ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 15 November 2018

Addressee: [redacted]

Decision number: CCH-D-2114449965-32-01/F
Substance name: CETRIMONIUM BROMIDE
EC number: 200-311-3
CAS number: 57-09-0
Registration number: [redacted]
Submission number: [redacted]
Submission date: 11.05.2016
Registered tonnage band: 1 – 10 T (latest submission tonnage band, [redacted])
Registered jointly: 10 – 100 T (latest submission tonnage band, [redacted])

DECISION ON A COMPLIANCE CHECK

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

1. **Spectral data (Annex VI, Section 2.3.5) of the registered substance;**
   - **Infra-red spectrum, Nuclear magnetic resonance or mass spectrum**

2. **High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6);**
   - **Identification and quantification of the constituents, as specified in section 2 of Appendix 1.**

3. **Description of the analytical methods (Annex VI, Section 2.3.7);**
   - **Identification and quantification of the bromide counter-ion**

4. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) with the registered substance;**

5. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: EU B.10./OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

6. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance provided that both the studies requested under 4 and 5 have negative results;**

7. **Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD [421/422]) in rats, oral route with the registered substance;**

8. **Classification and labelling (Annex VI, Section 4.): apply classification and labelling on the registered substance for chronic aquatic hazard or provide a justification for not classifying.**

You are required to submit the requested information in an updated registration dossier by ***22 May 2020***. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Kevin Pollard, Head of Unit, Evaluation E1

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

IDENTIFICATION OF THE SUBSTANCE

Pursuant to Article 10(a)(ii) of the REACH Regulation, the technical dossier shall contain information on the identity of the substance as specified in Annex VI, Section 2 of the REACH Regulation. In accordance with Annex VI, Section 2 the information provided shall be sufficient to enable the identification of the registered substance.

1. Spectral data (Annex VI, Section 2.3.5.)

Spectral data are a formal information requirement as laid down in Annex VI, Section 2.3.5 of the REACH Regulation. Adequate information needs to be present in the technical dossier for the registered substance to meet this information requirement.

ECHA notes that the registration dossier does not contain full set of analytical data for the registered substance. No Ultra-violet spectrum (UV), Infra-red (IR) spectrum, nuclear magnetic resonance (NMR) spectrum or Mass spectrum (MS), as required under Annex VI Section 2.3.5 of the REACH Regulation have been submitted. Moreover, a scientifically based justification for not including this information has not been included.

ECHA regards this required information scientifically necessary for the identification of the registered substance. The IR spectrum displays characteristic vibration bands for the covalent bonds of organic compounds such as the registered substance. Moreover, NMR spectroscopic analyses such as a 1H-NMR or a 13C-NMR are powerful tools for structure characterisation and elucidation due to characteristic chemical shifts and spin-spin coupling which also reflect the relative abundance of individual atoms. Alternatively, a mass spectrum which is an appropriate analytical method to characterise the substance and determine its elemental composition, can be provided. Although the UV spectrum is a REACH requirement as well, because of the lack of chromophore in the molecule, no significant additional information is expected from this analysis and therefore it can be omitted.

Accordingly, you are requested to provide the missing IR spectral data as well as a NMR spectrum, such as a 1H-NMR or a 13C-NMR or, alternatively, a mass spectrum including the corresponding interpretation of the fragmentation scheme.

You shall ensure that the description of the analytical methods used for recording the spectra is specified in the dossier in such detail to allow the methods to be reproduced, in line with the requirements under Annex VI Section 2.3.7 of the REACH Regulation. You shall ensure that the information is consistent with the information provided throughout the dossier.

In your comments submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.

Regarding how to report the spectral data, the information shall be attached in section 1.4 of the IUCLID dossier.

2. High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6.)

"High-pressure liquid chromatogram, gas chromatogram" is an information requirement as laid down in Annex VI, Section 2.3.6. of the REACH Regulation. Adequate information needs to be present in the registration dossier to meet this information requirement.

In IUCLID section 1.4, you have provided a potentiometric titration method with silver nitrate for the quantification of the quaternary ammonium compounds. Furthermore for the determination of free amines and amine salts a potentiometric method with back titration was performed. In addition, a Thin-Layer Chromatogram was performed on the sample. No HPLC or GC as required by REACH Annex VI, section 2.3.6 was provided for the quantification of the main constituent and the impurities.

The potentiometric titration methods are not specific for the main constituent "cetrimonium bromide", as other quaternary ammonium compounds can be quantified by the titration method. Furthermore, the provided Thin-Layer Chromatogram is not a specific method for the quantification of the main constituent and the impurities, as it is based of visual detection (colour and position). Therefore, the analytical data provided is not sufficient to support the quantification of the constituents required to be reported in the IUCLID dossier

You are accordingly requested to submit an appropriate chromatographic analysis including the chromatogram and a peak table containing the retention times, peak areas and peak area % of the constituents. If other analytical methods are more suitable for quantification of the constituents required to be reported in section 1.2, such methods may also be used. You should also provide a description of the analytical methods used for the identification and quantification of the constituents and impurities required to be reported in the composition of the registered substance. The description shall be sufficient for the methods to be reproduced and shall therefore include details of the experimental protocol followed, any calculation made, and the results obtained.

In your comments submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.

As for the reporting of the data in the registration dossier, the information should be included in section 1.4 of the IUCLID dossier.

3. Description of the analytical methods (Annex VI, Section 2.3.7.)

Annex VI, section 2.3.7 of the REACH Regulation requires that each registration dossier contains a sufficiently detailed description of the analytical method used for establishing the composition of the registered substance and therefore its identity. This information shall be sufficient to allow the method to be reproduced.

You have identified your substance as "N,N,N-trimethylhexadecan-1-aminium bromide", which indicates that bromide is present as a counter-ion in your substance and must be identified and quantified. You have not provided a description of the analytical method used to identify and quantify the bromide counter-ion.

Therefore, your dossier does not have sufficient information to establish the composition of the registered substance and therefore its identity.

In your comments submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.

Accordingly, you are required to provide the description of the analytical method for the identification and quantification of the bromide counter-ion.

As for the reporting of the data in the registration dossier, the information should be included in section 1.4 of the IUCLID dossier.

TOXICOLOGICAL INFORMATION

Your registration dossier contains adaptation arguments in form of a read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual endpoints.

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 10 to 100 tonnes per year must contain, as a minimum, the information specified in Annexes VII to VIII to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Your registration dossier contains for multiple endpoints adaptation arguments in the form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual endpoints.

Grouping of substances and read-across approach

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

- 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)

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

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

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

account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

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

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

Information provided for the read-across approach

The registered substance is N,N,N-trimethylhexadecan-1-aminium bromide (EC No 200-311-3), hereinafter referred to as 'cetrimonium bromide' or the 'the target substance'. The structurally similar substance (hereafter the 'source substance' or 'cetrimonium chloride') is 1-Hexadecanaminium, N,N,N-trimethyl-, chloride (EC No 203-928-6; CAS No 112-02-7).

There is an additional source substance, identified as 1-Dodecanaminium, N,N,N-trimethyl-, chloride (EC No 203-927-0 CAS no 112-00-5, also called 'dodecyltrimethylammonium' by you) which is used to predict the properties of the in vitro cytogenicity study in mammalian cells or in vitro micronucleus study and of the in vitro gene mutation study in mammalian cells. This source substance has chloride as anion and dodecyltrimethylammonium as cation.

You provide the following reasoning for the proposed adaptation:
"Reasoning for read-across from cetrimonium chloride (CAS 112-02-7) to cetrimonium bromide (CAS 57-09-0):

- *the substances contain the same cationic surfactant: the C16-trimethylammonium-structure*
- *comparable measured physical-chemical properties for the substances*
- *no significant toxicological difference between the chloride and the bromide salt can be expected as the toxicity is driven by the cationic surfactant part of the molecule.*

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

"Reasoning for read-across from dodecyltrimethylammonium chloride (CAS 112-00-5) to cetrimonium bromide (CAS 57-09-0):

- *Due to high similarity in chemical structure, physical-chemical and toxicological properties."*

No further justification or supporting data is provided for the use of 1-Dodecanaminium, N,N,N-trimethyl-, chloride as source substance.

You propose on this basis that the source and registered substances have similar properties for the above-mentioned information requirements.

ECHA considers this as the hypothesis under which you make predictions for the properties listed above.

Support of the grouping and read-across approach

You have provided the read-across justification quoted above as a separate attachment in the corresponding endpoint summary sections in the dossier registration. Furthermore you have provided QSAR documentation for the properties related to genetic toxicity. Finally, you quote in the endpoint study summaries from two documents:

- the Scientific Committee on Consumer safety (SCCS) opinion document [redacted] (2009)
- the assessment report for American Chemistry Council, Fatty Nitrogen Derivatives Panel, Cationics Task Group [redacted] (2001)

Furthermore you claim that the SCCS evaluation of alkyl (C16; C18; C22) trimethylammonium chloride uses read-across from data on cetrimonium bromide in their evaluation of the alkyl (C16; C18; C22) trimethylammonium chlorides, that Cosmetic Regulation (EC) No 1223/2009 uses a common entry for the limit value for cetrimonium chloride and bromide (Alkyl (C 12-22) trimethylammonium bromide and chloride and that the American College of Toxiciology uses a category approach and read-across in their "Final report on the safety assessment of cetrimonium chloride, cetrimonium bromide and steartrimonium chloride", Int J Toxicol 16, 195-220 (1997).

With regard to the cited references above ECHA points out that in the context of this decision it is analysed whether the criteria set out in Annex XI, Section 1.5 of the REACH Regulation are met.

ECHA analysis of the grouping and read-across approach

With regard to the proposed predictions ECHA has the following observations:

(a) Substance characterisation of source and target substances

The substance characterisation of the source substances need to be sufficiently detailed in order to assess whether the attempted prediction is not compromised by the composition and/or impurities. In the ECHA's Practical Guide on "How to use alternatives to animal testing to fulfil your information requirements" (chapter 4.4), it is recommended to follow the ECHA *Guidance for identification and naming of substances under REACH and CLP* also

for the source substances. This ensures that the identity of the source substance and its impurity profile allows an assessment of the suitability of the substances for read-across purposes.

The registered substance and proposed source substances are quaternary ammonium salts used as [redacted].

ECHA notes the following observations:

- For N,N,N-trimethylhexadecan-1-aminium chloride (cetrimonium chloride); EC number 203-928-6; and CAS number 112-02-7, no information has been provided on its identity and impurity profile.
- For 1-Dodecanaminium, N,N,N-trimethyl-, chloride, no information has been provided on its identity and impurity profile.

Currently the identity of the source substances and their impurity profile cannot be assessed using the information provided in the registration dossier and the suitability of the substances for read-across purposes cannot be verified. Therefore ECHA cannot verify whether the source substances can be used to predict properties for the registered substance.

(b) Explanation on why and how the structural similarities allow predictions

In order to meet the provisions in Annex XI, Section 1.5. to predict human health effects from data for a reference substance within the group by interpolation to other substances in the group, ECHA considers that structural similarity alone is not sufficient. It has to be justified why such prediction is possible in view of the identified structural differences and the provided evidence has to support such explanation. In particular, the structural similarities must be linked to a scientific explanation of how and why a prediction is possible.

ECHA understands that your read-across approach is based on the dissociation of the cetrimonium salts into an cation which is common to both substances (N,N,N-trimethylhexadecan-1-aminium or C16-trimethylammonium) and the corresponding counter-ions (Cl^- or Br^-), which are different in source and target substance. No documentation on the speed and extent of the dissociation of these salts has been provided. However, ECHA considers the dissociation into the mentioned ions as plausible.

ECHA notes the following observations:

- The statement "*no significant toxicological difference between the chloride and the bromide salt can be expected as the toxicity is driven by the cationic surfactant part of the molecule*" is not supported by data. More specifically, the toxicological properties of bromide has not been taken into account in your read-across approach. Bromide has pharmaceutical properties, mostly acting at central nervous system level. In human medicines it has been used as sedative and anti-epileptic. In veterinary medicine bromide is still used to treat seizures in dogs. From its medicinal uses it has been identified bromide may cause nausea and vomiting, abdominal pain, coma and paralysis. In addition, high doses of bromide in plasma can produce bromism. The symptoms of bromism relate to the nervous system, skin, glandular secretions and gastrointestinal tract. Further, prenatal exposure to sodium bromide affects the postnatal growth and brain development.

- In the absence of a scientific justification, ECHA cannot verify that 1-Dodecanaminium, N,N,N-trimethyl-, chloride can be used to predict properties for the registered substance.

ECHA concludes that you have not addressed the obvious structural differences between the source substance and the target substance and did not explain why those differences would not lead to differences in the toxicity profile of target and source substances. The provided explanation is not considered as valid to establish a scientific credible link between the structural similarity and the prediction.

(c) Support of a similar or regular pattern as a result of structural similarity

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

You did not provide a data matrix for the source and target substance which would allow side-by-side comparison of the properties and to determine whether they have similar properties or follow a regular pattern.

There are results obtained in repeated dose toxicity studies for the source and target substances. However due to the insufficient reporting for the source substance this information does not allow conclusions. In addition, in the absence of data on the registered substance for the properties related to genetic toxicity and toxicity to reproduction, it is also not possible to reach conclusions for these properties.

ECHA concludes that the presented evidence does not support a similar or regular pattern of toxicity as a result of structural similarity. Therefore it cannot be verified that the proposed source substance can be used to predict properties of the registered substance.

(d) Reliability and adequacy of the source studies

Annex XI, Section 1.5 provides with regard to the reliability and adequacy of the source studies that in all cases the results of the read-across should:

- *be adequate for the purpose of classification and labelling and/or risk assessment,*
- *have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3),*
- *cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter, and*
- *adequate and reliable documentation of the applied method shall be provided.*

ECHA observes that you have not submitted robust study summaries of the experimental data on the source substances in your dossier (see also endpoint specific sections). As explained above, your read-across hypothesis is mainly supported by references to two scientific reports. As specified in the Article 40(3) of the REACH Regulation, study results shall be provided in form of a robust study summary if required by Annex I. Pursuant to Annex I, Section 1.1.4. of the REACH Regulation if there is one study available, this has to be reported in form of a robust study summary. Article 3(28) of the REACH Regulation defines robust study summaries as: "*a detailed summary of the objectives, methods, results*

and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report". Therefore, in the absence of this information an independent assessment on whether the source studies meet the REACH requirements in terms of reliability and adequacy as requested for any key study is not possible.

ECHA concludes that currently the proposed source studies cannot be independently assessed in the dossier on the registered substance. ECHA therefore cannot verify whether the study design is adequate and reliable for the purpose of the prediction, whether the test material used represents the source substance as described in the justification documents, and whether the results are adequate for the purpose of classification and labelling and/or risk assessment.

Conclusion on the read-across approach

The adaptation of the standard information requirements for the properties investigated by in vitro gene mutation study in bacteria, in vitro cytogenicity study in mammalian cells and in vitro gene mutation study in mammalian cells in the technical dossier is based on the proposed read-across approach examined above. ECHA does not consider the read-across justification to be a reliable basis to predict the properties of the registered substance for the reasons set out above. Thus, the adaptation does not comply with the general rules of adaptation as set out in Annex XI, 1.5. Therefore, ECHA rejects the above adaptations in the technical dossier that are based on Annex XI, 1.5.

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

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid down in Annex VII, Section 8.4.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

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

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

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

Your technical dossier contains the following information:

i. Key study, read-across from supporting substance (structural analogue or surrogate), reliability 2 (reliable with restrictions), 1980, GLP, non-guideline (test performed only with *Salmonella typhimurium* TA98 and TA 100), Test

material: cetrimonium chloride. Test concentrations 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg/plate. No study report has been provided, only the two references to SCCS opinion document and [redacted] Your interpretation of the results: negative without metabolic activation.

ii. Key study, (Q)SAR prediction, reliability 2 (reliable with restrictions), 2012: "QSAR Toolbox 2.2.1.1120 prediction for "Gene Mutation" read across evaluation for 57-09-0". Your interpretation of the results: gene mutation predicted from the OECD QSAR Toolbox, for salmonella typhimurium with S9 is negative.

iii. Supporting study, (Q)SAR prediction, reliability 2 (reliable with restrictions), 2012: "QSAR Toolbox 2.2.1.1120 prediction for "Gene Mutation" read across evaluation for 57-09-0". Your interpretation of the results: gene mutation predicted from the OECD QSAR Toolbox, for salmonella typhimurium with S9 is negative.

As explained in the section on "Grouping of substances and read-across approach" above, your adaptation of the information requirement using the source substance cetrimonium chloride (see i) cannot be accepted.

Furthermore, the study referred to (i) above does not provide the information required by Annex VIII, Section 8.4.1., because the test used only two different strains of *S. typhimurium* TA (TA98 and T100). According to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used: S. typhimurium TA1535; TA1537 or TA97a or TA97; TA98; TA100; S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). This includes four strains of S. typhimurium (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four S. typhimurium strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by E.coli WP2 strains or S. typhimurium TA102 which have an AT base pair at the primary reversion site. Therefore, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Article 13(3) and Annex XI, 1.1.2. of the REACH Regulation. Hence, the study provided cannot be considered as a valid source study in your read-across approach.

You have also sought to adapt this information requirement according to Annex XI, Section 1.3. of the REACH Regulation by providing two (Q)SAR predictions for Salmonella (with and without S9 activation) (see ii. and iii. above). Those predictions cannot be accepted because in the provided documentation, the prediction reports, it is not described how the aggregated result was obtained and the strains are not specified, so the endpoint is not clearly defined. Therefore, the criteria set out in Annex XI, section 1.3, which concern the "reliable documentation of the applied method" is not met. The applicability domain is determined mainly as a range of hydrophobicity descriptor. There are functional groups that differ significantly between the target and the source substances. Therefore, ECHA considers that you have not shown that QSAR results are also applicable to the registered substance. The choice of analogues seems almost random with respect to chemical structure. These could not be used to derive reliable predictions. Further, for both predictions, the substance includes several impurities in the composition [redacted], which have to be characterised if the substance is not tested as such.

Therefore, your adaptation of the information requirement is rejected.

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

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

In your comments submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.
Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471).

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

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

Your technical dossier contains the following information:

i. Key study, read-across from supporting substance (structural analogue or surrogate), reliability 2 (reliable with restrictions), 1989, GLP, performed following OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test). Test material: cetrimonium chloride (24-26% in water). Test concentrations with metabolic activation: up to 6.0 µg/ml; and without metabolic activation: up to 10.0 µg/ml. Your interpretation of the results: negative for Chinese hamster lung fibroblasts (V79).
ii. Supporting study, (Q)SAR prediction, reliability 2 (reliable with restrictions), 2012: "QSAR Toolbox 2.3.0.1132 prediction for "Chromosome Aberration" read across evaluation for 8001-54-5". Your interpretation of the results: *QSAR prediction according to read across results for in vitro mammalian chromosome aberration test is negative for benzalkonium chloride. It is evaluated that same result is also applicable for cetrimonium bromide*.
iii. Supporting study, read-across from supporting substance (structural analogue or surrogate), reliability 2 (reliable with restrictions), 1982, bone marrow cytogenetic assay, GLP, no guideline followed. Test material: [redacted] 24.7% aqueous solution. Test concentrations: 16, 53.3, 160 mg/kg. Your interpretation of the results: negative.

As explained in the section on "Grouping of substances and read-across approach" above, your adaptation of the information requirement using the source substance cetrimonium chloride (see i.) cannot be accepted.

Furthermore, the reporting of the study (i) above lacks relevant elements, for instance, no tabulated data has been provided, no information whether negative control is compatible with the historical control, no specification on how the concentrations tested were chosen.

In addition, the test deviates from the version of the guideline indicated because, instead of 200 metaphases, only 100 metaphases were scored. Therefore, the information provided cannot be considered as a valid source study in your read-across approach as it has been presented.

You have also provided (Q)SAR predictions for chromosome aberration (see ii above). The prediction is defined only as "*Chromosome Aberration*" and the applicability domain is determined mainly as a range of hydrophobicity descriptor. There are functional groups that differ significantly between the target and the source substances. Therefore, ECHA considers that you have not shown that QSAR results are also applicable to the registered substance. The choice of analogues seems almost random with respect to chemical structure. These could not be used to derive reliable prediction. In addition, the input structure is not that of the registered substance. For all predictions, the substance includes several impurities in the composition [redacted], which have to be characterised if the substance is not tested as such.

Further, you provided an *in vivo* bone marrow cytogenetic assay as a supporting study (see iii above). As explained in the section on "Grouping of substances and read-across approach" above, the prediction based on 1-Dodecanaminium, N,N,N-trimethyl-, chloride cannot be accepted. Furthermore, the study was not conducted according to the current test guideline. The reporting lacks relevant elements, for instance, no tabulated data has been provided.

Therefore, your adaptation of the information requirement is rejected.

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

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

In your comments submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

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

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained.

Your technical dossier contains the following information:

i. key study, read-across from supporting substance (structural analogue or surrogate), reliability 2 (reliable with restrictions), 2001, GLP, performed following OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test). Test material: [redacted]. Test concentrations with metabolic activation: 0.012 to 0.16 µl/ml (10 concentrations); and without metabolic activation: 0.0038 to 0.050 µl/ml (10 concentrations). Your interpretation of the results: negative without metabolic activation.
ii. supporting study, read-across from supporting substance (structural analogue or surrogate), reliability 2 (reliable with restrictions), 2001, GLP, performed following an equivalent or similar to OECD guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro). Test material: [redacted], 24.7% aqueous solution. Test concentrations: 0.004 to 0.1 µl/ml. Your interpretation of the results: genotoxicity was inactive.

In the technical dossier you have provided a study record for an *in vitro* gene mutation study in mammalian cells (see i). As explained in the section on "Grouping of substances and read-across approach" above, the prediction based on 1-Dodecanaminium, N,N,N-trimethyl- chloride cannot be accepted.

Furthermore, the reporting of study i referred to above lacks relevant elements, for instance, no tabulated data has been provided. Therefore, the information provided cannot be considered as a valid source study in your read-across approach as it has been presented.

You also provided a "DNA damage and repair, unscheduled DNA synthesis in mammalian cells *in vitro*" assay as a supporting study (ii). As explained above, the prediction based on 1-Dodecanaminium, N,N,N-trimethyl- chloride cannot be accepted. Furthermore, the reporting of the assay lacks relevant elements, for instance, no tabulated data has been provided. Therefore, the information provided cannot be considered as a valid source study in your read-across approach as it has been presented.

Therefore, your adaptation of the information requirement is rejected.

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

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

In your comments on the draft decision submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that both studies requested under 4. and 5. have negative results.

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

"Screening for reproductive/developmental toxicity" (test method OECD TG 421 or 422) is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of a screening for reproductive/developmental toxicity in the dossier that would meet the information requirement of Annex VIII, Section 8.7.1.

In your adaptation argument, you first explain the results of developmental toxicity study via dermal route, of the sub-acute study and of the one-year study, and summarise that the substance has local adverse effects. You conclude that at tolerable dose level in a screening study OECD 421 or 422 (45-100 mg/kg) "*effects on fertility/development can hardly be expected. Due to the potent local toxicity of the substance and due to animal welfare reasons, it does not seem justified to use higher exposure levels and thus, a screening study with such low systemic exposure levels that may be obtained is, not considered scientific justified to perform.*" ECHA notes this is not an adaptation provision in Annex VIII Section 8.7.1, column 2, nor in Annex XI. Therefore, your adaptation of the information requirement is rejected.

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

According to the test methods OECD TG 421/422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

ECHA Secretariat (ECHA-S) acknowledges your comments submitted during the 30-day commenting period on the draft decision.

In your comments you claim that doses higher than 100 mg/kg cannot be applied, "*because of severe gastric irritation of cetrimonium bromide in experimental animals*".

ECHA notes that the evidence of gastric irritation was obtained with the source substance of the read-across, cetrimonium chloride, not with the registered substance, cetrimonium bromide, and in the draft decision ECHA has rejected that read-across. Only an old (1975) non-guideline developmental toxicity study via intraperitoneal route with a maximum dose of 35 mg/kg has been provided of the registered substance. Therefore, you have not proven that gastric irritation would prevent or interfere with carrying out the *in vivo* tests that are requested.

Recognising that the registered substance is self-classified as Skin Irritant 2, you are advised to examine whether the concentration of the test substance, as administered to the test animals, can be adjusted to avoid corrosion and irritation allowing at the same time the detection of potential systemic toxicity effects of the substance. ECHA notes that the maximum volume i.e. 10 ml was administered to the control animals in the 28 day study made with cetrimonium chloride, while the volume administered to the three dosed groups is not reported.

Furthermore, you have provided the results of a few reproductive and developmental toxicity studies made with bromide compounds, (ammonium bromide and sodium bromide). Some of these studies gave evidence of developmental toxicity (e.g. increased litter loss, increase in pup mortality and incomplete ossification of ribs).

While ECHA considers that in your comments, you made a preliminary attempt to build a weight of evidence case by submitting data on cetrimonium chloride and on two different bromide compounds in order to address to effects of cetrimonium bromide, ECHA concludes that:

Since there is evidence of developmental toxicity from several studies made with read-across substances, but no adequate study with the registered substance has been provided, the developmental and reproductive toxicity of cetrimonium bromide has not been adequately addressed. ECHA notes that the registered substance has not been self-classified for reproductive or developmental effects.

Above, the read-across from cetrimonium chloride has been rejected by ECHA. In your comments, you have provided data on other substances, which are bromides. However, you have not justified and explained how the read-across from these substances could be made, in order to meet the relevant information requirements, as set out in Annexes VIII and IX. As one observation, since the counter-ion of these compounds (cetrimonium versus ammonium or potassium) may effect on bioavailability and toxicity, the data provided of the other bromide compounds is not conclusive. Therefore, with the current data in the dossier and in the comments, characterisation of reproductive and developmental effects of the registered substance and the calculation of the DNEL is considered inadequate.

ECHA Secretariat (ECHA-S) acknowledges your comments submitted during the 30-day commenting period on the draft decision.

In your comments on the draft decision you claim that doses higher than 100 mg/kg cannot be applied, "because of severe gastric irritation of cetrimonium bromide in experimental animals".

ECHA notes that the evidence of gastric irritation was obtained with the source substance of the read-across, cetrimonium chloride, not with the registered substance, cetrimonium bromide, and in the draft decision ECHA has rejected that read-across. Only an old (1975) non-guideline developmental toxicity study via intraperitoneal route with a maximum dose of 35 mg/kg has been provided of the registered substance. Therefore, you have not demonstrated that gastric irritation would prevent or interfere with carrying out the *in vivo* tests that are requested.

Recognising that the registered substance is self-classified as Skin Irritant 2, you are advised to examine, whether the concentration of the test substance, as administered to the test animals, can be adjusted to avoid corrosion and irritation allowing at the same time the detection of potential systemic toxicity effects of the substance.

Furthermore, you have provided the results of a few reproductive and developmental toxicity studies made with bromide compounds (ammonium bromide and sodium bromide). Some of these studies gave evidence of developmental toxicity (e.g. increased litter loss, increase in pup mortality and incomplete ossification of ribs).

ECHA considers that in your comments on the draft decision, you made a preliminary attempt to build a weight of evidence case by submitting data on cetrimonium chloride and on two different bromide compounds in order to address to effects of cetrimonium bromide. ECHA concludes that since there is evidence of developmental toxicity from several studies made with read-across substances, but no adequate study with the registered substance has been provided, the developmental and reproductive toxicity of cetrimonium bromide has not been adequately addressed. ECHA notes that the registered substance has not been self-classified for reproductive or developmental effects.

Above, the read-across from cetrimonium chloride has been rejected by ECHA. In your comments on the draft decision, you have provided data on other substances, which are bromides. However, you have not justified and explained how the read-across from these substances could be made, in order to meet the relevant information requirements, as set out in Annexes VIII and IX. ECHA observes that since the counter-ion of these compounds (cetrimonium versus ammonium or potassium) may effect on bioavailability and toxicity, the data provided of the other bromide compounds is not considered conclusive.

Overall, ECHA takes note of your intention to improve the read-across documentaion and justification, but based on the information available at present it cannot be accepted. Therefore, with the current data in the dossier and in the comments, characterisation of reproductive and developmental effects of the registered substance and the calculation of the DNEL is considered inadequate.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision:
Reproductive/developmental toxicity screening test (test method: OECD TG 421) *or*
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

8. Hazard classification and resulting hazard label for chronic aquatic hazard (Annex VI, 4.)

Pursuant to Article 10(a)(iv) of the REACH Regulation the technical dossier shall contain information on classification and labelling of the substance as specified in Annex VI, Section 4 of the REACH Regulation in conjunction with Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation). Annex VI, Section 4.1. of the REACH Regulation clarifies that the hazard classification of the substance shall result from the application of Title I and II of the CLP Regulation. In addition, for each entry, the scientifically justified reasons why no classification is given for a hazard class or differentiation of a hazard class should be provided. According to Article 5(1) of Title I and recitals 20 and 21 of the CLP Regulation, a substance shall be classified on the basis of available information.

Furthermore, the technical dossier must include the resulting hazard label for the substance in line with Title III of the CLP Regulation (Annex VI, Section 4.2 of the REACH Regulation) and the specific concentration limits and M-factors, where applicable, resulting from the application of Article 10 of the CLP Regulation (Annex VI, Section 4.3 of the REACH Regulation).

ECHA notes that while you have self-classified the registered substance as 'category acute 1' for acute (short-term) aquatic hazard, your dossier does not contain any classification for chronic aquatic hazard. As a reason for not classifying the registered substance for chronic aquatic hazard, you have indicated that the data were "conclusive but not sufficient for classification".

However, ECHA considers that the registered substance also needs to be classified for chronic aquatic hazard.

Your dossier contains chronic aquatic toxicity study results based on OECD TG 201, indicated by you as reliable studies, showing NOECs which are below 0.01 mg/L:

- 72h-NOEC of 1.1 µg/L on algae (*Pseudokirchneriella subcapitata*), based on growth rate [redacted], 2007)[4],
- 72h-NOEC = 1.8 µg/L on algae, based on growth rate (Ministry of the Environment in Japan, 2010)[5],

In addition, ECHA notes that the registered substance is readily biodegradable.

Pursuant to Title I and II of Regulation (EC) No 1272/2008 (CLP Regulation) and the criteria set out in Part 4 of Annex I of the CLP Regulation, as amended by Commission Regulation (EU) No 286/2011 of 10 March 2011 (Tables 4.1.0. (a) and/or (b) and 4.1.4), substances that are rapidly degradable and for which chronic NOEC or ECx for fish or crustacean or algae are ≤ 0.01 mg/L shall be classified as 'category chronic 1' for long-term aquatic hazard, with hazard statement "H410: Very toxic to aquatic life with long lasting effects".

In your comments on the draft decision submitted during the 30-day commenting period, you agreed to comply with this request in the draft decision.

[4] [redacted].

[5] Ministry of the Environment in Japan, 2010. Results of Eco-toxicity test of chemicals conducted by Ministry of the Environment in Japan (-March 2010). www.env.go.jp/chemi/

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to provide adequate hazard classification and the resulting hazard label for chronic aquatic hazard for the registered substance subject to the present decision taking into account the information above. In the alternative, you are required to provide scientifically justified reasons why no such classification is given. You are reminded that also for a differentiation of a hazard class, scientifically justified reasons need to be provided.

Deadline to submit the requested information in this decision

In the draft decision communicated to you the time indicated to provide the requested information was 30 months from the date of adoption of the decision. This period of time took into account the fact that the draft decision also requested a Pre-natal developmental toxicity study (Annex IX, Section 8.7.2). As this study is not addressed in the present decision, ECHA considers that a reasonable time period for providing the required information in the form of an updated registration is 18 months from the date of the adoption of the decision. The decision was therefore modified accordingly.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation. However, following your comments on the draft decision indicating a tonnage band downgrade, you have updated your dossier tonnage band in submission number [redacted] submission date 22 August 2018, only. ECHA has taken into account the updated tonnage band, as outlined in this submission. No assessment of the updated registration dossiers has occurred. Based on the average production and/or import volumes for the three preceding calendar years, ECHA has changed the tonnage band as basis for the draft decision from 100 - 1000 tonnes per year (submission number: [redacted] (submission date 11 May 2015)) to 10 - 100 tonnes per year (submission number: [redacted]).

The compliance check was initiated on 10 October 2017.

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

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

ECHA took into account your comments and your information about a tonnage band downgrade.

This has resulted in the removal of the following decision request: Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route.

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

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

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.