ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 19 July 2017

Addressee: [redacted]

Decision number: CCH-D-2114366617-39-01/F
Substance name: Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides
List number: 931-292-6
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 16.09.2014
Registered tonnage band: 1000+T

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. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102 with the with the registered substance;**

2. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

3. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rabbit), oral route with the registered substance;**

4. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./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 some 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;**
   - **Ophthalmological examination of the parental animals (P0) prior to sacrifice and the F1 animals prior to sacrifice.**

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 **27 January 2020**. 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 E2

[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 response to the draft decision, you provided for the endpoints In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.); Pre-natal developmental toxicity study (Annex X, Section 8.7.2.); Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.) adaptation arguments in form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual endpoints (sections 1, 3 and 4).

Grouping of substances and read-across approach for toxicological information

You have sought to adapt the information requirements for In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.); Pre-natal developmental toxicity study (Annex X, Section 8.7.2.); Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.) by applying a read-across approach in accordance with Annex XI, Section 1.5. 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 aspects the chemical structures have in common and the differences between the structures of 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. Thus physicochemical properties influence the human health and environmental properties of a substance and should be considered in read-across assessments.
However, the information on physicochemical 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.

[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.

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) and (2) Different compounds have the same type of effect(s).

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

Description of the grouping and read-across approach proposed by the Registrant

You seek to adapt the human health information requirements for In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.); Pre-natal developmental toxicity study (Annex X, Section 8.7.2.); Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.) by applying a read-across approach according to Annex XI, Section 1.5.

You propose read-across between the structurally similar substance, dodecyldimethylamine oxide, EC No 216-700-6 (CAS No 1643-20-5) as source substance and the substance subject to this decision, amines, C12-14 (even numbered)-alkyldimethyl, N-oxides (EC 931-292-6) as target substance for the endpoints: In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.); Pre-natal developmental toxicity study (Annex X, Section 8.7.2.); Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.) . Furthermore, you propose read-across between the structurally similar substances decyldimethylamine oxide EC 220-020-5 (CAS 2605-79-0) and N,N, dimethyltetradecylamine N-oxide EC 222-059-3 (CAS 3332-27-2) for the endpoint In vitro gene mutation study in bacteria (Annex VIII, Section 8.4.1). You have also made reference to other members of a putative category of N-oxides, with EC numbers 220-020-5, 222-059-3, 230-429-0, 219-919-5, 274-687-2, 287-010-0, 263-016-9, 938-774-5, 931-341-1, and 938-679-9.

Your comment contains read-across documentation in a separate zip file. You have provided a read-across justification, general and specific comments on the draft decision, data matrices, robust study summaries of endpoint study records, and scientific papers.

You use the following arguments to support the prediction of properties of the registered substance from data for reference substance(s) within the group by interpolation to other substances in the group: that the registered substance is part of a category of related substances, and that as a result of structural and toxicological similarity, different category members have the same effects. According to you the source and registered substances have the same effects and potency for the above-mentioned information requirements.

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

ECHA analysis of the grouping and read-across approach

ECHA notes that the source substances include components of the registered substance, and substances with closely related composition. On the basis of the information provided in the comment, ECHA considers that the proposed read-across is plausible. However, this information, and the studies to be relied upon, have not been provided in the IUCLID dossier for the registered substance and so ECHA cannot conclude that the dossier is compliant. The information provided as part of your comments to the draft decision must be

[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).

included in an updated dossier in order to consider the dossier compliant. Therefore, the proposed read-across proposition does not comply with the requirement to provide adequate and reliable documentation (REACH, Annex XI, Section 1.5, last indent) in the IUCLID dossier.

In addition, ECHA notes that while you have provided study summaries for Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) and Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.), and these summaries are based on the SIDS Initial Assessment Report for Amine Oxides, you also indicate that updated robust study summaries will be prepared and used to update the dossier. As these robust study summaries have not been provided and ECHA has not been able to evaluate those studies; ECHA reserves the right to consider whether those studies are compliant. The evaluation of those studies might lead to the conclusion that the proposed read-across and provided robust study summaries are compliant or not. Information on how to report robust study summaries can be found in the ECHA Practical Guide "How to report robust study summaries" at https://echa.europa.eu/practical-guides.

Therefore, while the proposed adaptation is plausible, the information currently available in the dossier cannot be accepted to adapt the standard information requirements in quesstion.

1. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102**

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.

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid down in Annex VII, Section 8.4.1. 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.

According to Article 13(3) of the REACH Regulation, tests required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods recognised by the Commission or ECHA.

Other tests may be used if the conditions of Annex XI are met. More specifically, Section 1.1.2 of Annex XI provides that existing data on human health properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) may be used if the following conditions are met:

(1) Adequacy for the purpose of classification and labelling and/or risk assessment;
(2) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
(3) Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and
(4) adequate and reliable documentation of the study is provided.

According to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used: S. typhimurium TA1535; TA1537 or TA97a or TA97; TA98; TA100; S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). This includes four strains of *S. typhimurium* (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by E.coli WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site.

You have provided a test from the year 1989 according to OECD TG 471 and GLP with an assigned reliability score of 1. The test used five different strains of *S. typhimurium* TA 1535, TA 1537, TA 1538 TA 98 and TA 100 and it did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). However, since the test was conducted, significant changes have been made to OECD TG guideline 471 so that additionally testing with S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101) is now required.
Therefore, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

ECHA concludes that a test using *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 has not been submitted and that the test using one of these is required to conclude on *in vitro* gene mutation in bacteria.

Therefore, your adaptation of the information requirement is rejected.

After receipt of the draft decision, you provided comments including a proposed read-across, which is considered above under "Grouping of substances and read-across approach for toxicological information". ECHA considers that the read-across is plausible but this adaptation and the proposed studies are not present in the dossier, and for this reason, ECHA cannot conclude that the information in the dossier is compliant.

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 considers that the bacterial reverse mutation test (test method EU B.13/14. / OECD TG 471) is appropriate to address the standard information requirement of Annex VII, Section 8.4.1. of the REACH Regulation.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to complete following information derived with the registered substance subject to the present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102.

2. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

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.

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. 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 an *in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study in the dossier that would meet the information requirement of Annex VIII, Section 8.4.2.

Instead, you have provided the following information:

a) An adaptation according to Annex VIII, Section 8.4.2, column 2. This adaptation relies on a further adaptation based on Annex XI, Section 1.5 (grouping of substances and read-across approach-see point b)
b) An adaptation according to Annex XI, Section 1.5, using information from the source substance N,N-Dimethyl-1-methyldodecylamine oxide, (CAS no 60729-78-4)
c) An *in vivo* dominant lethal assay, OECD TG 478, on the registered substance, used as a supporting study

The adequacy of these information is analysed below:

a) Adaptation according to Annex VIII, Section 8.4.2, column 2.

You have sought to adapt this information requirement according to Annex VIII, Section 8.4.2., column 2 . You provided the following justification for the adaptation "In accordance with REACH Annex VIII section 8.4.2, an in vitro cytogenicity study in mammalian cells or in vitro micronucleus study is required as part of the standard information. However, according to the Column 2 adaptation, the study does not usually need to be conducted if adequate data from an in vivo cytogenicity test are available. Information from in vivo studies performed on a close structural analogue has been provided and hence it is considered that an in vitro study is not required."

However, ECHA notes that your adaptation does not meet the specific rules for adaptation of Annex IX, Section 8.7.2., column 2 because the available has been performed on a rejected read-across adaptation from an analogue substance according to Annex XI, Section 1.5 of the REACH regulation. The reason for rejecting this read-across adaptation is explained below.

b) Adaptation according to Annex IX, Section 1.5 (grouping of substances and read-across approach)

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing the following study records

1. An *in* vivo micronucleus assay (Szavbova, E. and Devinsky, F. 1988), no guideline followed, not according to GLP, with the source substance N,N-Dimethyl-1-methyldodecylamine oxide, (CAS no 60729-78-4).
2. An *in* vivo chromosome aberration and micronucleus assay (Karasova, M., Catar, G., Kieferova, L., Devinsky, F., and Leitmanova, A., 1987) , according to OECD TG 475, not according to GLP, with the analogue substance N,N-Dimethyl-1-methyldodecylamine

oxide, (CAS no 60729-78-4).

According to Annex XI, Section 1.5. there needs to be structural similarity among the substances within a group or category and furthermore, 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). Furthermore, Annex XI, Section 1.5. lists several additional requirements, including that adequate and reliable documentation of the applied method have to be provided.

You consider to achieve compliance with the REACH information requirements for the registered substance amines, C12-14 (even numbered)-alkyldimethyl, N-oxides using data of structurally similar substance N,N-Dimethyl-1-methyldodecylamine oxide (CAS No 60729-78-4) (hereafter the 'source substance').

You propose to predict the properties of the registered substance from data for source substances within the group on the basis of structural similarity. You propose that the source and registered substances have similar properties for the above-mentioned information requirement. You have not provided any additional read-across documentation.

ECHA considers that this information is your read-across hypothesis.

ECHA's evaluation and conclusion on the read-across approach

Your proposed adaptation argument is that the structural similarity between the source and target substance is a sufficient basis for predicting the properties of the substance. Structural similarity is a prerequisite for applying the grouping and read-across approach, but not per se sufficient to enable the prediction of human health properties of a substance.

This is because structural similarity does not always lead to predictable or similar human health properties. Further elements are needed[4], such as a well-founded hypothesis of (bio)transformation to a common compound(s), or that the target and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties that does not underestimate risks. You have not provided such elements in your dossier.

On that basis, the requirement of Annex XI, Section 1.5., that human health effects may be predicted from data for reference substance(s) within the group, has not been met.

Therefore, your adaptation of the information requirement is rejected.

c) Information from an *in vivo* dominant lethal assay, OECD TG 478,

You have provided information from an *in vivo* dominant lethal assay, OECD TG 478, done on the registered substance. ECHA notes that according to the first indent of column 2 of Annex VIII, Section 8.4.2, an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study does not need to be conducted if adequate data from an *in vivo* cytogenicity test are available.

[4]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 and 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).

First, ECHA notes that the study in question does not meet the requirements of the OECD TG 478 for the following reasons:

- The OECD TG 478 requires the use of concurrent positive control animals, unless the laboratory has demonstrated proficiency in the conduct of the test and has used the test routinely in the recent past (e.g. within the last 5 years). However, the study did not use concurrent positive controls

- The OECD TG 478 states the following regarding the study design "The exposure and mating regimen used is dependent on the ultimate purpose of the DL study. If the goal is to determine whether a given chemical induces DL mutations *per se*, then the accepted method would be to expose an entire round of spermatogenesis (e.g. 7 weeks in the mouse, 5-7 treatments per week) and mate once at the end. However, if the goal is to identify the sensitive germ cell type for DL induction, then a single or 5 day exposure followed by weekly mating is preferred." The study in question employed a five day exposure rather than an exposure duration for the entire round of spermatogenesis.

  Therefore, the study design employed is not suitable for identifying if the substance dominant lethal mutations per se, rather it is useful for identifying sensitive germ cells types for dominant lethal induction.

Second, ECHA notes that while the dominant lethal assay is designed to investigate if chemicals produce mutations due to chromosome aberrations, this investigation is limited to germ cells, and does not examine somatic cells. Therefore, a dominant lethal assay cannot be considered as an adequate *in vivo* cytogenicity study for the purpose of replacing an *in vitro* cytogenicity or *in vitro* micronucleus assay. ECHA concludes that this study is not sufficient for adapting the information requirement in question based on the specific rules for adaptation in Column 2 of Annex VIII, 8.4.2

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.

In your comments on the draft decision, you agreed with ECHA's decision.

ECHA considers that the *in vitro* mammalian chromosome aberration test (test method OECD TG 473) and the *in vitro* mammalian cell micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

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: *In vitro* mammalian chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

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

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.

Pre-natal developmental toxicity studies (test method EU B.31./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 contains the following endpoint study records on the endpoint for pre-natal developmental toxicity:

1. A pre-natal developmental toxicity study done according to OECD TG 414, on rats, on the registered substance
2. A screening study on reproductive/developmental toxicity done according to OECD TG 422 on the registered substance
3. A pre-natal developmental toxicity study done according to EPA OPP 83-3 (Prenatal Developmental Toxicity Study), on rabbits, done on the analogue substance cis-9-octadecenylamine, CAS 112-90-3, with an assigned reliability score of 4 (reliability not assignable)
4. A pre-natal developmental toxicity study done according to EPA OPP 83-3 (Prenatal Developmental Toxicity Study), on rabbits, done on the analogue substance 1-Octadecanamine, N-methyl-N-octadecyl-, CAS 4088-22-6, with an assigned reliability score of 4 (reliability not assignable)
5. An endpoint study record, waiving the study based on a weight of evidence adaptation, according to Annex XI, Section 1.2

Therefore, the technical dossier contains information on a pre-natal developmental toxicity study in rats by the oral route using the registered substance as test material.

However, there is no information provided for a pre-natal developmental toxicity study in a second species on the registered substance.

Instead, you have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this adaptation. You have provided the following justification for the weight of evidence adaptation for the information requirement on a pre-natal developmental toxicity study in a second species: "The absence of developmental toxicity effects at dose levels below those at which maternal systemic toxicity was observed in studies performed in rats using C12-14 AO and in rabbits using cis-9-octadecenylamine or N-methyl-N-octadecyl-1-octadecanamine (surrogates for the metabolic intermediates of amine oxides) indicates that there is no concern for developmental toxicity from amine oxides. On this basis, and taking account of REACH Annex XI, Section 1.2, no further pre-natal developmental toxicity studies on C12-14 AO are required."

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance with respect to a pre-natal developmental toxicity study (EU B.31/OECD TG 414). Relevant elements are in particular information on a second species, exposure route, duration and levels, sensitivity and depth of investigations to

detect pre-natal developmental toxicity (including growth, survival, external, skeletal and visceral alterations) and maternal toxicity.

Furthermore, the relative values/weights of different pieces of the provided information needs to be assessed as indicated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.4.4. In particular relevance, reliability and adequacy for the purpose as well as consistency of results/data need to be considered.

Evaluation of the provided information

This adaptation itself relies on information obtained from an analogue substance, and therefore can be considered as an adaptation according to Annex XI, Section 1.5 (grouping of substances and read-across approach). Therefore ECHA will first evaluate the read-across adaptation before concluding on the weight of evidence approach.

Information obtained using a read-across approach

You have sought to adapt the information requirements for this endpoint by applying a read-across approach in accordance with Annex XI, Section 1.5. According to Annex XI, Section 1.5. there needs to be structural similarity among the substances within a group or category and furthermore, 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). Furthermore, Annex XI, Section 1.5. lists several additional requirements, including that adequate and reliable documentation of the applied method have to be provided.

You consider to achieve compliance with the REACH information requirements for the registered substance using data of structurally similar substances cis-9-octadecenylamine, CAS 112-90-3 and 1-Octadecanamine, N-methyl-N-octadecyl-, CAS 4088-22-6 (hereafter the 'source substances').

You have provided read-across documentation in the endpoint summary as well as the CSR. You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group:

- You consider that the registered substance has been shown to undergo "rapid" metabolism

- You consider that some of the metabolites identified indicate that alkyl amines are an intermediate formed during the metabolism of the registered substance. Therefore, you consider that it is justified to rely on information from alkyl amines to fulfil this information requirement.

- You state that the metabolism of amine oxides is "considered to be likely to be similar between different mammalian species including the rabbit and the rat"

- Information on the pre-natal developmental toxicity of two alkyl amines (cis-9-octadecenylamine, CAS 112-90-3 and N-methyl-N-octadecyl-1-octadecanamine CAS 4088-22-6) is available, and this information is read-across to the registered substance.

Based on the above, you propose that the source and registered substances have similar properties for the above-mentioned information requirement.ECHA considers that this information is your read-across hypothesis.

ECHA's evaluation and conclusion on the read-across approach

ECHA has identified the following shortcomings in your arguments:

1. Although the information available from the toxicokinetic information on the registered substance does indicate that metabolism occurs, the metabolism is not sufficiently rapid to exclude exposure of animals to the registered susbtance prior to metabolism. The available information on the toxicokinetics of the registered substance do not provide information on how rapidly the parent substance is metabolised. The information only indicates that the maximum concentration of radioactivity occurs at approximately 90 minutes following administration. This information does not indicate whether the measured radioactivity is due to the registered substance, its metabolites, or a combination of these. Contrary to your assertion that metabolism is rapid, the information in the dossier indicates that there is a likelihood of exposure to the registered substance prior to its metabolism.
2. Even assuming sufficiently rapid metabolism occurs, the analogue substances used for read-across are not metabolites of the registered substance. In your technical dossier, you indicated that the toxicokinetic studies available identified 10 metabolites, of which two are identified in the dossier, and these are indicates 2 (,N-dimethyl-N-oxide-4-aminobutyric acid) and E (N,N-dimethyl-4-aminobutyric acid). Neither of those identified metabolites are the analogue substances used for read-across. In fact, the structure of the analogue substances indicates that they are unlikely to be formed as a result of the metabolism of the registered substance. Both analogues in question have C-18 carbon side chain. The composition of the registered substance does not include any C-18 chains, and the metabolic pathway proposed cannot result in the formation of the analogue substance from the registered substance. Rather, the source substances used here are possible analogues of some of the potential metabolites of the registered substance. Therefore, the source substances cannot predict the properties of the registered substance based on the presence of common metabolites.
3. While you consider that the metabolism of amine oxides is likely to be similar between the rat and the rabbit, the available toxicokinetic information, as well as your conclusion on the toxicokinetics of the registered substance contradict this conclusion. Your conclusion on the metabolism of the substance states the following: "Qualitatively, the metabolic profiles (from urine) were similar for rat, rabbit and man but quantitatively there were differences. The identity of the major human metabolite of the test substance was proposed as *N*-methyl-4-aminobutyric acid N-oxide (~60% of urinary profile). This metabolite was also the most abundant metabolite in rabbit urine but not for rats. The major metabolite in rats resulted from hydroxylation of the side chain. The proportion of this metabolite in rat urine doubled when the dose of the test substance was increased from 1 to 100 mg/kg. Another substantial (~30%) metabolite seen in rats, rabbits and humans was proposed to be *N, N*-dimethyl-4-aminobutyric acid. The presence of this metabolite would indicate that a dodecyldimethylamine (DDA) was a likely metabolic intermediate. Further examination of the urinary metabolic profiles indicated that the rat excreted >12% of the dose as long-chain compounds (at least half of these were C-hydroxylated amino-alcohols), which was in contrast to the rabbit and man. As such, it appears that the metabolism of the test substance by the rabbit is closer to that of man than the rat." (emphasis added).

These differences in the metabolism of the substance between rat, rabbit, and man indicate that the substance may behave differently in pre-natal developmental toxicity study in rabbit, compared to the rat.

4. Finally, even if the source substances are considered to be analogues of some of the metabolites, or metabolic intermediates of the registered substance, you have not considered the impact of other metabolites of the registered substance on its toxicity. In your justification for the adaptation, you note that several different metabolic pathways are proposed, including:

   • Oxidative degradation of the alkyl side chain by ω-oxidation to form a carboxylic acid followed by the loss of two-carbon fragments by sequential β-oxidation. This route of metabolism is frequently observed in similar substances to the test substance.
   • Hydroxylation of the alkyl side chain at a position four or five carbons from the end of the chain.
   • Reduction of the amine oxide group to the parent amine.

   However, the source substance you have used would only account for some of the metabolites resulting from the third pathway (reduction of the amine oxide group), and would not address the potential toxicity of any other metabolites from other metabolic pathways.

On that basis, the requirement of Annex XI, Section 1.5., that human health effects may be predicted from data for reference substance(s) within the group, has not been met and the proposed read-across is rejected.

Finally, ECHA notes that you assigned reliability 4 (not assignable) to both of the studies on the source substances. In view of the reliability you assigned, this information cannot be used as reliable source of information within your weight of evidence adaptation.

Hence, the studies on these source substances cannot be considered as reliable evidence as part of a weight of evidence approach, and cannot be used to assume/conclude that the substance does not have a particular dangerous (hazardous) property with respect to the information requirement for Annex X, Section 8.7.2.

Evaluation of other sources of information

In addition to the studies obtained from read-across from structural analogues, your weight of evidence approach relies on the information obtained from the pre-natal developmental toxicity study on the rat (first species), and the screening study for reproductive/developmental toxicity, both performed on the registered substance. However, ECHA points out that neither of these studies provides any information on pre-natal developmental toxicity in a second (non-rodent) species. Therefore, they cannot be considered to address the key elements of this information requirement. In addition, ECHA points out that the screening study for reproductive/developmental toxicity does not provide information on key parameters of a pre-natal developmental toxicity study.

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.

After receipt of the draft decision, you provided comments including a proposed read-across, which is considered under "Grouping of substances and read-across approach for toxicological information". ECHA considers that the read-across is plausible, but there is insufficient information provided for ECHA to conduct an independent assessment of this study to determine if it is compliant. This adaptation and the proposed studies are not present in the dossier, and ECHA cannot conclude that the information in the dossier is compliant.

The test in the first species was carried out by using a rodent species (rat). According to the test method EU B.31./OECD 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbit as a second 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 5.0, December 2016) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 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: EU B.31./OECD TG 414) in a second species (rabbit) by the oral route.

4. 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 EU B.56./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* R.7a, chapter R.7.6 (version 5.0, December 2016).

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.

In the technical dossier you have provided a study record for a screening study for reproductive/developmental toxicity using OECD TG 422 on the registered substance.

However, this study 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.

In addition, you have sought to adapt this information requirement . You have provided a statement to justify the absence of information on this endpoint "The results available from the long term repeat dose studies show no effects on the reproductive organs of the animals, where examined. In the prenatal developmental toxicity study, no evidence of embryotoxic or teratogenic potential was obtained independent of maternal toxicity. In the OECD 422 study there no adverse effects on reproduction or development of offspring. Based on the experimental evidence a 2 generation study is not necessary."

While you have not explicitly claimed an adaptation based on weight of evidence, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this adaptation. You have not provided an explanation or justification on how the sources of information/studies, which you have provided enable an assumption or conclusion that the registered substance does or does not have a dangerous property with respect to the extended one generation reproductive toxicity study. ECHA understands that you conclude that the registered substance does not have a dangerous (hazardous) property with respect to this endpoint.

To support your weight of evidence adaptation you have provided the following sources of information:

- Key study: screening study for reproductive/developmental toxicity, rat, oral route (OECD TG 422; GLP) with the registered substance, [redacted] 2008.),
- Information on pre-natal developmental toxicity. Although you do not refer to specific studies in your justification, ECHA understands that you refer to all the information available on pre-natal developmental toxicity (as described in point 3 above),
- Information on sub-chronic toxicity study. Although you do not refer to specific studies in your justification, ECHA understands that you refer to all the available information on repeated dose toxicity in your dossier. There are a total of five repeated dose toxicity studies via the oral route as well as two repeated dose toxicity studies via the dermal route in your dossier.

ECHA's evaluation and conclusion of the provided information

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property with respect to the information requirement in question including an adequate and reliable documentation.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance at equivalent level as investigated in an extended one-generation reproductive toxicity study (EU B.56./OECD TG 443) as requested in this decision. ECHA considers that this study provides relevant information on two aspects,

namely on sexual function and fertility in P0 and F1 generations (further referred to as 'sexual function and fertility') and on developmental toxicity observable peri- and postnatally in the F1 generation (further referred to as 'post-natal developmental toxicity'). Relevant elements for 'sexual function and fertility' are in particular functional fertility (mating behaviour, conception, pregnancy, parturition, and lactation) in the parental generation after sufficient pre-mating exposure and histopathological examinations of reproductive organs in both P0 and F1 generations. Relevant elements for 'post-natal developmental toxicity' are in particular peri- and post-natal investigations of the F1 generation up to adulthood.

Sexual function and fertility

With respect to the aspect of sexual function and fertility of P0 and F1 generation, you have provided reliable information on histopathological changes in major reproductive organs (OECD TG 422 screening study). You have also provided reliable information on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition (OECD TG 422 screening study). However, ECHA notes that the statistical power of OECD TG 422 study is lower than that of the extended one-generation reproductive toxicity study, and certain investigations are not included, such as functional fertility after 10 weeks premating exposure to cover spermatogenesis and folliculogenesis before mating, histopathology of the reproductive organs in F1 animals in adulthood, sexual maturation, oestrous cycle measurements in F1 animals, and investigations related to hormonal modes of action. Furthermore, you did not provide information on sperm parameters in P and F1 generations. You claim that the available information from the long term repeated dose toxicity studies (including sub-chronic toxicity (90-days) study) in the rat confirm that the reproductive organs are not affected after repeated exposure to the registered substance. ECHA notes that of these studies, 4 studies included examination of reproductive organs. These studies are:

1) A 13 week sub-chronic toxicity study on the registered substance, in rats, via the oral route, performed with "methods comparable to OECD guideline 408", [redacted] 1974, non-GLP
2) A 13 week sub-chronic toxicity study on the registered substance, in rats, via the oral route, performed with "methods comparable to OECD guideline 408", [redacted], 1980, non-GLP
3) A 32 week study on the registered substance, in rabbits, via the oral route, performed with "methods similar to OECD 408", [redacted] 1977, non-GLP
4) A 91 day repeated dose toxicity study on the registered substance, in mice, via the dermal route, performed with "methods comparable to OECD guideline 411", [redacted], 1975, non-GLP. This study included pathological examination of some reproductive organs but no histopathological examination.

ECHA notes that all these studies have been conducted prior to approval of current guidelines and GLP. With regard to the examination of reproductive organs this means that there is limited evidence from these studies that such reproductive organs/tissues are not affected. In comparison to current methods the past histopathological methods used fixation methods not-recommended anymore and furthermore lack the sensitivity of the modern methods.

Thus, the information you provided does not support your conclusion that the substance does not have a dangerous property with respect to sexual function and fertility.

Post-natal developmental toxicity

ECHA notes that your adaptation justification does not address the post-natal developmental toxicity. The provided information does also not cover the key elements which need to be investigated in this regard. The OECD TG 422 screening study investigates developmental toxicity only until postnatal day 4. The studies according to OECD TG 414 provide information only on pre-natal developmental toxicity. ECHA notes that the PNDT study in a second species is also missing for the reasons explained in request 3 above. These data do not allow to conclude that developmental effects are not observed at all in studies conducted with the registered substance, and in particular do not cover the peri-and postnatal developmental toxicity. Thus, the information you provided does not support the conclusion that the substance does not have a hazardous property with respect to postnatal developmental toxicity.

Conclusion

Hence, the information you provided to support you adaptation, considered individually or together, do not allow to assume/conclude that the substance does not have a particular dangerous (hazardous) property with respect to the information requirement for Annex X, Section 8.7.3.

Therefore, 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.

Hence, 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.

After receipt of the draft decision, you provided comments including a proposed read-across, which is considered under "Grouping of substances and read-across approach for toxicological information". ECHA considers that the read-across is plausible, but there is insufficient information provided for ECHA to conduct an independent assessment of this study to determine if it is compliant. This adaptation and the proposed studies are not present in the dossier, and ECHA cannot conclude that the information in the dossier is compliant.

The following refers to the specifications of this required study.

a) 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* R.7a, chapter R.7.6 (version 5.0, December 2016).

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of 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 existing 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.

Ophthalmological examinations

The OECD TG 443 does not include ophthalmological examination of animals as part of the required observations. However, ECHA notes that the registered substance induces cataracts in a number of repeated dose toxicity studies on the registered substance. Due to these findings in repeated-dose toxicity studies, the Registrant is required to include ophthalmological examination of the P0 animals prior to sacrifice. In addition, in order to examine whether the substance causes developmental toxicity in the form of cataracts, you are required to include ophthalmological examination of the F1 animals prior to sacrifice.

Species and route selection

According to the test method EU B.56./ 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 5.0, December 2016) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

b) 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 EU B.56./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 some 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;
- Ophthalmological examination of the parental animals (P0) prior to sacrifice and the F1 animals prior to sacrifice.

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 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* R.7a, chapter R.7.6 (version 5.0, December 2016). 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.

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 20 December 2016.

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.

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. The substance subject to the present decision is provisionally listed in the Community rolling action plan (CoRAP) for start of substance evaluation in 2019.

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

3. 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.

4. 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.