EUROPEAN CHEMICALS AGENCY

Helsinki, 31 August 2018

Addressee: [redacted]

Decision number: CCH-D-2114440636-48-01/F
Substance name: 1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-C8-18(even numbered) acyl derivs., hydroxides, inner salts
EC number: 931-296-8
CAS number: -
Registration number: [redacted]
Submission number: [redacted]
Submission date: 02/05/2017
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

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

1. **High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6.) of the registered substance;**
   - **Peak table**
2. **Description of the analytical methods (Annex VI, Section 2.3.7.) of the registered substance;**
   - **Identification and quantification of the constituents**
3. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species rabbit, oral route with analogue substance (carboxymethyl)dimethyl-3-[(1-oxododecyl)amino]propylammonium hydroxide (C12 AAPB, CAS no 4292-10-8, EC no 224-292-6);**
4. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the analogue substance (carboxymethyl)dimethyl-3-[(1-oxododecyl)amino]propylammonium hydroxide (C12 AAPB, CAS no 4292-10-8, EC no 224-292-6) specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI to the REACH Regulation. To ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective annex, and adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by ***8 March 2021***. You also have to update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

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

Appeal

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

Authorised[1] by Ofelia Bercaru, Head of Unit, Evaluation E3

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

Appendix 1: Reasons

0. Grouping and read-across approach for toxicological information

Article 13(1) of the REACH Regulation provides that information on intrinsic properties of substances may be generated by means other than tests. Such other means include the use of information from structurally related substances (grouping of substances and read-across), "provided that the conditions set out in Annex XI are met".

In the registration dossier, you have adapted the standard information requirements by applying a read-across adaptation following REACH Annex XI, Section 1.5. for

- Acute dermal toxicity (Annex VIII, Section 8.5.3.)
- Skin sensitisation (Annex VII, Section 8.3.)
- *In vitro* gene mutation in bacteria (Annex VII, Section 8.4.1.)
- *In vivo mammalian gene mutation assay (*Annex VIII, Section 8.4.3)

Annex XI, Section 1.5. requires a structural similarity among the substances within a group or category such that relevant properties of a substance within the group can be predicted from the data on reference substance(s) within the group by interpolation. The following analysis presents your justification for the proposed grouping approach and read-across hypothesis, together with ECHA's analysis concerning the justification in both a generic and a endpoint-specific context.

A. Description of the grouping and read-across approach proposed by you

You have provided a read-across justification document entitled [redacted].

The AAPBs considered within this read-across approach include the following substances registered under REACH:

1. **C12 AAPB** (Reference Substance Name: (carboxymethyl)dimethyl-3-[(1-oxododecyl)amino]propylammonium hydroxide), CAS number: 4292-10-8, EC number: 224-292-6
2. **C12-18 AAPB** (Reference Substance Name: 1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-(C12-18(even numbered) acyl) derivs., hydroxides, inner salts), CAS number: -, EC number: 931-513-6
3. **C8-18 AAPB** (Reference Substance Name:1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-C8-18(even numbered) acyl derivs., hydroxides, inner salts), CAS number: 97862-59-4, EC number: 931-296-8
4. **C8-18 and C18 unsatd. AAPB**, (1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-(C8-18(even numbered) and C18 unsaturated acyl) derivs., hydroxides, inner salts), CAS number:-, EC number: 931-333-8

In your read-across justification, you also include the following substance:

5. **C12-14 AAPB**, (Reference Substance Name: 1-Propanaminium, 3-amino-N-(carboxymethyl)- N,N-dimethyl-, NC12-14 acyl derivs., hydroxides, inner salts), EC: not available

ECHA notes that the latter substance is characterised by its name only, and the read-across justification document contains no other identifiers such as EC or CAS numbers that would allow ECHA to verify its identity and hence its suitability for the read-across. In addition, there are no experimental data available with this substance regarding its physico-chemical, environmental and toxicological properties, neither in the read-across justification document nor attached to the technical dossiers of the other 4 substances. As a consequence, since there are no source data available with this substance, ECHA does not consider it as a source or target substance for the purpose of this read-across. In conclusion, ECHA has assessed the read-across only for the first 4 substances listed above.

You have provided a hypothesis for grouping alkylbetaines on the basis of structural similarity and the presence of same functional groups.

You have provided the following hypothesis: "*the substances under evaluation have similar physicochemical, toxicological and ecotoxicological properties because they share structural similarities with common functional groups: quaternary amines, amide bonds, carboxymethyl groups, and fatty acid chains, differing in length and degree of saturation. This prediction is supported by physicochemical, toxicological and ecotoxicological data on the substances themselves.*"

You have explained structural differences in relation to toxicological properties that could be attributed to:

1. Differences in the fatty acid moiety that would relate to the degree of saturation and/or alkyl chain length. In particular you indicated that "*the AAPBs differ by their carbon chain length distribution and the degree of unsaturation in the fatty acid moiety. However* [redacted] *is the major ingredient of all AAPBs".*
   You further state that "*Higher amounts of higher chain lengths and corresponding lower amounts of lower chain length could result in a rising average lipophilicity*".

2. Different amounts of unsaturated fatty ester moieties: "*Effects may be expected for e.g. physical state and for some toxicological endpoints, mainly local effects (e.g. irritation)".*

You have further addressed the impact of impurities: "*Due to the lack of differentiation between constituents and impurities, the terms "main constituents" and "impurities" are not regarded as relevant for UVCB substances".* You have provided a table of "*minor constituents*" present in the composition of the substances used in the read-across approach.

You have also provided data matrix for physicochemical and (eco)toxicological properties to further support the mutual read-across of the AAPBs to one another regarding presence or absence of (eco)toxicological effects.

You further state that the read-across approach is justified due to following reasons:
a) "All AAPBs are similar in structure, since they are manufactured from similar resp. identical precursors under similar conditions and all contain the same functional groups. Thus a common mode of action can be assumed.

b) The content of minor constituents in all products are comparable and differ to an irrelevant amount.

c) The only deviation within this group of substances is a minor variety in their fatty acid moiety, which is not expected to have a relevant impact on intrinsic toxic or ecotoxic activity and environmental fate. Potential minor impact on specific endpoints will be discussed in the specific endpoint sections".

B. ECHA analysis of the grouping and read-across approach in light of the requirements of Annex XI, 1.5.

B1. Grouping – Structural Similarity

In order to meet the provisions in Annex XI 1.5 to predict physicochemical and toxicological properties 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 you intend to use a read-across approach where structurally similar substances have the same type and strength of effects.

ECHA agrees that the constituents of the four substances (i.e. C8 to C18 AAPB) share the same functional groups, namely: quaternary amines, amide bonds, carboxymethyl groups, and fatty acid chains. ECHA considers that the common functional groups support the read-across approach on the basis of structural similarity. ECHA further notes that the main constituents of the four substances exhibit the following structural differences: length of the C-chain and the degree of saturation in the fatty acid moiety.

ECHA notes that the four substances used in the read-across approach differ in their composition, i.e. in the distribution of the fatty acid moiety chain length, as shown in the table below with the information you provided in the read-across justification document.

ECHA agrees that the C12 (C12 carbon chain length distribution) is the main common fatty acid moiety for all substances ranging from [redacted] %, with the remaining constituents composing mostly of higher chain lengths in the fatty acid moiety (i.e. C14, C16, and C18, concentrations [redacted] %) and [redacted] % of C8 and C10. The unsaturated fatty acid moieties are mostly present in the **C8-18 AAPB** (< [redacted] %) and **C8-18 and C18 unsaturated AAPB** ([redacted] %).

Carbon chain length distribution of Alkylamidopropyl betaines (AAPBs) as described in the read-across justification document submitted by you

C12 AAPB	C12-18 AAPB	C8-18 AAPB	C8-18 and C18 unsatd. AAPB	C12-14 AAPB
C10: < [redacted] %, C12: > [redacted] %, C14: < [redacted] %	C8 + C10: </= [redacted] C12: [redacted] %, C14: [redacted] %, C16: [redacted] %, C18 [redacted] %, C18 unsatd.:	C8: <= [redacted] %, C10: <= [redacted] %, C12: [redacted] %, C14: [redacted] %, C16: [redacted] %, C18: [redacted] %,	C6: <= [redacted] %, C8: <= [redacted] %, C10: <= [redacted] %, C12: [redacted] %, C14: [redacted] %, C16: [redacted] %,	C10: < [redacted] %, C12: [redacted] %, C14: [redacted] %

	<[redacted]%	C18 unsatd.: <= [redacted]	C18: [redacted]%, C18 unsatd.: [redacted] [redacted]%	

You have addressed the differences in the structure of the constituents of the four substances and state that "*The only deviation within this group of substances is a minor variety in their fatty acid moiety, which is not expected to have a relevant impact on intrinsic toxic or ecotoxic activity and environmental fate.*" Furthermore, you have addressed the differences in the composition of the four substances and state that "*The content of minor constituents in all products are comparable and differ to an irrelevant amount.*"

ECHA observes that the differences in composition are covered with experimental data on **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB** addressing the impact of carbon chain length and unsaturation in the toxicological profile of the four substances used in the read-across approach.

Regarding similarities and/or differences for the presence of impurities you state that "*The content of minor constituents in all products are comparable and differ to an irrelevant amount* ". ECHA observes that all substances contain [redacted]
The impurity profile of **C8-18 AAPB** differs from the other substances used in the read-across approach as it contains also [redacted]. ECHA considers that this difference is unlikely to affect the toxicological properties of the substance.

Based on the above ECHA considers that the structural similarity and the dissimilarities of the analogues are sufficiently explained with a view to considering the possibility of prediction.

B2. Predictions for toxicological properties

ECHA considers that the experimental studies conducted with the substances used in a read-across approach need to sufficiently cover the structural differences of the substances with regard to carbon chain length and unsaturation. This is needed to present a robust justification which meets the requirements of Annex XI, Section 1.5. that toxicological properties may be predicted from data for target substances. ECHA has therefore assessed the adequacy and reliability of the experimental studies provided and how the structural differences are covered by these studies.

As support for the proposed predictions for the read-across approach, you have provided:

- *In vivo* toxicokinetic data conducted with **C12 AAPB** (oral and dermal route) and *in vitro* dermal absorption study with **C8-18 and C18 unsatd. AAPB**;

- Experimental physico-chemical data conducted with **C12 AAPB**, **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB**. You state that "*Similar physicochemical properties are expected for the other members of this group for which no experimental data are available based on structural similarity with differences only in the fatty acid chain length distribution*";

- Experimental data on toxicological properties and conclude that the fatty acid moiety is not expected to "*be relevant to the intrinsic systemic toxicity of the compounds*", and not to have any influence on sensitisation. You have used **C8-18 and C8 unsatd. AAPB** as a worst case for skin and eye irritation and genotoxicity because it contains short chain fatty acid moieties and unsaturated fatty acid moieties. In particular, you have provided experimental data from **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB** regarding acute toxicity, skin and eye irritation, skin sensitisation and genotoxicity. You have also provided two sub-chronic toxicity (90-day) studies conducted with **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB** and a sub-acute (28-day) study conducted with **C8-18 and C18 unsatd. AAPB**, and a pre-natal developmental toxicity study in rats with **C8-18 AAPB**. You use this data to predict the toxicological properties of the other substances in the read-across approach.

You further conclude that "*The read-across hypothesis is based on structural similarity of target and source substances. Based on the available experimental data, including key physico-chemical properties and data from toxicokinetic, acute toxicity, irritation, sensitisation, genotoxicity and repeated dose toxicity studies, the read-across strategy is supported by a quite similar toxicological profile of all five substances*".

ECHA observes that the experimental studies provided in the read-across approach have been conducted with **C8-18 AAPB** and **C8-18 and C18 unsaturated AAPB** (with one supporting skin sensitisation study conducted with **C12 AAPB**).

ECHA notes that the composition of the test substances in the available experimental studies (namely: **C8-18 AAPB** and **C8-18 and C18 unsaturated AAPB)** are similar. The only difference is the concentration of the constituent C18 unsaturated, which is reported to be < [redacted] % and [redacted]% in these substances, respectively. ECHA further notes that in addition to the C12 fatty acid moiety these substances contain both the lower (C8 and C10) and higher (C14, C16, C18) carbon chain lengths and unsaturated C18 carbon chains.

ECHA has assessed the experimental data available and considers them adequate and reliable.

ECHA considers that structural and compositional variations of all the read-across substances are sufficiently covered with experimental data from **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB** regarding acute toxicity, skin and eye irritation, skin sensitisation, genotoxicity, repeated dose and prenatal developmental toxicity. ECHA notes that although no experimental studies are available for the **C12 AAPB** and **C12-18 AAPB** substances, the toxicological properties can be predicted from the common constituents with the **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB** substances that have adequate experimental data.

Conclusion on the grouping and read-across approach for toxicological properties:

Based on the reasons presented above, ECHA considers that the available studies and information are adequate and reliable and support the read-across approach as presented in the justification document for the endpoints that are not addressed with requests in this decision.

ECHA concludes that the read-across approach for these endpoints is plausible taking into account the toxicokinetic data (absorption, distribution, metabolism, elimination) and similar

physico-chemical properties of the substances and the analysis of structural similarity presented in Section B1 above.

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

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

According to Annex VI Section 2.3.6, chromatographic data is required be reported in a registration dossier and this information is required to be sufficient to enable the identity of the substance to be verified. This means that the information included in the analytical report needs to enable understanding how the constituents required to be reported in the composition section of the IUCLID dossier have been identified and quantified.

In the present dossier you have provided the description of the chromatographic method used to quantify your substance together with the corresponding chromatogram. However the peak table with the peak identification, retention times, peak area and area % was not included.

Without the peak table information it is not possible to confirm the identity and concentration levels of the constituents as reported in section 1.2 of your dossier.

You are accordingly requested to provide the peak table including peak identification, retention times, peak area and area % corresponding to the chromatographic analysis used to verify the composition of the registered substance as reported in section 1.2.

You shall ensure that the composition reported in section 1.2 of the dossier is consistent with the analytical results reported in section 1.4.

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

2. **Description of the analytical methods (Annex VI, Section 2.3.7.) of the registered substance;**

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

According to Annex VI Section 2.3.7, description of analytical methods are required to be reported in a registration dossier for the identification and quantification of the substance enabling the identity of the substance to be verified. This means that the information included in the analytical report needs to be sufficient to verify the identity and quantity of the constituents reported in section 1.2 of the IUCLID dossier.

In the present dossier you reported [redacted] as a constituent of your substance with typical concentration of [redacted]% and concentration range of [redacted]%. However, no analytical data was provided to verify this compositional information.

Without the description of the analytical method used to identify and quantify the [redacted] in your substance it is not possible to verify the identity and quantity of this constituent as reported in section 1.2 of your dossier.

You are accordingly requested to provide the description of the analytical method(s) used to identify and quantify the [redacted] present in your substance together with the corresponding analytical results.

You shall ensure that the composition reported in section 1.2 of the dossier is consistent with the analytical results reported in section 1.4.

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

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Pre-natal developmental toxicity studies (test method EU B.31./OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

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

You have sought to adapt this information requirement according to Annex X, Section 8.7.2., column 2. You provided the following justification for the adaptation:

"*In accordance with Annex X column 2 of the REACH Regulation (EC) No 1907/2006, the performance of a Prenatal developmental toxicity study in a second species (non-rodent) is not required. AAPB is of low systemic toxicity as indicated by a LD50 > 2000 mg/kg bw. No indication of any systemic toxicity of AAPBs relevant in view of a potential health risk for humans was found in the sub-chronic studies, including reproductive organs. From developmental toxicity data, there is no evidence for teratogenic effects. AAPBs have no genotoxic properties as proven in the full data set including in vivo data. The use profile of the substance indicates that relevant exposure to humans occurs via the dermal route. Reliable, relevant and adequate toxicokinetic data from an in vitro study on human skin showed a dermal resorption rate of 0 %. Based on the above specified toxicological and toxicokinetic data, it can be proven that the substance is of low toxicological activity and that no systemic absorption occurs via the relevant route of exposure. Therefore, further reproductive toxicity studies do not need to be conducted.*

Further, in accordance with Annex XI, section 1.2 of the REACH Regulation (EC) No 1907/2006, the performance of a Prenatal developmental toxicity study in a second species (non-rodent) is scientifically unjustified. As indicated above there is no indication of any systemic toxicity of AAPBs relevant in view of a potential health risk for humans, neither from sub-chronic data nor from developmental toxicity data. In conclusion, further testing on vertebrate animals in a Prenatal developmental toxicity study in a second species (non-rodent), is unjustified".

ECHA understands that your adaptation is based on Annex X, column 2, 8.7, third indent: "*the substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) and there is no or no significant human exposure*".

ECHA has analysed these three conditions as specified in Annex X, column 2, 8.7, third indent.

a) Low toxicological activity:

As the read-across approach is considered acceptable (see Section 0 above) ECHA considers that data from the substances used in the read-across approach can be used:

ECHA agrees that the acute oral and dermal toxicity of the category members is low (LD50 > 2000 mg/kg bw/day) and no major systemic adverse effects were observed in the sub-chronic toxicity study (90-day, gavage, OECD TG 408) with the registered substance and sub-chronic and sub-acute studies (90-day, in diet, OECD TG 408, and 28-day, gavage) with **C8-C18 and C18 unsatd. AAPB** (CAS no 147170-44-3, EC no 931-333-8). However, ECHA notes that the highest doses used in these studies are 300 (90-day, gavage) and 247/300 mg/kg bw/day (90-day in diet/28-day, gavage) and thus it cannot be excluded that toxicity would be seen with higher doses.

ECHA further notes that in the pre-natal developmental toxicity study (OECD TG 414) conducted with the registered substance effects on foetuses have been observed.

ECHA considers that the effects observed in the foetuses cannot be explained solely due to maternal toxicity. The available evidence indicates that the effects can also be attributed to the substance and therefore indicative for toxicological activity of the substance. Hence ECHA considers that the criteria of Annex IX, Column 2, 8.7. "*low toxicological activity (no evidence of toxicity seen in any of the tests available)*" are not met.

b) Toxicokinetic data

In your justification you state that "*in vitro study on human skin showed a dermal resorption rate of 0 %*" and "*no systemic absorption occurs via the relevant route of exposure*". ECHA notes that in the chemical safety report you also conclude that "*Absorption after oral or dermal exposure in the described reliable experimental study on rats reached a maximum of 10 %. In an reliable in vitro study on dermal resorption on human skin, the resorption rate for Coco AAPB was even 0 %*".

ECHA agrees that based on the *in vitro* dermal absorption study conducted with **C8-C18 and C18 unsatd. AAPB** (CAS no 147170-44-3, EC no 931-333-8) dermal absorption is indeed 0 %. However, ECHA notes that *in vivo* dermal absorption study conducted with **C12 AAPB** (CAS no 4292-10-8, EC no 224-292-6) shows 3.5 – 6% (females) and 2 – 3.5 % (males) absorption. Further, based on the *in vivo* toxicokinetic study the same substance (**C12 AAPB**) is absorbed via oral route (*"approximately 5 % of the 14C dose was excreted in urine and < 2 % in expired air and < 2 % remained in the carcass"*).

ECHA therefore considers that there is evidence from reliable toxicokinetic data that systemic absorption occurs via relevant routes of exposure, e.g. dermal and oral and thus the criteria of Annex IX, Column 2, 8.7. *"no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air"* are not met.

ECHA observes that you further refer to the adaptation based on Annex XI, Section 1.2. Weight of Evidence: *"no indication of any systemic toxicity of AAPBs relevant in view of a potential health risk for humans, neither from sub-chronic data nor from developmental toxicity data".*

ECHA notes that according to Annex XI, Section 1.2. *"There may be sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion".*

As stated above in section a) Low toxicological activity, there is evidence from the pre-natal developmental toxicity study conducted with the registered substance that the substance(s) have toxicological activity.

ECHA observes that the information from the Chemical Safety Report and the exposure scenarios indicate potential for exposure from the oral, dermal and inhalation routes.

ECHA concludes that the substance(s) cannot be considered as having low toxicological activity and that no systemic exposure occurs. Your adaptation therefore does neither meet the specific rules for adaptation of Annex IX, Section 8.7., column 2, third indent, nor those of the general rules for adaptation of Annex XI; Section 1.2.

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.

The test in the first species was carried out by using a rodent species (rat). According to the test method EU B.31./OECD 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbit as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction

as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

ECHA further considers that the test needs to be performed with the registered substance **C12 AAPB (**CAS number 4292-10-8, EC number: 224-292-6), taking into account animal welfare considerations as well as because:

1. The **C12 AAPB** is the major constituent of all AAPBs used in the read-across approach

2. The **C12 AAPB** has the highest concentration of this constituent,

3. The **C12 AAPB** does not have experimental data covering systemic toxicity, developmental/reproductive toxicity

4. The higher and lower molecular weight constituents are covered by the available toxicity studies with the other substances used in the read-across approach.

In addition, **C12 AAPB** is considered suitable to be tested since the tests can be used as bridging studies to further strengthen the read-across approach.

In your comments to the draft decision you have addressed low toxicity and low/no absorption of the AAPB substances. However, since you propose to conduct a preliminary test in rabbits to consider preliminary studies in rabbit to examine the hypothesis of gastrointestinal specific sensitivity of this species for testing prenatal developmental toxicity, ECHA understands that you agree to conduct the pre-natal developmental toxicity study in a second species.

ECHA notes that the ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017, R.7a, chapter R.7 6.4.2.2) indicates that "*if both or one of the default species (the rat or the rabbit) are not suitable species for prenatal developmental toxicity testing, a more suitable species considering the human relevance should be selected for testing. An adequate justification must be provided for other species other than the rat or the rabbit*".

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the analogue substance (carboxymethyl)dimethyl-3-[(1-oxododecyl)amino]propylammonium hydroxide), (**C12 AAPB**, CAS no 4292-10-8, EC no 224-292-6): Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species rabbit by the oral route.

Notes for your consideration

ECHA notes that a revised version of OECD TG 414 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA Guidance on information requirements and chemical safety assessment Chapter R.7a, Section R.7.6 (version 5.0, December 2016).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information provided

You have sought to adapt this information requirement according to Annex X, Section 8.7., column 2 and Annex XI, section 1.2. You provided the following justifications for the adaptation:

"In accordance with Annex X column 2 of the REACH Regulation (EC) No 1907/2006, the performance of an EOGRTS is not required. AAPB is of low systemic toxicity as indicated by a LD50 > 2000 mg/kg bw. No indication of any systemic toxicity of AAPBs relevant in view of a potential health risk for humans was found in the sub-chronic studies, including reproductive organs. From developmental toxicity data, there is no evidence for teratogenic effects. AAPBs have no genotoxic properties as proven in the full data set including in vivo data. The use profile of the substance indicates that relevant exposure to humans occurs via the dermal route. Reliable, relevant and adequate toxicokinetic data from an in vitro study on human skin showed a dermal resorption rate of 0 %. Based on the above specified toxicological and toxicokinetic data, it can be proven that the substance is of low toxicological activity and that no systemic absorption occurs via the relevant route of exposure. Therefore, further reproductive toxicity studies do not need to be conducted. Further, in accordance with Annex XI, section 1.2 of the REACH Regulation (EC) No 1907/2006, the performance of an EOGRTS is scienftifically unjustified. As indicated above there is no indication of any systemic toxicity of AAPBs relevant in view of a potential health risk for humans, neither from sub-chronic data nor from developmental toxicity data. In conclusion, further testing on vertebrate animals in an EOGRTS is unjustified.

Further, in accordance with Annex XI, section 1.2 of the REACH Regulation (EC) No 1907/2006, the performance of a two-generation reproductive toxicity study is scienftifically unjustified. As indicated above there is no indication of any systemic toxicity of AAPBs relevant in view of a potential health risk for humans, neither from sub-chronic data nor from developmental toxicity data.
In conclusion, further testing on vertebrate animals in a 2-generation reproductive toxicity study or extended one generation reproductive toxicity study is unjustified".

ECHA observes that you have provided the same justification for the pre-natal developmental toxicity and the extended one-generation reproductive toxicity endpoints.

As explained in section 3 above, your adaptation does neither meet the specific rules for adaptation of Annex X, Section 8.7., column 2 nor those of the general rules for adaptation of Annex XI, Section 1.2.

You have also provided study records for a sub-chronic (90-day) toxicity feeding study conducted with **C8-C18 and C18 unsatd. AAPB** (Coco AAPB, CAS no 147170-44-3, EC no 931-333-8) and conclude that "*The results from the evaluation of reproductive organs, especially organ weights of ovary and testis and histopathology of gonads from this 90 day rat feeding study with 38 day recovery revealed no indications of any substance-related effects up to and including the highest test dose of 1% in diet, corresponding to 247 mg a.i./kg bw/day*", and a sub-chronic (90-day) toxicity gavage study conducted with the registered substance and conclude that "*The results from the evaluation of reproductive organs, especially organ weights of ovary and testis and histopathology of gonads from this 90 day rat gavage study revealed no indications of any substance-related effects up to and including the highest test dose of 300 mg a.i./kg bw/d (= 1000 mg product (30.3% a.i.)/kg bw/d)*".

You claim that the available information from the repeated dose toxicity studies in the rat confirm that the reproductive organs are not affected after repeated exposure to the registered substance. ECHA notes that histopathological data alone does not adequately address all relevant elements with respect to sexual function and fertility.

ECHA further notes that your adaptation justification does not fully address the effects on offspring. The study according to OECD TG 414 in the rat provide information only on effects observable pre-natally and not effects on offspring observable and/or due to postnatal exposure. In particular, essential information on offspring toxicity observable and/or due to the peri-and postnatal exposure up to the adulthood is missing.

Thus, the information you provided does not adequately address all relevant elements with respect to effects on fertility and offspring. As explained above, the information you provided is not sufficient to support your conclusion that the substance does not have a dangerous property with respect to sexual function and fertility.

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. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the required study

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects

to be considered. According to the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017), the starting point for deciding on the length of the premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Species and route selection

According to the test method EU B.56/ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

ECHA further considers that the test needs to be performed with the analogue substance **C12 AAPB (**CAS number 4292-10-8, EC number: 224-292-6), taking into account animal welfare considerations as well as because:

1. The **C12 AAPB** is the major constituent of all AAPBs used in the read-across approach

2. The **C12 AAPB** has the highest concentration of this constituent,

3. The **C12 AAPB** does not have experimental data covering systemic toxicity, developmental/reproductive toxicity

4. The higher and lower molecular weight constituents are covered by the available toxicity studies with the other substances used in the read-across approach.

In addition, **C12 AAPB** is considered suitable to be tested since the tests can be used as bridging studies to further strengthen the read-across approach.

In your comments to the draft decision you have submitted the following new data:

1. "General justification for read-across / grouping between different alkylaminopropyl betaines (AAPB's)", in which you have included two new substances: C8-10 AAPB (EC list No. 944-170-2) and Formamidopropylbetain (EC No. 480-680-7);

2. OECD TG 407 study conducted with C8-10 AAPB. You conclude that no adverse effects were observed in this study up to 500 mg/kg bw/day (the highest dose tested).

3. OECD TG 408 and OECD TG 414 studies conducted with Formamidopropylbetain. You conclude that no adverse effects were observed in the OECD TG 408 study and no developmental toxicity was observed in the OECD TG 414 study up to 1000 mg/kg bw/day (the highest dose tested).

ECHA acknowledges the information provided and understands that you attempted to use specific rules for adaptation according to Annex X, 8.7, Column 2.: "*the substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure....and there is no or no significant human exposure*".

ECHA acknowledges the additional information provided from the OECD TG 408 and OECD TG 414 studies performed with formamidopropylbetain (C1 AAPB). ECHA does not consider C1 AAPB a suitable analogue that belongs to the original category addressed in the draft decision. The substance, although it contains similar functional groups to the other category members, has significant difference in alkyl chain length that might contribute to different metabolism and bioavailability.

ECHA acknowledges the additional sub-acute study with C8-10 AAPB in which no adverse effects were observed. However, the highest dose used in this study is 500 mg/kg bw/day and thus it cannot be excluded that toxicity would be seen with higher doses.

You also provided additional data on the pre-natal developmental toxicity study conducted with C8-C18 AAPB, and explain that the adverse effects observed in foetuses are due to maternal toxicity. You further explain that no adverse developmental toxicity effects were observed in the OECD TG 414 study with C1 AAPB and in general, some quaternary ammonium compounds are not developmental toxicants.

ECHA acknowledges the additional explanatory arguments provided regarding the PNDT study performed with C8-C18 AAPB. ECHA notes that there is still equivocal evidence on whether toxicological activity was evident in this study. The conclusion reached by you are not in line with the conclusion of Study author that considered maternal and foetal effects observed as substance related effects. ECHA does not consider that the maternal body weight changes were severe enough to explain solely the total post-implantation loss.

Regarding toxicokinetic data you further explained that the *in vivo* dermal absorption study "has to be considered as an unrealistic worst case" and conclude that "*for dermal penetration the resorption rate of 0% based on the in vitro study on human skin should be the starting point for risk assessment*". In addition, you state that the most relevant route of exposure for workers is the dermal route and the oral route is relevant only for consumers.

ECHA agrees that no dermal absorption is expected when the in vitro human skin data is used for risk characterisation. ECHA notes that the available toxicokinetic oral gavage study available in the registration dossier indicates oral absorption up to 10%.

Based on the information provided in the Chemical Safety Reports, ECHA observes that indeed workers are mainly exposed via dermal route and consumer exposure (including oral route) is likely. However, inhalation exposure has also been identified both for workers and consumers. ECHA therefore notes that no or no significant human exposure cannot be excluded based on the information provided in the Chemical Safety Report.

ECHA further stresses that oral route is the most appropriate route of exposure for detection of hazardous properties on reproduction (ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2).

ECHA concludes that
- the pre-natal developmental toxicity study shows evidence of toxicity, and
- the toxicokinetic information indicates potential for systemic absorption, and
- significant human exposure is likely.

Therefore, the adaptation of the information requirement according to Annex X, Section 8.7, Column 2, is not fulfilled.

c) Outcome

Based on the available information, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the analogue substance (carboxymethyl)dimethyl-3-[(1-oxododecyl)amino]propylammonium hydroxide), (C12 AAPB, CAS no 4292-10-8, EC no 224-292-6): Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;

While the specifications for the study design are given above, you shall also submit with the new endpoint study record a scientific justification on each of the following aspects: 1) length of the premating exposure duration and dose level selection, 2) reasons for why or why not Cohort 1B was extended, 3) termination time for F2 generation, and 4) reasons for why or why not Cohorts 2A/2B and/or Cohort 3 were included.

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the available information, together with the new information shows triggers which are described in column 2 of Section 8.7.3., Annex IX and further elaborated in ECHA Guidance on information requirements and chemical safety assessment R.7a,

chapter R.7.6 (version 6.0, July 2017). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 14 June 2017.

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

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

You provided comments on the draft decision.

ECHA took into account your comments and amended the request(s).

The request "Classification and labelling (Annex VI, Section 4): Apply classification and labelling on the registered substance for acute aquatic hazard or provide a justification for not classifying" was removed from the draft decision considering your comments on the draft decision and the outcome of the evaluation under Article 42(2) following up on dossier evaluation decision CCH-D-0000005226-77-02/F.

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) for amendment.

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

Appendix 3: Further information, observations and technical guidance

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

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

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

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

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

4. If the required tests are conducted with an analogue substance in the context of a read-across approach, the identity of the test material used to perform the test should be specified in line with ECHA's Practical Guide on "How to use alternatives to animal testing to fulfil your information requirements" (chapter 4.4). This is required to show that the test material is representative of the analogue substance identified in the read-across approach and used to predict the properties of the registered substance.