EUROPEAN CHEMICALS AGENCY

Helsinki, 13 December 2018

Addressee: [redacted]

Decision number: CCH-D-2114453649-36-01/F
Substance name: 3-C12-18-(even numbered)-alkylamido-N,N-dimethylpropan-1-amino oxide
EC number: 939-581-9
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 26/09/2017
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. **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;**

2. **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;**

3. **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;**

4. **Update of the technical dossier and the Chemical Safety Report using the study *"Short term to aquatic invertebrates_Ninox HCDO_Stepan_2000 ([redacted] 2000); performed according to OECD TG 202"* as key study showing the highest concern according to Annex I section 3.1.5. for the endpoint of Short-term toxicity testing on invertebrates (Annex VII section 9.1.1.) *or* provide a detailed justification for not using this study as giving rise to the highest concern.**

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 ***21 June 2021***. You also have to update the chemical safety report, where relevant.

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 Ofelia Bercaru, Head of Unit, Evaluation E3

[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

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

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.

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.

While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement based on the absence of adverse effects on the reproductive organs and tissues in a reproduction/developmental toxicity screening study (OECD TG 421) and in 28-day and 90-day repeated dose toxicity studies (OECD TGs 407 and 408, respectively) conducted with the registered substance. You also mentioned that a testing proposal for a prenatal development toxicity study in rats according to OECD guideline 414 had been submitted to ECHA. On that basis you concluded that "*based on the above, the test substance is considered not to be tested in an extended one generation reproductive toxicity study*". However, ECHA notes that your adaptation does neither meet the specific rules for adaptation of Annex X, Section 8.7., column 2 nor the general rule for adaptation of Annex XI, Section 1.2.

Specifically, the specific rules for adaptation of Annex X, Section 8.7., column 2 specify that the studies listed under section 8.7 of Annex X do not need to be conducted if "*the substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) and there is no or no significant human exposure*".
This specific rule for adaptation of the standard information requirement requires all three conditions to be met to waive the studies:

i. The substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available): evidence of toxicity has been reported in the 28-day and 90-day repeated dose toxicity studies. The forestomach, the kidney and urinary bladder

have been identified as target organs in these studies. Therefore, ECHA considers that the criterion for absence of evidence of toxicity in any of the tests available is not met.

ii. No systemic absorption occurs via relevant routes of exposure as demonstrated by toxicokinetic data: based on physico-chemical properties of the registered substance and taking into account the available toxicological data you concluded in your assessment of the toxicokinetic properties of the registered substance that "*oral absorption is set to 100%*". ECHA considers that the effects observed in the repeated dose toxicity studies conducted with the registered substance demonstrate that the substance is systemically available after oral administration. Therefore, ECHA is of the opinion that the criterion for no systemic absorption occurs via relevant routes of exposure is not met.

iii. No or no significant human exposure occurs: based on the information on the uses of the registered substance included in the technical dossier, workers and consumers exposure may occur. However, all risk characterisation ration reported in the technical dossier are inferior to [redacted]. Whilst this suggests that worker and consumer exposure may occur, the significance of such exposure with regard to the safe levels identified for this substance is questionable. Therefore ECHA considers that the criterion for no or no significant human exposure is met.

For the reasons presented above, ECHA considers that the three conditions of the specific rule for adaptation of Annex X, section 8.7 column 2 are not all met. Therefore, ECHA concludes that the adaptation of the information requirement for a reproductive toxicity study according to Annex X, section 8.7 column 2 cannot be accepted.

Furthermore, the absence of adverse effects in repeated dose toxicity studies or in a screening study for reproductive and developmental toxicity do not allow to conclude on whether the registered substance has or has not reproductive toxicity properties. Repeated dose toxicity studies do not investigate the reproductive function. The "reproduction/developmental toxicity screening test" (test method: OECD TG 421) does not provide the information required by Annex X, Section 8.7.3. because it does not cover key elements, such as exposure duration, life stages and statistical power of an extended one-generation reproductive toxicity study. More specifically, the main missing key elements are: 10 weeks pre-mating exposure duration, at least 20 pregnant females per group, and an extensive postnatal evaluation of the F1 generation. Therefore, ECHA considers that the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and your adaptation of the information requirement is rejected. 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.

Ten weeks premating exposure duration is required because 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.

If there is no relevant data to be used for dose level setting, it is recommended that results from a 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 solid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you suggested a tiered testing strategy including pre-natal developmental toxicity studies (requested in a separate testing proposal decision), and an intermediate dossier update for ECHA to review.

ECHA stresses that the sequence for performing the tests requested in this decision and the pre-natal developmental toxicity studies requested in a separate testing proposal decision is at your discretion. The extended one-generation reproductive toxicity study requested in this decision and the pre-natal developmental toxicity study in a first species requested in a separate testing proposal decision are standard information requirements at your tonnage level. Therefore, the regulatory obligation to perform these studies is not influenced by the outcome of the other study. ECHA will only evaluate the received information when the deadline in the decision has passed.

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 the extension of Cohort 1B, 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.

ECOTOXICOLOGICAL INFORMATION

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.

Your registration dossier contains for multiple ecotoxicological endpoints adaptation arguments in the form of a grouping and read-across approach according to Annex XI, 1.5. of the REACH Regulation. ECHA has assessed first the scientific and regulatory validity of your Grouping and read-across approach for ecotoxicological endpoints in general before the individual endpoints (sections 2. and 3. below).

Grouping and read-across approach for ecotoxicological information

You have sought to adapt information requirements by applying a read-across approach in accordance with Annex XI, Section 1.5, for the endpoints:

- Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

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 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.

You consider to achieve compliance with the REACH information requirements for the registered substance 3-C12-18-(even numbered)-alkylamido-N,N-dimethylpropan-1-amino oxide (C12-C18 AAO, EC number: 939-581-9) using data of structurally similar substance Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides (C12-C14 AO, CAS: 308062-28-4, EC: 931-292-6) (hereafter the 'source substance').

You have provided a read-across documentation as a separate attachment in section 13 of the IUCLID registration dossier "[redacted]".

You indicate that you have followed Scenario 2 of the ECHA RAAF Guidance whereby read-across is based on different compounds having similar properties.
You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group:

- *"the effects of the target substance predicted to be quantitatively equal to those of the source substance or prediction based on a worst-case approach".*
- *"Based on the similarity of the chemical structural composition and physicochemical*

[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.
[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).

properties, a predictable pattern in environmental effects is anticipated."

- *"The justification for read-across is based on their structural and functional similarity. Both substances are surfactants, and have a polar "head" (the amine oxide) and a relatively inert hydrophobic "tail" (the long alkyl substituent)."*

In your justification you also state that both substances are UVCBs that have [redacted] and [redacted] as main components in the mixture at very similar levels. As a structural difference you identify "*the nature of the third substituent on the amine, the presence of an additional amide group in C12-C18 AAAO* [the target] *and the number of carbon atoms in the alkyl chain*."

Concerning physicochemical properties you state that "*Although the carbon chain length differ, they are expected to have essentially similar physicochemical properties."* However you identify at the same time that "*The solubility could potentially differ based on the alkyl chain length, usually decreasing with increasing chain length."*

ECHA considers that this information is your read-across hypothesis, which provides the basis whereby you predict the properties of the registered substance from the source substances.

In the following, ECHA examines whether the substances have indeed similar properties and whether long-term aquatic toxicity for the target substance can be predicted from the source substance.

Structural (dis)similarities

Structural similarity is a prerequisite for applying the grouping and read-across approach, but ECHA does not accept in general or this specific case that structural similarity per se is sufficient to enable the prediction of ecotoxicological properties of a substance. It has to be justified why such prediction is possible in view of the identified structural differences and the provided evidence has to support such explanation. In particular, the structural (dis)similarities must be linked to a scientific explanation of how and why a prediction is possible.

You have described that the target and the source are "*surfactants with a polar "head" (the amine oxide) and a relatively inert hydrophobic "tail" (the long alkyl substituent)."* You consider that due to the structural similarities "*predictable pattern in environmental effects is anticipated".* You have at the same time identified several structural differences noting that "*an increase in the alkyl chain lengths can increase the ecotoxicity of the substance, on the other hand it can be observed that an amide bond in the fatty alkyl chain (such as for C12-C18 AAAO* [the target]) *decreases the aquatic toxicity of the amine oxide based surfactant."* You have in particular indicated that "*acute ecotoxicity is affected by chain length for fish and invertebrates*".

ECHA notes that you have sufficiently described in your read-across justification document the structural (dis)similarities between the target and source substances. However ECHA observes that you provide only limited considerations on the impact of these structural dissimilarities on the aquatic ecotoxicity of the substance without addressing in a scientific and substantiated manner how the structural differences may impact the possibility to predict, in particular chronic aquatic ecotoxicity, between the target and source substances.

The provided explanation is therefore not sufficient to establish a scientifically credible link between the structural similarity and the prediction, as discussed further in the following sections.

Similar properties or regular pattern as a result of structural similarity

Annex XI, Section 1.5. provides that *"substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances"*. One prerequisite for a prediction based on read-across therefore is that the substances involved are structurally similar and are likely to have similar properties. One important aspect in this regard is the analysis of the data matrix to compare the properties of source and target substances and to establish whether indeed they are similar or follow a regular pattern.

In your read-across justification you state that you consider the physicochemical properties to be similar. However, you also note that *"the chemical properties are directly related to the length of the aliphatic or hydrophobic chain"*.

ECHA notes that the physicochemical properties of the target and the source differ significantly which may influence their potential for chronic toxicity. The water solubilities and vapour pressures differ by more than one order of magnitude (water solubility: target 1.05 g/L, source 409.5 g/L; vapour pressure: target 60 Pa, source calculated to be between 1.7E-06 and 7.5E-05 Pa).

Based on the data provided it seems that the water solubilities differ and that the solubility decreases with increasing chain length as while both substances have similar content of [redacted] and [redacted], the target substance contains also a significant amount of [redacted] (<[redacted]%), [redacted] (<[redacted]%) and the higher chain lengths of [redacted] ([redacted]%) and [redacted] ([redacted]%). In this respect, you provide contradictory information on the impact of the water solubility on the potential ecoxtoxicity as on one hand you consider that due to the higher solubility the source substance would have *"higher potential environmental toxicity"* however you also note that increase in chain length can increase the ecotoxity of the substance. Also the presence of the additional amide group in the target substance and the nature of the third substituent in the amine might influence the physico-chemical properties of the registered substance and you provide no discussion on this.

Based on the above, ECHA considers that you have not explained how the observed difference in physicochemical properties, especially water solubility, would affect the possibility to predict the environmental intrinsic properties of the registered substance.

Further, regarding the environmental fate properties, ECHA observes that based on the information provided in the technical dossiers, both the target and source substance can be considered as being readily biodegradable and hydrolytically stable with potentially low partition coefficient.

Regarding bioaccumulation potential, you note that due to *"relatively low measured octanol/water partition coefficients"* both substances are unlikely to be bioaccumulative. However, ECHA notes that as the substances have surface active properties, logKow values may not be suitable for assessing their bioaccumulation potential and cannot be used for calculation of a (reliable) BCF value. Information on bioaccumulation of such substances should take account of other descriptors or mechanisms than hydrophobicity. ECHA hence

considers that based on the information provided the bioaccumulation potential of the two substances is unclear.

Regarding ecotoxicology, you consider that the target and the source substances are similar. At the same time you explain that the acute toxicity is affected by chain length for fish and invertebrates. You also claim that "*an amide bond in the fatty alkyl chain (such as for C12-C18 AAAO* [the target]) *decreases the aquatic toxicity of the amine oxide based surfactant*". However, ECHA considers that your claim of similar toxicity is not justified by the acute ecotoxicity data available as discussed below.

Firstly, based on the information provided the target is acutely more toxic to fish (key study 96 h LC50 of 0.68 mg/L for target, 96 h LC50 of 2.67 -3.46 mg/L for the source). In case of invertebrates you provide a 48 h EC50 of 3.1 mg/L for the source substance. For the source substance you provide results obtained from three different studies with 48 h EC50 values of 0.96 mg/L, 16 mg/L and 19.9 mg/L. You have marked the study with the result of 19.9 mg/L as the key study and consider the study with the result of 0.96 mg/L as an "*outlier*", however you provide no justification for ignoring the lowest effect value. ECHA considers this study as valid and, based on the available information, considers that it should be set as the key study as further discussed in request 4. below. ECHA hence considers that also for daphnids, the target substance is acutely more toxic. There is hence no support for your claim that the amide bond in the fatty alkyl chain of the target substance decreases the aquatic toxicity. You have not in your justification discussed why and how the different functional groups may affect the mode of action for toxicity of the substances. The information provided does however support the hypothesis that an increase in the alkyl chain length results in an increase of ecotoxicity as the target substance has a substantial amount of [redacted] (up to [redacted]%) and [redacted] (up to [redacted]%) not present in the source substance. Finally, 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. However, your read-across justification is lacking the relevant reasoning specific to the endpoints under consideration as no discussion on chronic aquatic toxicity specifically is provided. You have not sufficiently explained how the observed differences, together with the structural differences and the differences in physico-chemical parameters would not affect the prediction for long-term toxicity on *Daphnia,* and to fish.

Conclusion on the read-across approach

To conclude, ECHA has taken into account all of your arguments together and considers that this grouping and read-across approach does not provide a reliable basis whereby the chronic aquatic effects of the registered substance may be predicted from data for the target substance. Hence, this approach does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation.

2. 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 XI, Section 1.5. of the REACH Regulation by providing a study record for a Dapnia magna reproduction test (OECD TG 211) with the analogue substance Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides (CAS: 308062-28-4, EC: 931-292-6). However, as explained above in the section 'Grouping of substances and read-across approach for ecotoxicological information', your adaptation of the information requirement cannot be accepted.

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).

3. 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 XI, Section 1.5. of the REACH Regulation by providing a study record for a long-term toxicity to fish (performed according to a test guideline equivalent to EPA OPPTS 850.1500) with the analogue substance Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides (CAS: 308062-28-4, EC: 931-292-6). However, as explained above in the section 'Grouping of substances and read-across approach for ecotoxicological information', your adaptation of the information requirement cannot be accepted.

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) 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) can be performed 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 (version 4.0, June 2017), Chapter R7b, Section R.7.8.4.1.

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).

4. **Update of the technical dossier and the Chemical Safety Report using the study "Short term to aquatic invertebrates_Ninox HCDO_Stepan_2000 ([redacted] 2000); performed according to OECD TG 202" as key study showing the highest concern according to Annex I section 3.1.5. for the endpoint of Short-term toxicity testing on invertebrates (Annex VII section 9.1.1.) or provide a detailed justification for not using this study as giving rise to the highest concern.**

In accordance with Articles 10(b) and 14(1) of the REACH Regulation, the registration must contain a chemical safety report (CSR) which documents the chemical safety assessment (CSA) conducted in accordance with Article 14(2) to (7) and with Annex I to the REACH Regulation.

Annex I, Section 3.1.5. of the REACH Regulation requires that the study or studies giving rise to the highest concern shall be used to draw a conclusion and a robust study summary shall be prepared for that study or studies and included in the technical dossier. In addition, Annex I, Section 3.1.5. requires that if a study giving rise to the highest concern is not used, then this shall be fully justified.

You have provided the following three study summaries to fulfill the Annex VII section 9.1.1. information requirement of Short-term toxicity testing on invertebrates (IUCLID section 6.1.3):

1. Key study on the registered substance according to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test): *"Short term to aquatic invertebrates_[redacted]_2013 [redacted], 2013)"*. Reliability 1, result: 48-h EC50 of 19.9 mg a.i./L;
2. Supporting study on the registered substance according to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test): *"Short term to aquatic invertebrates_Ninox HCDO_Stepan_2000 ([redacted] 2000)"*. Reliability 2, result 48-h EC50 of 0.96 mg a.i./L;
3. Supporting study on the registered substance according to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test): *"Short term to aquatic invertebrates_Aminoxid_[redacted]1997 ([redacted] 1997)"*. Reliability 1, result 48-h EC50 of 16 mg a.i./L.

In the endpoint summary of IUCLID section 6.1.3. Short-term toxicity testing on aquatic invertebrates you have provided the following explanation for the selection of the key study *"Three studies were available for this endpoint on the test substance. A new study was selected as key study [([redacted] 2013)]. This study was run according to the OECD guideline 202 and EU guideline C.2."..."Two other studies are available which gave a 48 -*

Hour EC50 of 16 and 0.96 mg a.i./L respectively [[redacted] 1997) and [redacted] (2000)]. The study of [redacted] (2000) was rated Klimisch 2 and considered a supporting study. The study of [redacted] was rated Klimisch 1. Although the obtained EC50 value was clearly lower than the EC50 value obtained in the study from [redacted] (2013), this value was considered an outlier and therefore not used for the PNEC derivation."

ECHA notes that study no 2 carried out on the registered substance is the one showing the highest concern. As indicated above, if the study showing the highest concern is not used to draw conclusion for an endpoint, a full justification shall be provided. You have merely stated that you have chosen the newest study as the key study and that the study showing the highest concern is an "*outlier*". Moreover you have assigned the Klimisch 2 reliability score (reliable with restrictions) to the study showing the highest concern without explaining the reasons. ECHA considers that merely stating that a study is an "*outlier*" is not an appropriate justification especially as only three studies have been provided and when no clear reason for discrediting a study is given in the endpoint study record. Moreover in the technical dossier you have indicated that all three studies fulfill the validity criteria and are reliable.
ECHA hence considers that you did not provide sufficient justification for not using the study showing the highest concern as the key study.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to update the technical dossier and the Chemical Safety Report using this study showing the highest concern as key study according to Annex I section 3.1.5. for the endpoint of Short-term toxicity testing on invertebrates (Annex VII section 9.1.1.) or provide a detailed justification for not using the study giving rise to the highest concern.

Note for your consideration for requests 2-3

Before conducting the tests requested above under points 2. and 3., you shall consult the ECHA Guidance on information requirements and chemical safety assessment (version 3.0, February 2016), Chapter R7b, Section R.7.8.5 to determine the necessity to conduct the long-term toxicity testing on aquatic invertebrates and on fish.

Concerning the order of studies to be conducted, you may first complete the requirements on short-term toxicity testing on invertebrates requested under point 4 in this decision, and subsequently update the CSA according to Annex I of the REACH Regulation.
According to ECHA Guidance on information requirements and chemical safety assessment (version 4.0, June 2017), 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. However, if a risk is indicated, the long-term fish study needs to be conducted.

Deadline to submit the requested information in this decision

In the draft decision communicated to you the time indicated to provide the requested information was 24 months from the date of adoption of the decision. In your comments on the draft decision, you requested an extension of the timeline to 36 months. You sought to justify this request by suggesting a tiered testing strategy including an intermediate

evaluation by ECHA and studies requested in a parallel testing proposal decision. You also provided documentary evidence from testing laboratories with indicative timelines for the performance of the requested extended one-generation reproductive toxicity study in this decision and of the pre-natal developmental toxicity studies requested in a separate testing proposal decision.

The extended one-generation reproductive toxicity study requested in this decision and the pre-natal developmental toxicity study in a first species, requested in a parallel testing proposal decision, can be performed simultaneously. However, considering the indicative timelines provided by the testing laboratories, ECHA has modified the deadline of the decision from the original 24 months to 30 months. The deadlines in this decision and the parallel testing proposal decision are set to 30 months, each.

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 22 September 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.

ECHA took into account your comments and did not amend the requests, but amended the deadline in the decision.

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 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.