EUROPEAN CHEMICALS AGENCY

Helsinki, 23 November 2018

Addressee: [redacted]

Decision number: CCH-D-2114447545-44-01/F
Substance name: Fatty acids, C16-18 (even numbered) and C18 unsatd., reaction products with triethanolamine, di-Me sulfate-quaternized
List number: 931-203-0
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 16/12/2013
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;**

2. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: OECD TG 414) in a second species (rat or rabbit), oral route with the registered substance;**

3. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce systemic toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.**

4. **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;**

5. **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) with the registered substance;**

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 to the REACH

Regulation. 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 adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by ***31 May 2021***. You also have to 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 and 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, has to 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 **Claudio Carlon**, Head of Unit, Evaluation

[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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

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

A "pre-natal developmental toxicity study" (test method OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of a pre-natal developmental toxicity study in the dossier that would meet the information requirement of Annex IX, Section 8.7.2.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a pre-natal developmental toxicity study (OECD TG 414; GLP compliant) ([redacted] study report) with the analogue substance alkylesterquat (EC no 267-382-0).

Grouping of substances and read-across approach

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances[2]. This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability

[2] Please see for further information ECHA *Guidance on information requirements and chemical safety assessment* (version 1, May 2008), Chapter R.6: QSARs and grouping of chemicals.

of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis[3]- (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

ECHA's evaluation and conclusion

ECHA notes that you have provided a read-across justification document for the grouping of the TEA-esterquats. However, this read-across documentation (including the rationale) does not include the specific analogue substance alkylesterquat (EC no 267-382-0) (hereafter the 'source substance') for the grouping of substances. Hence there is no documentation explaining the read-across, which could be assessed by ECHA. In the absence of this information, ECHA cannot verify that the human health properties of the registered substance can be predicted from the data on the source substance. Since your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5., it is rejected and it is necessary to perform testing on the registered substance.

In the absence of such information, ECHA can only observe the dissimilarities of the source and registered substances. Even though the source substance is also an ethanaminium, there are some structural differences when compared to the registered substance, such as: two methyl groups attached to the central nitrogen atom and thus only two side carbon chains (while the registered substance is a mixture of substance having only one methyl; the other positions are filled by one to three side C16-C18 C-chains or hydroxyethyl); the two carbon-chains are saturated C18 while the C-chain of the registered substance is C16-C18 and C18 unsaturated; the counter anion is different (methylsulfate versus chlorine); and the source substance has no free ethanol groups in this structure while the target substance is a mixture of mono-/di-/tri ester of triethanolamine, and thus there are 0 to 3 ethanol groups (3 ethanol groups in the unreacted impurity).

In any case, structural similarity per se would not be sufficient to enable the prediction of human health properties of a substance. As explained above, further elements are needed to establish a reliable prediction for a toxicological or ecotoxicological property, based on recognition of the structural similarities and differences between the source and registered substances. This could be achieved (if it is possible) by a well-founded hypothesis of (bio)transformation to a common compound(s), or that the registered and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties.

[3] Please see ECHA's Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to the test method OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits 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 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a waxy viscous solidified liquid, ECHA concludes that testing should be performed by the oral route.

In your comments on the draft decision, you acknowledged the fact that the current read-across adaptation cannot be accepted by ECHA due to the absence of justification. You have also indicated that you still intend to use the read-across approach to cover this information requirement. In your comments, you have provided tables with the substance identities and the toxicological data matrix for the registered and analogue substances. However, you only intend to provide a full read-across justification in future dossier updates(s). Hence, currently ECHA cannot assess the read-across approach. However, as already indicated above, under this section, ECHA can already point out that similarity in chemical structure and similarity of some of the toxicological properties does not necessarily lead to predictable or similar human health properties in other endpoints. In the justification you will need to establish why the prediction is reliable for this particular endpoint for which the read across is claimed.

With reference to the future dossier update(s) and in line with the decision making process, ECHA emphasizes that the current decision will not take into account any updates submitted after 19 October 2017 (i.e., the date when the draft decision was notified to you). However, new information from later update(s) of the registration dossier will be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation (i.e., after the deadline set out in the final decision has passed).

In your comments you have also stated that REACH regulation "*does not require to carry out the test with a rodent and a non rodent species*" and "*it lies within the responsibility of the registrant to choose the appropriate species*". ECHA notes that indeed the REACH Regulation does not specify the type of species for the pre-natal developmental toxicity (PNDT) studies. However, according to the test method OECD TG 414 "*The preferred rodent species is the rat and the preferred non-rodent species is the rabbit. Justification should be provided if another species is used*". Moreover, according to ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, when choosing the appropriate species or strain of animal, "*consideration must be given to the suitability of the species and strain for the test protocol, and the availability of background information on the species and strain for the test protocol. The species/strain selection should be justified if the default species referred to in a test method is not used.*"
Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the

present decision: Pre-natal developmental toxicity study (test method: OECD TG 414) in a first species (rat or rabbit) by the oral route.

Note for your consideration

ECHA notes that a revised version of OECD TG 414 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).
The above note also applies to Appendix 1, section 2 (pre-natal developmental toxicity study in a second species request), of the present decision.

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

Pre-natal developmental toxicity studies (test method OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier does not contain information on a pre-natal developmental toxicity study with the registered substance.

You have sought to adapt this information requirement according to Annex XI, Section 3.2 (a). You provided the following justification for the adaptation:

"*(I) The results of exposure and risk assessments covering all relevant exposures throughout the life cycle of the substance demonstrate a very low exposure and a RCR value about* [redacted] *and even lower in all scenarios of manufacture, formulation and professional use even without implementing any RMMs such as gloves. Already the use of gloves (80%) as generally recommended in an industrial and professional setting, would lead to RCR values even below 0.01 for workers. For consumer use and indirect exposure of humans via the environment the worst case RCR values are well below 0.05…*

(II) …The DNELs fertility have been derived from results of the sub-chronic repeated dose toxicity study, taking full account of the potential increased uncertainty resulting from the omission of the information requirement by applying an additional assessment factor…The potential embryotoxicity/teratogenicity of TEA-Esterquats has been evaluated in a reliable OECD 414 guideline compliant prenatal developmental toxicity study in the rat with the source substance MDEA-Esterquat…A DNEL derived from these studies will in any case be higher than the DNEL derived for repeated dose toxicity…Therefore the DNELs for repeated dose toxicity (oral, dermal and inhalation) are also protective for developmental toxicity.

(III) …Comparison of all the derived DNELs with the results of the exposure assessment shows that exposures in all life cycle stages of the substance are well below the derived DNELs even under the precautionary assumptions applied…"

However, ECHA notes that your adaptation does not meet the general rule for adaptation of Annex XI, Section 3.2.(a), because the DNEL derived from the available data must be relevant and appropriate both to the information requirement to be omitted and for risk

assessment purposes. In the technical dossier the DNEL value has only been "*derived from results of the sub-chronic repeated dose toxicity study"* as you claim that they are also "*protective for developmental toxicity.*" The derived DNEL is based on the highest dose level administered in that study showing no adverse effects on reproductive organs. However, ECHA notes that the DNEL derived from a sub-chronic toxicity (90-day) study cannot be used to omit the pre-natal developmental toxicity study because the sub-chronic repeated dose toxicity study does not provide relevant and appropriate information on prenatal developmental toxicity; in fact, it does not provide any information on developmental toxicity. According to the ECHA Guidance document[4], a repeated-dose toxicity study "*showing no adverse effects on reproductive organs is **not** considered to provide sufficient information for a DNEL calculation for fertility or other reproductive effects*". Hence, in absence of relevant and appropriate data for DNEL derivation, the criterion 3.2(a)(ii) is not met. For the adaptation set in Annex XI, Section 3.2.(a) to be fulfilled, all conditions (i) to (iii) need to be met. Therefore, your adaptation of the information requirement is rejected.

Furthermore, ECHA notes that in your adaptation you indicate that the "*potential embryotoxicity/teratogenicity of TEA-Esterquats has been evaluated in a...prenatal developmental toxicity study...with the source substance MDEA-Esterquat".* ECHA notes that since the read-across adaptation cannot be accepted (as explained above in Appendix 1, under section 1) you cannot derive a valid DNEL from the existing pre-natal developmental toxicity study with the proposed source substance. Hence, the only available DNEL is the one derived from the sub-chronic toxicity (90-day) study, which as explained above cannot be used to omit the pre-natal developmental toxicity study.

Therefore, your adaptation of the information requirement is rejected.

In your comments on the draft decision you indicated that you still intend to use the exposure-based adaptation, according to Annex XI, Section 3.2.(a), by deriving a DNEL from the pre-natal developmental toxicity study with the analogue substance. As already indicated above, in Appendix 1, section 1., since you did not provide a robust justification, the read-across approach could not be assessed by ECHA. Hence, currently the read-across adaptation cannot be accepted. As a consequence, the DNEL derived from the pre-natal developmental toxicity study in rats with the source substance (MDEA-EQ) cannot be considered as being valid. At this stage the exposure-based adaptation, according to Annex XI, Section 3.2.(a), cannot be accepted.

With reference to the future dossier update(s) and in line with the decision making process, ECHA emphasizes that the current decision will not take into account any updates submitted after 19 October 2017 (i.e., the date when the draft decision was notified to you). However, new information from later update(s) of the registration dossier will be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation (i.e., after the deadline set out in the final decision has passed).

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

According to the test method 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

[4] ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisations of dose [concentration]-response for human health (version 2.1, November 2012)

considers testing should be performed with rabbits or rats as a second species, depending on the species tested in the first pre-natal developmental toxicity study.

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 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a waxy viscous solidified liquid, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: OECD TG 414) in a second species (rabbit or rat) by the oral route.

Notes for your consideration
You are reminded that before performing a pre-natal developmental toxicity study in a second species you must consider the specific adaptation possibilities of Annex X, Section 8.7., column 2 and general adaptation possibilities of Annex XI. If the results of the test in the first species with other available information enable such adaptation, testing in the second species should be omitted and the registration dossier should be updated containing the corresponding adaptation statement.

ECHA notes that the timeline of 30 months allows for sequential testing of the pre-natal developmental toxicity study with a second species after the first species pre-natal developmental toxicity study and the extended one-generation reproductive toxicity study. Specifically, if (a) the extended one-generation reproductive toxicity study (section 3) and the first species pre-natal developmental toxicity study (section 1) are dosed up to the limit dose, and (b) there is no toxicity observed in these studies, then ECHA considers that you should carefully consider your possibilities for adaptation of this information requirement.

3. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

The basic test design of an extended one-generation reproductive toxicity study (test method OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information provided

You have not provided any study record of an extended one-generation reproductive toxicity study in the dossier that would meet the information requirement of Annex X, Section 8.7.3.

You have sought to adapt this information requirement according to Annex XI, Section

3.2.(a). You provided the following justification for the adaptation:

"...The results of exposure and risk assessments covering all relevant exposures throughout the life cycle of the substance demonstrate a low exposure and a RCR value below 1 in all scenarios of manufacture, formulation, professional use, consumer use and indirect exposure of humans via the environment..."

"...No substance-related adverse effects on reproductive endpoints were found in any of the tests conducted and the NOAELs used to derive the DNELs correspond to the limit doses tested. The DNELs fertility have been derived from results of the sub-chronic repeated dose toxicity study..."

"...Comparison of all the derived DNELs with the results of the exposure assessment shows that exposures in all life cycle stages of the substance are well below the derived DNELs even under the precautionary assumptions applied."

However, ECHA notes that your adaptation does not meet the general rule for adaptation of Annex XI, Section 3.2.(a), because the DNEL derived from the available data must be relevant and appropriate both to the information requirement to be omitted and for risk assessment purposes. In the technical dossier, the DNEL fertility value has only been "*derived from results of the sub-chronic repeated dose toxicity study*". The derived DNEL is based on the highest dose level administered in that study showing no adverse effects on reproductive organs. According to the ECHA Guidance document on information requirements and chemical safety assessment[5], a repeated-dose toxicity study "*showing no adverse effects on reproductive organs is **not** considered to provide sufficient information for a DNEL calculation for fertility or other reproductive effects*." Moreover, a sub-chronic repeated dose toxicity study provides only very limited information on reproductive toxicity (only on toxicity on gonads and lacking information on functional fertility (for example mating, pregnancy, delivery, litter size and survival of offspring, lactation and nursing of pups). Hence, in absence of relevant and appropriate data for DNEL derivation, the criterion 3.2(a)(ii) is not met. For the adaptation set in Annex XI, Section 3.2.(a) to be fulfilled, all conditions (i) to (iii) need to be met. Therefore, your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the study design

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

[5] ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisations of dose [concentration]-response for human health (version 2.1, November 2012)

Ten weeks premating exposure duration is required if there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

The highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels (Cf. OECD TG 443 para 21 & 22).

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a conducted range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Species and route selection

According to the test method OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

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 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a waxy viscous solidified liquid, ECHA concludes that testing should be performed by the oral route.

In your comments on the draft decision you informed ECHA that you are still trying to find relevant and additional data that could be considered to provide sufficient information for a DNEL calculation for fertility or other reproductive effects according to Annex XI, Section 3.2.(a). You have also indicated that you identified an OECD TG 422 study on a structurally related substance. ECHA notes that the DNEL derived from a screening test for reproductive/developmental toxicity (OECD TG 422) shall not be considered as appropriate to omit the extended one-generation reproductive toxicity study, as per footnote 1 of condition (ii) of Annex XI, Section 3.2.(a)(ii) . Moreover, to be able to use this adaptation, at least a one-generation reproductive toxicity study (OECD TG 415) would need to be available. However, the acceptability of such adaptation would still need to be considered on a case-by-case basis.

c) Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

While the specifications for the study design are given above, you shall also submit with the new endpoint study record a scientific justification on each of the following aspects: 1) length of the premating exposure duration and dose level selection, 2) reasons for why or why not Cohort 1B was extended, 3) termination time for F2 generation, and 4) reasons for why or why not Cohorts 2A/2B and/or Cohort 3 were included.

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including Cohorts 2A and 2B and/or Cohort 3 if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the available information, together with the new information shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

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

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex IX, Section 9.1., column 2. You provided the following justification for the adaptation: *"In accordance with REACH Annex IX, 9.1.5 column 2, long-term toxicity testing on aquatic invertebrates does not need to be conducted. A test for long-term toxicity on aquatic invertebrates is only required, if the chemical safety assessment according to Annex I indicates the need to investigate further the effects on aquatic organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment. As the registration substance does not need to be classified with regard to environmental effects, an exposure assessment is not required. The long-term exposure of aquatic organisms is unlikely as the substance is readily biodegradable. The ready biodegradability of the substance is proved in a number of tests conducted under different conditions (aerobic, anaerobic)."*

However, ECHA notes that your adaptation does not meet the specific rules for adaptation of Annex IX, Section 9.1., column 2 because your chemical safety assessment does not rule out the need to investigate further the effects on aquatic organisms.

In your technical dossier you provide a report of a water solubility test, which shows that the water solubility of the registered substance depends on pH and on the occurrence of counter ions in solution. While the solubility of the registered substance was high in pure water (2171 to 2359 mg/L at temperature ranging from 10 to 30°C), it was found to be only slightly soluble in buffered systems (5.30, 3.39 and 19.4 mg/L at pH 4, 7 and 9,

respectively). You then concluded that "*Based on the results in buffered systems it can be assumed that the water solubility is dependent on pH. However, due to the bipolarity of the molecules, it is noted that the counter ions phosphate, citrate and borate, respectively obviously have a more distinct influence on solubility than pH, since the solubility is almost three orders of magnitude below that in pure water*". While you state, in the robust study summary, that "*partially unsaturated TEA-Esterquat was identified by comparison of the retention time with a reference item and by fragment ions of characteristical masses of the mono-, di-, and triesters of the test item*", you did not provide individual water solubility for these constituents or for the other constituents of the registered substance.

You acknowledged that "*TEA-Esterquat are typical UVCB substances (Substances of Unknown or Variable composition, Complex reaction products or Biological materials), which are defined as reaction products of long chain fatty acids of different natural origin with substituted ammonium compound*" and you provided n-octanol/water partition coefficient estimates based on the prediction software ACD/Labs v12 for mono-, di- and triester of TEA-esterquat (C_{18}chain-length, saturated) of -2.95, 5.04 and 13.93, respectively. In an OECD TG 117 test report, you state that "*the test item was not eluted from the column using methanol/water as mobile phase. Based on the results with the reference substances (highest log Kow=6.5 for DDT) the log Kow of the test item was deduced to be >6.5*".

While you selected the results obtained in pure water to reflect the water solubility of the substance, ECHA considers that the values obtained in buffer systems shall be considered as more realistic estimates of the solubility of the registered substance in natural waters. In addition, based on the n-octanol/water partition coefficient data, the constituents of this complex substance are likely to have varying water solubility, some of which being likely poorly water soluble.

Poorly water soluble and adsorptive substances require longer time to be significantly taken up by the test organisms and, consequently, the duration of short-term toxicity test is likely to be insufficient to reach steady state conditions. For this reason, short-term tests may not give a true measure of toxicity for poorly soluble substances and, for some substances, toxicity may not even occur at the water solubility of the substance, if the test duration is too short. Accordingly, long-term toxicity cannot be excluded and should be investigated. Annex VII, section 9.1.1. and Annex VIII, section 9.1.3. of the REACH Regulation explicitly require to consider long-term aquatic toxicity tests if the substance is poorly water soluble. As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) *Daphnia magna* reproduction test (test method EU C.20. / OECD TG 211) is the preferred test to cover the standard information requirement of Annex IX, Section 9.1.5.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Daphnia magna reproduction test (test method: EU C.20./OECD TG 211).

In your comments on the draft decision, you agreed to conduct a long-term toxicity test on Daphnia.

Notes for your consideration:

Once results of the test on long-term toxicity to aquatic invertebrates are available, you shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation.

Due to the adsorptive properties and the relative sensitivity of the substance towards hydrolysis, 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 4.0, June 2017), Chapter R7b, Table R.7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested ecotoxicity test(s) and for calculation and expression of the result of the test(s).

However, please note that the Water Accommodated Fraction (WAF) approach is problematic when used with a test substance containing several constituents, as in the case of the registered substance. In such cases the toxicity cannot be allocated to specific constituents directly and interpretation of the results in the risk assessment requires careful consideration taking into account differences in fate of the constituents in the environment. When constituents of varying solubility are present there can be partitioning effects which limit dissolution in the water. These effects should be minimised and appropriate loadings selected accordingly to allow an appropriate determination of the toxicity of the different constituents. In that respect, it is critical that a robust chemical analysis is carried out to identify those constituents present in the water to which the test organisms are exposed. Additionally, chemical analysis to demonstrate attainment of equilibrium in WAF preparation and stability during the conduct of the test is required. Methods capable of identifying gross changes in the composition of WAFs with time are required such as ultra-violet spectroscopy or total peak area have been used successfully for this purpose. The method used to prepare the WAF should be fully described in the test report and evidence of its compositional stability over time should be provided.

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

"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. Adequate information on Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.), or Fish, short-term toxicity test on embryo and sac-fry stages (Annex IX, 9.1.6.2.), or Fish, juvenile growth test (Annex IX, 9.1.6.3.) needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex IX, Section 9.1., column 2. You provided the following justification for the adaptation: "*In accordance with REACH Annex IX, 9.1.6, column 2, long-term toxicity testing on fish does not need to be conducted. A test for long-term toxicity on fish is only required, if the chemical safety assessment according to Annex I indicates the need to investigate further the effects on aquatic organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment. As the registration substance does not need to be classified with regard to environmental effects, an exposure assessment is not required. The long-term exposure of aquatic organisms is unlikely as the substance is readily biodegradable. The ready biodegradability of the substance is proved in a number of tests conducted under different conditions (aerobic, anaerobic).*"However, ECHA notes that your adaptation does

not meet the specific rules for adaptation of Annex IX, Section 9.1., column 2 because your chemical safety assessment does not rule out the need to investigate further the effects on aquatic organisms.

As explained in request 4, the constituents of the registered substances are likely to have varying water solubility, some of which being likely poorly water soluble and adsorptive. Poorly water soluble and adsorptive substances require longer time to be significantly taken up by the test organisms and, consequently, the duration of short-term toxicity test is likely to be insufficient to reach steady state conditions. For this reason, short-term tests may not give a true measure of toxicity for poorly soluble substances and, for some substances, toxicity may not even occur at the water solubility of the substance if the test duration is too short. Accordingly, long-term toxicity cannot be excluded and should be investigated. Annex VII, section 9.1.1. and Annex VIII, section 9.1.3. of the REACH Regulation explicitly require to consider long-term aquatic toxicity tests if the substance is poorly water soluble.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA further considers that the aquatic ITS (ECHA *Guidance on information requirements and chemical safety assessment, Chapter R7b, section R.7.8.5.3.,* version 4.0, June 2017) is not applicable, because you cannot demonstrate that there is a species sensitivity difference between invertebrates and fish. As discussed above, due to the likely low solubility of at least some constituents of the registered substance, the short-term data cannot serve as compelling evidence to predict relative differences (or lack of) in species sensitivity required to apply the aquatic ITS (ECHA *Guidance on information requirements and chemical safety assessment, Chapter R7b, section R.7.8.5.3.,* version 4.0, June 2017).

Regarding the choice of test investigating long-term toxicity on fish, according to ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017) fish early-life stage (FELS) toxicity test (test method OECD TG 210), fish short-term toxicity test on embryo and sac-fry stages (test method EU C.15. / OECD TG 212) and fish juvenile growth test (test method EU C.14. / OECD TG 215) are the preferred tests to cover the standard information requirement of Annex IX, Section 9.1.6.

However, the FELS toxicity test according to OECD TG 210 is more sensitive than the fish, short-term toxicity test on embryo and sac-fry stages (test method EU C.15 / OECD TG 212), or the fish, juvenile growth test (test method EU C.14. / OECD TG 215), as it covers several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (see ECHA Guidance on information requirements and chemical safety assessment, Chapter R7b (version 4.0, June 2017).

Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are expected to cause effects over a longer exposure period, or which require a longer exposure period of time to reach steady state (ECHA Guidance Chapter R7b, version 4.0, June 2017).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

In your comments on the draft decision, you agreed conduct a long-term test on fish.

Notes for your consideration:

Once results of the test on long-term toxicity to fish are available, you shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation.

Due to the adsorptive properties and the relative sensitivity of the substance towards hydrolysis, 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 4.0, June 2017), Chapter R7b, Table R.7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested ecotoxicity test(s) and for calculation and expression of the result of the test(s).

However, please note that the Water Accommodated Fraction (WAF) approach is problematic when used with a test substance containing several constituents, as in the case of the registered substance. In such cases the toxicity cannot be allocated to specific constituents directly and interpretation of the results in the risk assessment requires careful consideration taking into account differences in fate of the constituents in the environment.

When constituents of varying solubility are present there can be partitioning effects which limit dissolution in the water. These effects should be minimised and appropriate loadings selected accordingly to allow an appropriate determination of the toxicity of the different constituents.

In that respect, it is critical that a robust chemical analysis is carried out to identify those constituents present in the water to which the test organisms are exposed. Additionally, chemical analysis to demonstrate attainment of equilibrium in WAF preparation and stability during the conduct of the test is required. Methods capable of identifying gross changes in the composition of WAFs with time are required such as ultra-violet spectroscopy or total peak area have been used successfully for this purpose. The method used to prepare the WAF should be fully described in the test report and evidence of its compositional stability over time should be provided.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 29 August 2017.

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

ECHA notified you of the draft decision and invited you to provide comments. As REACH-IT was closed from 31 October 2017 22:00 (EEST) to 7 November 2017 10:00 (EEST), the deadline for commenting on the draft decision was exceptionally extended to 5 December 2017.

ECHA took into account your comments and amended the request(s).

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendments were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-61 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests 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 tests 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 tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.