~~Confidential~~

Helsinki, 21 April 2022

Addressees
Registrants of JS-C18-DMA as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
08/04/2021

Registered substance subject to this decision ("the Substance")
Substance name: Dimantine
EC number: 204-694-8
CAS number: 124-28-7

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 ***27 July 2023***.

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 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)

2. If negative results are obtained in test performed for the information requirement of Annex VIII, Section 8.4.2. then: In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490)

3. Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.) to be combined with the Screening for reproductive/developmental toxicity below

4. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.64/OECD TG 422) by oral route, in rats

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 to VIII 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 and VIII to REACH, for registration at 10-100 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.

Authorised[1] 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 cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

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)
[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 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 / N,N-dimethyldecan-1-amine / CAS 1120-24-7, RA_C10DMA_KEY_471_1996_[redacted]
- Dodecyldimethylamine / N,N-dimethyldodecan-1-amine /CAS 112-18-5, RA_C12DMA_KEY_471_1988_[redacted] and RA_C12DMA_NON_KEY_471_1996_[redacted]
- Dimethyl(tetradecyl)amine / N,N-dimethyltetradecan-1-amine /CAS 112-75-4, RA_C14DMA_KEY_471_1996_[redacted]
- Hexadecyldimethylamine / N,N-dimethylhexadecan-1-amine /CAS 112-69-6, RA_C16DMA_KEY_471_1996_[redacted]
- Amines, C12-14-alkyldimethyl/N,N-dimethyl-C12-14-(even numbered)-alkyl-1-amines / CAS 84649-84-3, RA_C12-14 DMA_KEY_471_1996_[redacted]

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

- C12-18-alkyldimethyl / N,N-dimethyl-C12-18-(even numbered)-alkyl-1-amines /CAS 68391-04-8, RA_C12-18DMA_KEY_473_2007

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

- Amines, C12-18-alkyldimethyl / N,N-dimethyl-C12-18-(even numbered)-alkyl-1-amines /CAS 68391-04-8, RA_C12-18DMA_KEY_476, OECD 476 ([redacted]) and RA_C12-18DMA_KEY_476 ([redacted])

Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)

- N,N-dimethyl-C12-14-(even numbered)-alkyl-1-amines / CAS 84649-84-3 /RA_C12-14DMA_KEY_407_1995_ [redacted]
- N,N-dimethyl-C12-14-(even numbered)-alkyl-1-amines / CAS 84649-84-3 /RA_C12-14DMA_KEY_421_1995_ [redacted]
- Dimethyl(pentadecyl)amine oxide /CAS 68955-55-5/ Amines, C12-18-alkyldimethyl, N-oxides, [redacted], 2011, OECD 422
- Dimethyl(tridecyl)amine oxide /CAS 70592-80-2 / Amines, C10-16-alkyldimethyl, N-oxides / Petersen D.W. (C), 1988, OECD Guideline 410
- Dodecyl(dimethyl)amine oxide / CAS 1643-20-5/ Dodecyldimethylamine oxide/Pang S.N.J., 1994 ,sub-acute dermal study

Additional long term repeated dose toxicity studies provided

- Amines, C10-16-alkyldimethyl, N-oxides / dimethyl(tridecyl)amine oxide /CAS 70592-80-2 (EC 274-687-2),
  - Cardin et al, 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,
  - Petersen D.W., 1988, OECD Guideline 411 (Subchronic Dermal Toxicity: 90-Day Study),
- Dodecyldimethylamine oxide / dodecyl(dimethyl)amine oxide / CAS 1643-20-5 (EC 216-700-6),
  - Lijinsky, W., 1984 - combined repeated dose and carcinogenicity,
  - [redacted], 1979B, two-generations study,

Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

- N,N-dimethyl-C12-14-(even numbered)-alkyl-1-amines / CAS 84649-84-3 /RA_C12-14DMA_KEY_421_1995_ [redacted]
- Dimethyl(pentadecyl)amine oxide /CAS 68955-55-5/ Amines, C12-18-alkyldimethyl, N-oxides, [redacted], 2011, OECD 422
- Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides / CAS 308062-28-4 / EC 931-292-6, 1999_[redacted], according to EPA OTS 798.4900 (Prenatal Developmental Toxicity Study)
- Amines, C10-16-alkyldimethyl, N-oxides / dimethyl(tridecyl)amine oxide /CAS 70592-80-2 (EC 274-687-2), OECD SIDS [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 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 cytogenicity you provided one in vitro study with Amines, C12-18-alkyldimethyl / CAS 68391-04-8.

For gene mutation in bacterial cells there are seven Ames studies with six diferent substances. However, as explained in Appenix A.1 ,only the study performed with dodecyldimethylamine / CAS 112-18-5 is an adequate study performed with all the strains 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.

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

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.

You provided 10 toxicokinetic studies with DMAOs comparing their metabolite profiles and one study (Turan and Gibson, 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 (Turan and Gibson, 1981) provided for the toxicokinetics endpoint is oerformed 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 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 Turan and Gibson (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

Guidance[7] 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 toxicty 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-[redacted] 1979B) nor in a 3-generation study with Amines, C10-16-alkyldimethyl, N-oxides / CAS 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.

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

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_[redacted] with dimantine / N,N-dimethyloctadecan-1-amine / CAS 124-28-7 in strains S. typhimurium TA 98 and TA 100 which gave negative results.
ii. RA_C10DMA_KEY_471_1996_[redacted] with decyldimethylamine / CAS 1120-24-7 in strains S. typhimurium TA 98 and TA 100 which gave negative results
iii. RA_C12-14DMA_KEY_471_1996_[redacted]_96 with Amines, C12-14-alkyldimethyl / CAS 84649-84-3 in strains S. typhimurium TA 98 and TA 100 which gave negative results.
iv. RA_C12DMA_KEY_471_1988_[redacted] 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.
v. RA_C14DMA_KEY_471_1996_[redacted] with dimethyl(tetradecyl) / CAS 112-75-4 in strains S. typhimurium TA 98 and TA 100 which gave negative results.
vi. RA_C16DMA_KEY_471_1996_[redacted] with hexadecyldimethylamine / CAS 112-69-6 in strains S. typhimurium TA 98 and TA 100 which gave negative results.
vii. RA_C12DMA_NON KEY_471_1996_[redacted], 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 471[8] (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).

The reported data for the study (i.) performed with the substance did not 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 the studies (ii.,iii.,v.,vi. and vii.) are performed in only two and same strains (*S. thyphimurium* TA 98 and 100). Only the study iv. 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 perfomed 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 state that the *in vitro* gene mutation study in bacteria with C18-DMA is already performed and that C18-DMA is not mutagenic. You also

[8] ECHA Guidance R.7a, Table R.7.7–2, p.557

mention that you will provide this information in an updated of your registration dossier. However, the 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 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 cytogenicity study in mammalian cells or In vitro micronucleus study

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is an information requirement under Annex VIII to REACH (Section 8.4.2.).

You have provided an adaptation according to Annex XI, Section 1.5 (Grouping of substances and read-across approach) together with the following study:

i. *In vitro* chromosomal aberration study, OECD 473, [redacted], 2007 with the analogye C12-18-alkyldimethyl EC 269-923-6,

We have assessed this information and identified the following issue:

For the reasons explained in the "Appendix on Reasons common to several requests", your adaptations according to Annex XI, Section 1.5 is rejected for all the studies submitted.

Therefore, the information requirement is not fulfilled.

In the comments to the draft decision, you agree to perform the requested study and you state that the study is already ongoing.

2. 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 data for an *in vitro* gene mutation study in bacteria, and data for an in vitro cytogenicity study in mammalian cells.

The information for the *in vitro* gene mutation study in bacteria and for the *in vitro* cytogenicity study in mammalian cells provided in the dossier are rejected for the reasons provided in section 1 of the Appendix on Reasons common to several requests.

If the result of the requested information in sections A 1. and B 1. in Appendices A and B is negative, the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 will be 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 ([redacted], 2006),
ii. a Gene Mutation Assay in Chinese Hamster V79 Cells in vitro (V79/HPRT), according to OECD 476 ([redacted], 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 within the DMA category 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.

Consequently, you are required to provide information for this endpoint, if the *in vitro* gene mutation study in bacteria and the *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study provide a negative result.

In the comments to the draft decision, you agree to perform the requested study and you state that the study is already ongoing.

3. Short-term repeated dose toxicity (28 days)

A Short-term repeated dose toxicity study (28 days) is a standard information requirement in Annex VIII 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, [redacted] 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, [redacted] 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, [redacted] 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, Cardin 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, Lijinsky 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, [redacted] 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, [redacted] 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, [redacted] 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, Cardin 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, Petersen 1988, (supporting study)

xi. a 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, Petersen 1988

xii. short term repeated dose toxicity 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, Petersen 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, Pang 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.

Study design

Referring to the criteria in Annex VIII, Section 8.6.1, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the Substance is a liquid with very low vapour pressure.

Therefore, the short-term toxicity study must be performed according to the OECD TG 407, in rats and with oral administration of a neutralised form of the Substance.

When there is no information available neither for the 28-day repeated dose toxicity endpoint (OECD TG 407) nor for the screening study for reproductive/developmental toxicity (OECD TG 421) (see request B.4 below), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to avoid unnecessary animal testing and because it fulfils the information requirement in both Annex VIII, Section 8.6.1. and 8.7.1. of REACH (ECHA Guidance R.7.6.2.3.2.).

In the comments to the draft decision, you agree to perform the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422).

4. Screening for reproductive/developmental toxicity

A Screening for reproductive/developmental toxicity study (test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) is a standard information requirement under Annex VIII to REACH, if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant. There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

You have adapted the standard information requirement mentioned above according to Annex XI, Section 1.2. of REACH (weight of evidence).

In support of your adaptation, you have provided the following study records:

i. A 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 ([redacted], 1995),

ii. A 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, ([redacted], 2011),

iii. A 2 generation reproductive toxicity study (No guideline), in rats via oral route, with the analogue substance dodecyldimethylamine oxide EC No. 216-700-6, ([redacted], 1979B)

iv. A 3 generation study reproductive toxicity study (No guideline), in rats via orale route, with the analogue substance Amines, C10-16-alkyldimethyl, N-oxides EC No. 274-687-2. ([redacted], 1966)

We have assessed the information and identified the following issue:

Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has (not) a particular (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude whether the Substance has the (hazardous) property investigated by the required study.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence adaptation. However, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has (not) a particular hazardous property investigated by the required study.

Irrespective of the above mentioned deficiencies on the documentation, which in itself could lead to the rejection of the adaptation, ECHA has assessed the provided sources of information.

To fulfil this information requirement, normally a study performed according to EU B.63/OECD TG 421 or EU B.64/OECD TG 422 must be provided. OECD TGs 421/422 require to investigate the following key elements: 1) sexual function and fertility, 2) toxicity to offspring, and 3) systemic toxicity.

Key elements/key investigations: *sexual function and fertility, toxicity to offspring, and systemic toxicity*

1) Sexual function and fertility

Sexual function and fertility on both sexes must include information on mating, fertility, gestation (length), maintenance of pregnancy (abortions, total resorptions), parturition, lactation, organ weights and histopathology of reproductive organs and tissues, litter sizes, nursing performance and other potential aspects of sexual function and fertility.

The sources of information (i-ii) provide required information on mating, fertility, gestation (length), maintenance of pregnancy (abortions, total resorptions), parturition, lactation, organ weights and histopathology of reproductive organs and tissues, litter sizes, nursing performance. However, they provide only statement "no effects observed" on organ weights and histopathology of reproductive organs and tissues.

The source of information (iii-iv) provide very limited information on these key investigations. More specifically, they provide only statements such as for source (iii) "*No effects observed for the oestrous cycle and reproductive performance*" and no detailed and numerical information on results. Furthermore, the sources of information addressing the key investigations must follow the rules for setting the dose levels as required in the information requirement (OECD TG 421, paragraph 24; OECD TG 422, paragraph 29) and be adequate for hazard classification and/or risk assessment as required by REACH. However, the sources (iii, iv) show too low top dose

In addition, the reliability of these sources of information is significantly affected by the following deficiencies:

Information from source substance(s) can contribute to weight of evidence adaptation only if the read-across adaptation is acceptable. Studies (i-iv) are performed with analogue substances. However, for the reasons explained under in the Appendix on Reasons common to several requests, the provided studies performed on source substances cannot be considered reliable sources of information that could contribute to the conclusion on the key parameters investigated by the required OECD TG 421/422.

2) Toxicity to offspring

Information on pre- and perinatal developmental toxicity reflected by litter sizes, postimplantation loss (resorptions and dead foetuses), stillborns, and external malformations, postnatal developmental toxicity reflected by survival, clinical signs and body weights of the pups (or litters), and other potential aspects related to pre-, peri- and postnatal developmental toxicity observed up to postnatal day 13.

The sources of information (i-ii) provide required information on litter sizes, postimplantation loss,stillborns, postnatal developmental toxicity reflected by survival, clinical signs and body weights of the pups (or litters). However, the source (i) doest not provide information on the external malformations. The source of information (iii-iv) provide very limited information on these key investigations. More specifically, they provide only a high level statement on the results not allowing an independent assessment.

In addition, the reliability of these sources of information is significantly affected by the reliability issues as explained under section 1) above

3) Systemic toxicity

Information on systemic toxicity include clinical signs, survival, body weights, food consumption, haematology, clinical chemistry, organ weights and histopathology of non-reproductive organs and other potential aspects of systemic toxicity in the parental generation up to postnatal day 13.

The sources of information (i-ii) provide required information on these key investigations. The source of information (iii-iv) provide only a high level statement on clinical signs, body weights, food consumption, organ weights and histopathology of non-reproductive organs not allowing an independent assessment.

In addition, the reliability of these sources of information is significantly affected by the reliability issues as explained under section 1) above.

Conclusion

Taken together, even if these sources of information provide information on reproductive toxicity, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude based on any source of information alone or considered together, whether your Substance has the particular (hazardous) properties. Thus, your adaptation is rejected and the information requirement is not fulfilled.

Study design

A study according to the test method EU B.64/OECD TG 422 must be performed in rats with oral[9] administration of the Substance.

When there is no information available neither for the 28-day repeated dose toxicity endpoint (OECD TG 407) nor for the screening study for reproductive/developmental toxicity (OECD TG 421) (see request B.3), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to avoid unnecessary animal testing and because it fulfils the information requirement in both Annex VIII, Section 8.6.1. and 8.7.1. of REACH (ECHA Guidance R.7.6.2.3.2.).

In the comments to the draft decision, you agree to perform the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422).

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

Appendix C: 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 summaries[10].

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 dossiers[11].

[10] https://echa.europa.eu/practical-guides
[11] https://echa.europa.eu/manuals

Appendix D: Procedure

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.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the requests.

Extension of the Deadline

In the comments to the draft decision, you requested an extension of the deadline to provide information from 12 to 24 months from the date of adoption of the decision. You considered that the extension of 12 months is needed due to the limited capacity of the testing laboratories, but also due to the difficulties in obtaining properly characterized test materials, as well as time-consuming analytical preparation work for the animal testing and the need of respective agreements among registrants.

However, you state that the *in vitro* gene mutation in bacteria study according to OECD TG 471 is already performed and that the other studies on C18-DMA are on-going.

On this basis, ECHA has not modified the deadline.

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 E: List of references - ECHA Guidance[12] and other supporting documents

Evaluation 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 documents[15]

[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 F: 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]

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.