valid. 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 Amphoacetates C12 (EC No 271-794-6). However, ECHA notes that in the absence of reliable information on aquatic 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 12 December 2012.

ECHA held a third party consultation for the testing proposal(s) from 16 March 2015 until 30 April 205 and from 29 February 2016 until 14 April 2016. ECHA did not receive information from third parties.

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 has exceptionally granted you additonal two months for the update. Your update of 7 October 2016 with submission number CS643513-28 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.