

Helsinki, 08 February 2022

Addressees

Registrants of JS-UVCB-283-464-9-197 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision

01/11/2018

Registered substance subject to this decision ("the Substance")

Substance name: Amines, C12-14-alkyldimethyl

EC number: 283-464-9

CAS number: 84649-84-3

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)**DECISION ON A COMPLIANCE CHECK**Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of **13 November 2024**.

Requested information must be generated using the Substance unless otherwise specified.

A. Information required from all the Registrants subject to Annex VII of REACH

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: EU B.13/14. / OECD TG 471)

B. Information required from all the Registrants subject to Annex VIII of REACH

1. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490)

C. Information required from all the Registrants subject to Annex IX of REACH

1. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats
2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit)
3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211)
4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: OECD TG 210)

Reasons for the request(s) are explained in the following appendices:

- Appendix entitled "Reasons common to several requests";
- Appendices entitled "Reasons to request information required under Annexes VII and IX of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to <http://echa.europa.eu/regulations/appeals> for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised¹ under the authority of Mike Rasenberg, Director of Hazard Assessment

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 on Reasons common to several requests

1. Assessment of your read-across approach under Annex XI, Section 1.5.

You seek to adapt the information requirements for the following standard information requirements by grouping substances in the category and applying a read-across approach in accordance with Annex XI, Section 1.5:

- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

ECHA has considered the scientific and regulatory validity of your grouping and read-across approach in general before assessing the specific standard information requirements in the following appendices.

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. 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 (addressed under 'Scope of the grouping'). 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 (addressed under 'Assessment of prediction(s)').

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance R.6. and related documents^{2,3}.

A. Scope of the grouping

i. Description of the grouping

In your registration dossier you have formed a group (category) of 'dimethylalkylamines' (DMA Category). You have provided a read-across justification document in IUCLID Section 13 in your CSR.

You provide the following reasoning for the grouping the substances: "*all members share a very similar chemical structure, which is the basis for physical-chemical properties that are similar and follow a predictable trend with increasing alkyl chain length*" and "*due to the very similar structure, similar physico-chemical properties, environmental fate, ecotoxicity and mammalian toxicity of the DMAs under discussion, they can be accounted for in one category and fulfilment of data requirements by read-across from one category member to all other category members is justified.*"

You define the applicability domain of the category as follows:

'The category of dimethylalkylamines (DMAs) (i.e. N,N-dimethyl-Cx-(even numbered)-alkyl-1-amines) covers ten DMAs with alkyl chain lengths ranging from C10 to C18.'

² Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: [Read-Across Assessment Framework \(https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across\)](https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)

³ Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017 (March) ECHA, Helsinki. 40 pp. Available online: <https://doi.org/10.2823/794394>

ECHA understands that this is the applicability domain of the grouping and will assess your predictions on this basis.

ECHA notes the following shortcomings with regards to your grouping approach regarding toxicological properties.

You have provided the following reasoning for the prediction of toxicological properties:

"The actual toxicity profile of a substance is driven by its intrinsic properties and its toxicokinetic behaviour. Based on the close structural similarities of the DMAs under consideration, significant differences in their intrinsic properties as well as in their toxicokinetic behaviour are not to be expected."

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which is based on the formation of common (bio)transformation products. The properties of your Substance are predicted to be quantitatively equal to those of the source substance.

You intend to predict the properties for the category members from information obtained from the following source substances:

In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

- Decyldimethylamine / CAS 1120-24-7, RA_C10DMA_KEY_471_1996 [REDACTED]
- Dodecyldimethylamine / /CAS 112-18-5, RA_C12DMA_KEY_471_1988 [REDACTED]
- Dimethyl(tetradecyl)amine /CAS 112-75-4, RA_C14DMA_KEY_471_1996 [REDACTED]
- Hexadecyldimethylamine /CAS 112-69-6, RA_C16DMA_KEY_471_1996 [REDACTED]
- Dimantine /CAS 124-28-7, RA_C18DMA_KEY_471_1996 [REDACTED]

In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

- Amines, C12-18-alkyldimethyl / CAS 68391-04-8, RA_C12-18DMA_KEY_476, OECD 476 (1002200) and RA_C12-18DMA_KEY_476 (1046601)

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

- Dimethyl(pentadecyl)amine oxide (EC 273-281-2), [REDACTED] 2011, OECD 422
- Amines, C10-16-alkyldimethyl, N-oxides / CAS 70592-80-2 (EC 274-687-2),
 - [REDACTED], 1985, combined repeated dose and carcinogenicity equiv. to OECD 453,
 - [REDACTED] 1980, 13 weeks subchronic dietary study (EPA OPP 82-1 (90-Day Oral Toxicity)),
 - [REDACTED] 1977, 32-Week feeding study in rabbits,
 - [REDACTED], 1988, OECD Guideline 411 (Subchronic Dermal Toxicity: 90-Day Study),
 - [REDACTED], 1988, OECD Guideline 410 (Repeated Dose Dermal Toxicity: 21/28-Day Study) in rabbit,
- Dodecyldimethylamine oxide / CAS 1643-20-5 (EC 216-700-6),
 - [REDACTED], 1984 - combined repeated dose and carcinogenicity,
 - [REDACTED], 1979B, two-generations study,
 - [REDACTED], 1994 (sub-acute dermal study)

Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

- Amines, C12-18-alkyldimethyl, N-oxides / CAS 68955-55-5 , [REDACTED], 2011, OECD 422
- Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides / CAS 308062-28-4, 1999-[REDACTED], according to EPA OTS 798.4900 (Prenatal Developmental

- Toxicity Study)
- Dodecyldimethylamine oxide / CAS 1643-20-5, [REDACTED], 1979A, [REDACTED] 1979B, [REDACTED] 1980,
 - Amines, C10-16-alkyldimethyl, N-oxides / CAS 70592-80-2, OECD SIDS [REDACTED] [REDACTED] 1966, 3-generations study

For the endpoints of acute toxicity, skin/eye irritation and genotoxicity you invoke a read-across approach between DMA category members.

For the high-tier endpoints such as repeated dose toxicity and reproductive toxicity you use a read-across approach to several DMA oxides (DMAOs). The hypothesis for this read-across is also based on similar toxicokinetics due to interconvertibility *in vivo* between tertiary amines and corresponding amine oxides leading to comparable metabolic fates. Specifically, you argue the following:

- All DMAOs used for read-across differ from the DMA category only in the N-oxidation.
- Tertiary amines are easily oxidized to form the corresponding N-oxide, while these N-oxide are readily reduced to form the respective tertiary amine.
- The studies on toxicokinetics of dodecyldimethylamine oxide (C12-DMAO) provide evidence that C12-DMA is an intermediate in the metabolism of C12-DMAO.
- Practically identical NOELs (40-50 mg/kg bw/d) were observed for C12-14-DMA (OCD 407 and 421) and C12-18-DMAO (OECD 422) in (subacute) repeated-dose toxicity studies

ECHA has assessed each read-across approach and notes the following shortcomings with regards to predictions of toxicological properties.

1) *Specific issue for the read-across within DMA category*

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.*"

According to the ECHA Guidance, one of the factors in determining the robustness of a category is the density and distribution of the available data across the category.⁴ To identify a regular pattern and/or to derive reliable prediction of the properties of the members of the category, adequate and reliable information covering the range of structural variations identified among the category members needs to be available.

Furthermore in larger categories there may be breaks in trends which could affect the reliability of interpolation.⁵ To confirm that there are no such breakpoints, adequate and reliable information needs to cover also substances within a range of homologous series.

However, for *in vitro* gene mutation in mammalian cells and repeated dose toxicity/Reproduction/Developmental Toxicity Screening study you have provided data only for one DMA category member (substances Amines, C12-18-alkyldimethyl /CAS 68391-04-8 and C12-14-alkyldimethyl / CAS 84649-84-3, respectively).

For gene mutation in bacterial cells you provided seven Ames studies with six different substances. However, as explained in Appendix A1. only the study performed with dodecyldimethylamine / CAS 112-18-5 is an adequate study performed with all the strains

⁴ Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.1.5.

⁵ Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.2.

requested by the testing guideline. All the other studies are performed in only two and same strains (*S. thyphimurium* TA 98 and 100). Therefore, also for this endpoint it is considered that there is only one reliable study for the whole category.

Based on these studies you claim that there is a similar toxicity profile for all the members of the DMA category.

Information for one or two category members per endpoint is not sufficient to establish a trend across the category consisting of 10 substances. Furthermore, these substances cover only part of the category range and none of studies, for any of the above endpoints, covers the lower range of the category. In addition, for the high tier endpoints also the upper range of the category is not covered. In the absence of information on substances for all ranges of the category (lower border, between the upper and lower borders of the category and upper border of the category), it cannot be confirmed that there is no breakpoint in toxicity trend within the given range of chain length. Therefore, the information provided is not sufficient to conclude that toxicological properties are likely to follow a regular pattern.

2) Common issue for the read-across within DMA category and between DMA and DMAOs

Annex XI, Section 1.5 of the REACH Regulation states that "*physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)*". For this purpose "*it is important to provide supporting information to strengthen the rationale for the read-across*"⁶. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on other category members.

Supporting information must include toxicokinetic information on the formation of the common compound.

As indicated above, your read-across hypothesis is based on the (bio)transformation of the category members to a common compound(s). In this context, information characterising the rate and extent of the hydrolysis of the category members is necessary to confirm the formation of the proposed common hydrolysis product and to assess the impact of the exposure to the parent compounds.

However, you provided 10 toxicokinetic studies with DMAOs comparing their metabolite profiles and one study ([REDACTED], 1981) with dodecyldimethylamine (DDA) / EC 112-18-5 and its oxide comparing their metabolite profiles in urine.

You have not provided any reliable information regarding the claim of similar toxicokinetics of the DMA category members. Only one study ([REDACTED], 1981) provided for the toxicokinetics endpoint is performed with a DMA compound. This study, which was insufficiently reported for assessment (reliability 4) and performed in only one animal, found that the metabolite profile of DDA was the same as that of the 0-24 h urine from rats dosed with 14C-DDA oxide. However, there are no studies comparing the metabolite profile with other DMA category members. This study is neither sufficient to extrapolate a metabolic similarity profile between DMA category members and DMAOs.

Regarding the claim of similar toxicokinetic between the DMA category members and DMAOs due to an interconvertibility *in vivo* between tertiary amines and corresponding amine oxides, it is noted that N-oxidation is only one of the two principal pathways involved in the

⁶ Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

metabolism of tertiary aliphatic amines. There is no experimental evidence on the extent of contribution to metabolism of N-oxidation vs the N-dealkylation, which is another metabolic pathway. The presence of the metabolite N,N-dimethyl-4-aminobutyric acid in urine of animals dosed with DDAO was considered to indicate that DDA might be a metabolic intermediate. Nevertheless, the study of ██████████ (1981) states that "*N,N-Dimethyl-4-aminobutyric acid and its N-oxide accounted for 28% of the dose in humans, 28% in rabbits and 23% in rats. These species excreted 44, 51 and 60% of the dose, respectively, in 24 h.*" Thus, it seems that a large part of the dose was eliminated in the form of other metabolites.

While the formation of N-oxide from the various analogues of the DMA category is plausible and in line with available literature on the metabolism of tertiary amines, more information is necessary from other DMA compounds to make the argumentation robust. Most importantly, there is no information regarding other metabolic pathways (e.g. dealkylation, hydroxylation of alkyl chains) which should be considered since the oxidation, while an important pathway, is not the only one.

In the absence of this information, you have not provided supporting evidence establishing that common metabolites are formed as assumed in your read-across hypothesis. Therefore, you have not provided sufficient supporting information to strengthen the rationale for the read-across.

3) *Specific issue for the read-across between DMA category members and DMAOs*

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. The ECHA Guidance⁷ indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substances. The observation of differences in the toxicological properties among some members of a category is a warning sign. An explanation for such a difference resulting in a contradiction between the similarities in properties claimed in the read-across hypothesis and the observation of different properties needs to be provided and supported by scientific evidence.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar target and source substances cause the same type of effects.

The results of the information on reproductive toxicity obtained with a DMA category member vary from the effects seen in similar studies performed with DMAOs. Specifically, the following effects were seen at in an OECD 421 with Amines, C12-14-alkyldimethyl / CAS 84649-84-3):

- Influence on parturition: 2 dams did not deliver at all;
- High number of postimplantation losses;
- Stillbirths and low number of pups alive on day of delivery;
- Low male birth weight;
- Influence on nursing behaviour.

No such effects were seen at similar dose ranges (150-200 mg/kg bw/d) in an OECD 422 with Amines, C12-18-alkyldimethyl / CAS 68955-55-5. No such effects were seen either in a 2-generations study with dodecyldimethylamine oxide / CAS 1643-20-5 (OECD SIDS-██████████ 1979B) nor in a 3-generation study with Amines, C10-16-alkyldimethyl, N-oxides / CAS

⁷ Guidance on information requirements and chemical safety assessment (version 6.0, July 2017), Chapter R.6, Section R.6.2.2.1.f

70592-80-2 (OECD SIDS [REDACTED] 1966), albeit in these studies the highest dose was up to 50 mg/kg bw/d.

The available set of data on the target and source substances indicates differences in the toxicological properties of the substances. This contradicts your read-across hypothesis whereby the structurally similar target and source substances cause the same type of effect(s). Therefore you have not demonstrated and justified that the properties of the category members are likely to be similar despite the observation of these differences.

B. Conclusions on the grouping of substances and read-across approach

As explained above, you have not established that relevant properties of the Substance can be predicted from data on the analogue substance. Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. and your grouping and read-across approach is rejected.

In your comments on the draft decision you inform ECHA on your intention to improve the toxicological profile of the Substance and your intention to address all the deficiencies identified above. Your comments refer to data which is not yet available in the dossier, therefore your comments have no impact on the dossier's incompliances currently identified. Please note that this decision does not consider updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation"). You remain responsible for complying with this decision by the set deadline.

Appendix A: Reasons to request information required under Annex VII of REACH

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is an information requirement under Annex VII to REACH (Section 8.4.1.).

You have provided key studies and one supporting study in your dossier:

- i. KEY_471_1996_██████████_96 with Amines, C12-14-alkyldimethyl / CAS 84649-84-3 in strains *S. typhimurium* TA 98 and TA 100 which gave negative results.
- ii. RA_C10DMA_KEY_471_1996_██████████ with decyldimethylamine / CAS 1120-24-7 in strains *S. typhimurium* TA 98 and TA 100 which gave negative results.
- iii. RA_C12DMA_KEY_471_1988_██████████ with dodecyldimethylamine / CAS 112-18-5 in the strains *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 which gave negative results.
- iv. RA_C14DMA_KEY_471_1996_██████████ with dimethyl(tetradecyl) / CAS 112-75-4 in strains *S. typhimurium* TA 98 and TA 100 which gave negative results.
- v. RA_C16DMA_KEY_471_1996_██████████ with hexadecyldimethylamine / CAS 112-69-6 in strains *S. typhimurium* TA 98 and TA 100 which gave negative results.
- vi. RA_C18DMA_KEY_471_1996_██████████ with dimantine / N,N-dimethyloctadecan-1-amine / CAS 124-28-7 in strains *S. typhimurium* TA 98 and TA 100 which gave negative results.
- vii. RA_C12DMA_NON KEY_471_1996_██████████, supporting study with dodecyldimethylamine / CAS 112-18-5 in the strains *S. typhimurium* TA 98 and TA 100 which gave negative results.

We have assessed this information and identified the following issue(s):

1) Non-conformity with the applicable test guideline

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471⁸ (1997). One of the key parameters of this test guideline includes that the test must be performed with 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

Neither the reported data for the study (i.) performed with the substance nor the studies ii. and iv.-vii. performed with analogue substances, did include results for the appropriate 5 strains, that is in TA98/TA100/TA1535/TA1537 or TA97a or TA97/the required fifth strain, *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101). All these studies are performed in only two and same strains (*S. typhimurium* TA 98 and 100). Only the study iii. performed with analogue dodecyldimethylamine / CAS 112-18-5 is an adequate study performed with all the strains requested by the testing guideline.

2) Invalid read-across adaptation

The studies (ii) to (vii.) are performed with analogues substances. However, as explained in the Appendix on Reasons common to several requests, the read-across within the DMA category was rejected.

In your comments on the draft decision, you inform ECHA that the *in vitro* gene mutation study in bacteria with C12-14-DMA is ongoing. Nevertheless, currently this information in your comments is not sufficient for ECHA to make an assessment, because you did not provide

⁸ ECHA Guidance R.7a, Table R.7.7-2, p.557

the robust study summary of the study. Please note that this decision does not take into account updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation")."

Therefore, the information requirement is not fulfilled.

Study design

To fulfil the information requirement for the Substance, the *in vitro* gene mutation study in bacteria (OECD TG 471) is considered suitable.

Appendix B: Reasons to request information required under Annex VIII of REACH

1. In vitro gene mutation study in mammalian cells

An *in vitro* gene mutation study in mammalian cells is an information requirement under Annex VIII to REACH (Section 8.4.3.) in case of a negative result in the *in vitro* gene mutation test in bacteria and the *in vitro* cytogenicity test.

i. Triggering of the study

Your dossier contains (i) a negative result for *in vivo* cytogenicity study in mammalian cells or *in vitro* micronucleus study, and (ii) no data or inadequate data for the other study (*in vitro* gene mutation study in bacteria).

The information for the *in vitro* gene mutation study in bacteria is rejected for the reasons provided in section 1 of the Appendix on Reasons common to several requests.

The result of the request for information in section A 1. will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

ii. Assessment of information provided

You have adapted this information requirement under Annex XI, Section 1.5 (Grouping of substances and read-across approach). In support of your adaptation, you provided the following information with the analogue N,N-dimethyl-C12-18-(even numbered)-alkyl-1- amines EC No. 269-923-6:

- i. a cell Mutation Assay at the Thymidine Kinase Locus in Mouse Lymphoma L5178Y Cells, according to OECD 476 (██████████, 2006),
- ii. a Gene Mutation Assay in Chinese Hamster V79 Cells in vitro (V79/HPRT), according to OECD 476 (██████████, 2006).

We have assessed this information and identified the following issue:

You have submitted two gene mutation in mammalian cells studies on an analogue substance. However, for the reasons explained in the Appendix on Reasons common to several requests your read-across adaptation is rejected.

On this basis, the provided studies are not regarded as providing reliable information to inform on the properties of the Substance. Therefore, the information requirement is not fulfilled.

In your comments on the draft decision, you inform ECHA that the *in vitro* gene mutation study in mammalian cells with C12-14-DMA is ongoing. Nevertheless, currently this information in your comments is not sufficient for ECHA to make an assessment, because you did not provide the robust study summary of the study. Please note the dossier update policy as described in Appendix A Section 1.

Consequently, you are required to provide information for this endpoint, if the *in vitro* gene mutation study in bacteria provides a negative result.

Appendix C: Reasons to request information required under Annex IX of REACH

1. Sub-chronic toxicity study (90-day)

A Sub-chronic toxicity study (90 day) is a standard information requirement in Annex IX to REACH.

You have adapted this information requirement under Annex XI, Section 1.5 (Grouping of substances and read-across approach). In support of your adaptation, you provided the following information:

- i. short term repeated toxicity study according to OECD 407, in rats via oral route, with the analogue substance *cln_n,n-dimethyl-c12-14-(even numbered)-alkyl-1-amines*, EC No.283-464-9, ██████████1995, (key study)
- ii. reproduction/development toxicity screening study according to OECD 421, in rats via oral route with the analogue substance *cln_n,n-dimethyl-c12-14-(even numbered)-alkyl-1-amines*, EC No. 283-464-9, ██████████1995, (supporting study)
- iii. combined repeated dose and reproduction / developmental screening according to OECD 422, in rats via oral route with the analogue substance *Amines, C12-18-alkyldimethyl, N-oxides*, EC No. 273-281-2, ██████████2011, (key study)
- iv. combined repeated dose and carcinogenicity study according to OECD 453, in rats via oral route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides*, EC No. 274-687-2, ██████████1985, (key study)
- v. combined repeated dose and carcinogenicity study (no guideline), in rats via oral route, with the analogue substance *dodecyldimethylamine oxide* EC No. 216-700-6, ██████████1984, (supporting study)
- vi. sub-chronic study according to EPA OPP 82-1, in rats via oral route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides* EC No. 274-687-2, ██████████1980, (supporting study)
- vii. sub-chronic study (No guideline), in rabbits via oral route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides* EC No. 274-687-2, ██████████1977, (supporting study)
- viii. sub-chronic study (No guideline), in rats via oral route, with the analogue substance *dodecyldimethylamine oxide* EC No. 216-700-6, ██████████1979, (supporting study)
- ix. combined repeated dose and carcinogenicity study according to OECD 453, in mouse via dermal route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides*, EC No. 274-687-2, ██████████1985, (key study)
- x. sub chronic studies (A) equivalent or similar to OECD 411, in rabbits via dermal route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides*, EC No. 274-687-2, ██████████1988, (supporting study)
- xi. sub chronic studies (B) equivalent or similar to OECD 411, in rabbits via dermal route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides*, EC No. 274-687-2, ██████████1988, (supporting study)
- xii. short term repeated dose toxicity (C) equivalent or similar to OECD 410, in rabbits, via dermal route, with the analogue substance *Amines, C10-16-alkyldimethyl, N-oxides*, EC No. 274-687-2, ██████████1988, (supporting study)
- xiii. short term repeated dose toxicity (No guideline), in rabbits, via dermal route, with the analogue substance *dodecyldimethylamine oxide* EC No. 216-700-6, ██████████1994, (supporting study).

We have assessed this information and identified the following issue:

For the reasons explained under the "Appendix on Reasons common to several requests", your adaptation according to Annex XI, Section 1.5 is rejected for the DMA and DMAO categories.

Based on the above, the information you provided do not fulfil the information requirement.

In your comments to draft decision you inform ECHA of a strategy relying on the generation of additional supporting information though four OECD 422 studies on the analogue substances C10-, C12-14-, C16- and C18-DMA that should substantiate your read-across approach. Based on the outcome of those studies you indicate your intention to perform at least one OECD 408 study on C10-, C12- or C12-14-DMA in order to consolidate your read-across hypothesis. Nevertheless, currently this information in your comments is not sufficient for ECHA to make an assessment, as a robust study summary of the study is not available. Please note the dossier update policy as described in Appendix A Section 1. In addition, note that the requested information on this information requirement must be submitted by the deadline set out in the present decision.

Information on the design of the study to be performed (route/ species/ strain)

Referring to the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the Substance is a liquid of very low vapour pressure.

Therefore the sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and with oral administration of the Substance.

2. Pre-natal developmental toxicity study in one species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

You have provided key studies and supporting studies in your dossier:

- i. [REDACTED], 1995, . OECD 421, with Amines, C12-14-alkyldimethyl / CAS 84649-84-3 in rat, (key study)
- ii. [REDACTED], 2011, OECD 422 with Amines, C12-18-alkyldimethyl, N-oxides / CAS 68955-55-5 in rat, (key study)
- iii. 1999 [REDACTED], according to EPA OTS 798.4900 (Prenatal Developmental Toxicity Study), with Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides / CAS 308062-28-4 in rat, (key study)
- iv. OECD SIDS-[REDACTED]1979A, (secondary source) developmental study with dodecyldimethylamine oxide / CAS 1643-20-5 in rat, (supporting study)
- v. OECD SIDS-[REDACTED]1980, (secondary source) developmental study with dodecyldimethylamine oxide / CAS 1643-20-5 in rabbit, (supporting study)
- vi. OECD SIDS-[REDACTED]1979B, (secondary source), 2-generations study with dodecyldimethylamine oxide / CAS 1643-20-5 in rat, (supporting study)
- vii. OECD SIDS [REDACTED]1966, (secondary source), 3-generations study with Amines, C10-16-alkyldimethyl, N-oxides / CAS 70592-80-2 in feed in rat, (supporting study)

We have assessed this information and identified the following issue:

In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, information provided has to meet the requirements of OECD TG 414 in one species, e.g external, skeletal and visceral malformations and variations has to be investigated as described in OECD TG 414.

However, you have not provided information following OECD TG 414 with the Substance. Instead, you have provided an OECD TG 421 "reproduction/ developmental toxicity screening test" ([REDACTED], 1995). This study does not inform on skeletal and visceral malformations and variations as required by OECD TG 414.

Therefore, the study provided with the Substance does not fulfil the information requirement.

You also provided several studies (ii. to vii.) performed with analogue substances. However, as explained in the Appendix on Reasons common to several requests, the read-across within the DMA category was rejected.

Therefore, the information requirement is not fulfilled.

In your comments to draft decision you inform ECHA on a strategy relying on the generation of additional supporting information though four OECD 422 studies on the analogue substances C10-, C12-14-, C16- and C18-DMA that should substantiate your read-across approach. Based on the outcome of those studies you agree to perform at least one OECD 414 study on C10-, C12- or C12-14-DMA. Nevertheless, currently this information in your comments is not sufficient for ECHA to make an assessment, as a robust study summary of the study is not available. Please note the dossier update policy as described in Appendix A Section 1.

A PNMT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral⁹ administration of the Substance.

3. Long-term toxicity testing on aquatic invertebrates

Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

You have provided the following information:

- i. 'Daphnia magna Reproduction Test (21d) with Natural River Water', on the Substance: [REDACTED], 2001.
- ii. 'Daphnia magna Reproduction Test with Natural River Water (Semi-Static, 21 d)' on the source substance N,N-dimethyl-C16-18-(even numbered)-alkyl-1-amines (EC 269-915-2): [REDACTED], 2012.

We have assessed this information and identified the following issues for both studies (i) and (ii):

To fulfil the information requirement, a study must comply with the OECD TG 211 (Article 13(3) of REACH). Therefore, the following specifications must be met:

Technical specifications impacting the sensitivity/reliability of the test

- the test medium fulfils the following conditions: total organic carbon (TOC) ≤ 2 mg/L, dissolved oxygen concentration ≥ 3 mg/L, hardness ≥ 140 mg/L (as CaCO₃), pH between 6 and 9;

However, your registration dossier provides two studies (i) and (ii) where the test was conducted with a test medium (river water) having the following characteristics: study (i) particulate matter of approximately 19 mg/L, dissolved organic carbon (DOC) of 6.6 mg/L, hardness of > 90 to ≤ 111 mg/L (as CaCO₃), pH of 7.3 to 7.96; and study (ii) particulate matter of 14.0 mg/L, dissolved organic carbon (DOC) of 14 mg/L, hardness of 271 mg/L (as CaCO₃), pH of 7.93.

In your comments to the draft decision, you explain that "OECD guideline 211 states that:

- "It is recommended that TOC levels in the medium be below 2mg/L."
- "Hardness above 140 mg/L (as CaCO₃) is recommended. Tests at this level and

⁹ ECHA Guidance R.7a, Section R.7.6.2.3.2.

above have demonstrated productive performance in compliance with the validity criteria"

- *"The guideline does not instruct to comply with the values presented to describe the test medium but recommends it. Furthermore, it is stated that:*
- *"...other media are acceptable provided the performance of the Daphnia culture is shown to meet the validity criteria for the test"*

In addition you state that *"the medium was changed to natural river water because of the difficulties in testing such substance. The deviations, especially in TOC, has not happened accidentally but were planned to provide an appropriate test environment for a cationic substance combined with a suitable medium for the daphnia organism. Therefore, the deviations from the recommendations are within the general experimental boundaries of the guideline and no methodological deficiencies can be identified. Therefore, the deviations from the recommendations are within the general experimental boundaries of the guideline and no methodological deficiencies can be identified."*

ECHA disagrees with your conclusions for the following reasons:

- OECD test guideline's recommendations on the maximum DOC concentrations aims at ensuring that the substance is available to the organisms in the testing media; especially when the Substance that has a high potency to bind on DOC;
- since the applied DOC concentration in the tests is 3-7 times higher than the maximum value recommended by the guideline (i.e. 2 mg/L), the binding to DOC may have limited bioavailability of the test substance and reduced exposure of test organisms to the dissolved fraction of the Substance.

Therefore, the reported effective concentrations may underestimate the toxicity of the substance and cannot be considered as reliable values for hazard assessment (i.e classification and labelling and PNEC derivation).

Based on the above,

- there are critical methodological deficiencies resulting in the rejection of both the study results. More, specifically the test medium for both studied (i) and (ii) was river water containing particulate matter with TOC > 2mg/l (as indicated by DOC values) and the water hardness for study (i) was outside the specification of OECD TG 211.

Therefore, the information requirement is not fulfilled.

4. Long-term toxicity testing on fish

Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

You have provided the following information: a justification to omit the study which you consider to be based on Annex IX, Section 9.1., Column 2. In support of your adaptation, you provided the following justification: *'Fish is clearly not the most sensitive organism group. Invertebrates are of higher sensitivity, and of special sensitivity are algae with EC50-values in the low µg/L range. Therefore, no further relevant information could be expected from tests on chronic fish toxicity.'*

We have assessed this information and identified the following issue:

Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on long-term toxicity to fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

Your adaptation is therefore rejected.

On this basis, the information requirement is not fulfilled.

In the comments to the draft decision you agree with the request.

Study design

To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (ECHA Guidance R.7.8.2.).

Appendix D: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

2. Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.
3. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.
4. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries¹⁰.

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

The Test Material used to generate the new data must be selected taking into account the following:

- the variation in compositions reported by all members of the joint submission,
- the boundary composition(s) of the Substance,
- the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

2. Information on the Test Material needed in the updated dossier

- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers¹¹.

¹⁰ <https://echa.europa.eu/practical-guides>

¹¹ <https://echa.europa.eu/manuals>

Appendix E: General recommendations when conducting and reporting new tests for REACH purposes

A. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in ECHA Guidance R.11 (Section R.11.4.2.2), you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the “known constituents approach” (by assessing specific constituents), or
- the “fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the “whole substance approach”, or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

Appendix F: Procedure

The information requirement for an Extended one-generation reproductive toxicity study (EOGRTS; Annexes IX, Section 8.7.3.) is not addressed in this decision. This may be addressed in a separate decision once the information from the Sub-chronic toxicity study (90-day) requested in the present decision is provided; this is due to the fact that the results from the 90-day study is needed for the design of the EOGRTS.

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

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 18 November 2020.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and amended the deadline.

Extension of the Deadline

In the comments to the draft decision, you requested an extension of the deadline to provide information, from 18 to 30 months from the date of adoption of the decision. You provided documentary evidence from the selected test laboratory indicating the scheduling timelines for the OECD 211 and OECD 210 studies to justify the extension.

On this basis, ECHA has granted the request and extended the deadline to 30 months.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix G: List of references - ECHA Guidance¹² and other supporting documentsEvaluation of available information

Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping

Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)¹³

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)¹⁴

Physical-chemical properties

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment

Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing

Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents¹⁵

¹² <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

¹³ <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

¹⁴ https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

¹⁵ <http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix H: Addressees of this decision and their corresponding information requirements

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.