EUROPEAN CHEMICALS AGENCY

Helsinki, 19 September 2018

Addressee: [redacted]

Decision number: CCH-D-2114440480-59-01/F
Substance name: (carboxymethyl)dimethyl-3-[(1-oxododecyl)amino]propylammonium hydroxide
EC number: 224-292-6
CAS number: 4292-10-8
Registration number: [redacted]
Submission number: [redacted]
Submission date: 23/08/2013
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. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rabbit), oral route with the registered substance;**
2. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**
3. **Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: Daphnia sp. Acute immobilisation test, EU C.2./OECD TG 202) with the registered substance;**
4. **Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: Fish, acute toxicity test, OECD TG 203) with the registered substance;**
5. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance;**
6. **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance;**

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 ***26 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 (eco)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 oral toxicity (Annex VII, Section 8.5.1.)
- Acute dermal toxicity (Annex VIII, Section 8.5.3.)
- Skin irritation (Annex VII, Section 8.1.)
- Eye irritation (Annex VII, Section 8.2.)
- *In vitro* gene mutation in bacteria (Annex VII, Section 8.4.1.)
- *In vitro* gene mutation in mammalian cells (Annex VIII, Section 8.4.3.)
- *In vivo* mammalian gene mutation assay ((Annex VIII, Section 8.4.3.)
- Sub-chronic toxicity (90-day) (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity, first species (Annex IX, Section 8.7.2.)
- Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
- Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)
- Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

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 an 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] as part of the Chemical Safety Report (CSR).

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 (eco)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 a 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.: < [redacted] %	C8: <= [redacted] %, C10: <= [redacted] %, C12: [redacted] %, C14 [redacted] %, C16: [redacted] %, C18: [redacted] %, C18 unsatd.: <= [redacted]	C6: <= [redacted] %, C8: <= [redacted] %, C10: <= [redacted] %, C12: [redacted] %, C14: [redacted] %, C16: [redacted] %, C18: [redacted] %, C18 unsatd [redacted] [redacted] %	C10: < [redacted] %, C12: [redacted] %, C14: [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 e.g. **C8-18 and C18 unsatd. AAPB** addressing the impact of carbon chain length and unsaturation in the (eco)toxicological profile of the four substances used in the read-across approach. However, due to the lack of aquatic toxicity data for **C12 AAPB**, ECHA notes that the impact of the differences in composition on the prediction of aquatic toxicity are not covered, as explained further in Section B.3 below.

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 (eco)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 C18 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 [redacted], 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.

B.3 Predictions for environmental endpoints

Annex XI, 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 structurally similar and are likely to have similar properties or follow a regular pattern. One important aspect in this regard is the data matrix comparing properties of source and target substances.

To substantiate the similarity in the ecotoxicological properties of the source and target substances, you have submitted the data on source substances for the following aquatic toxicity endpoints:

- short-term toxicity to fish, short-term toxicity to *Daphnia*, algae growth inhibition, long-term toxicity to *Daphnia* with **C8-18 AAPB** and **C8-18 and C18 unsatd. AAPB**;

- a long-term toxicity study on fish with **C8-18 AAPB**.

For the target substance **C12 AAPB** you have submitted a study record on algae growth inhibition in the registration dossier.

While you have not provided a description of the mode of toxic action of these substances, ECHA agrees that, since the AAPB constituents contain the same reactive functional groups (i.e. quaternary amines, amide bonds, carboxymethyl groups), a similar mode of action could be expected. However, ECHA notes that the four analogues have structural differences, namely the C-chain length of the AAPB constituents varies from C8 to C18 (including C18:1, even number) and the C-chain length distribution of the constituents varies among the four registered substances.

Regarding the difference in C-chain length among the AAPB constituents, ECHA considers that you have not adequately described the impact of this difference on the ecotoxicity predictions. You describe that the difference in C-chain length distribution is not expected to influence the ecotoxicity prediction since "*Aquatic toxicity is mainly determined by the C12 derivative, which represents the main constituent of all the substances within the scope of this assessment. The influence of the other constituents with shorter and longer chain lengths is considered to be subordinate.*" ECHA has assessed this statement and concludes the following:

1. You have not provided any justification why (e.g. from an absorption or accumulation point of view) the differences in chain length would not influence the toxicity.

2. Data on aquatic toxicity for the single constituents with different C-chain length (i.e. C8 to C18) is not available in the read-across justification or in the dossier, where there is only one study available on one single constituent: one algae growth inhibition study ([redacted] 2007) on **C12 AAPB** (EC 224-292-6). Hence,

no comparison between the toxicity of the different single constituents of the 4 analogues can be made.

ECHA notes that the compositions of **C12-18 AAPB, C8-18 AAPB** and **C8-18 and C18 unsaturated AAPB** are very similar and overlapping and the small differences in C-chain length distribution are not expected to influence their ecotoxicity. In contrast, the concentration of the C12 constituent in the target monoconstituent substance **C12 AAPB** is substantially higher, typically [redacted]% ([redacted]%).

Further, regarding the influence of the difference in the C-chain length distribution on the ecotoxity prediction for the target substance **C12 AAPB**, you indicate that "*comparable effect levels were observed in the available aquatic toxicity studies conducted with C12 AAPB, C8-18 AAPB and C8-18 and C18 unsatd. AAPB, demonstrating experimentally, that the differences in C-chain distribution and amount of unsaturated C18 chains are not relevant for ecotoxicity.*" ECHA has assessed this statement and concludes the following:

1. The algae toxicity endpoint is the only endpoint for which there is a bridging study available on **C12 AAPB** (EC 224-292-6), i.e. only for this endpoint it is possible to compare the ecotoxicity of the target C12 to those of the source substances. For the algae toxicity endpoint, studies are available for **C12 AAPB** ([redacted] 2007, 1 study: 72h-ErC50 = 3.15 mg a.i./L, NOErC = 0.3 mg a.i./L,), as well as for **C8-C18 AAPB** (1 study: 72h-ErC50 > 14.7 mg a.i./L) and **C8-18 and C18 unsatd. AAPB** (6 studies, result ranges 72h-ErC50 = 0.78-334 mg/L and NOErC = 0.36-3.86 mg/L). ECHA notes that the results of the algae studies are quite variable for **C8-18 and C18 unsatd. AAPB**, where ErC results vary up to four orders of magnitude, hence it is not possible to conclude that the toxicity of these substances is comparable. ECHA further notes that the RSSs of all the studies on algae do not report the composition of the test material, hence ECHA cannot verify whether the composition of the substances tested is comparable.

2. There are no studies available for fish and *Daphnia* with C12 only, hence it is not possible to verify whether studies on the source substances, where C12 is present only below [redacted]%, would not underestimate the predictions of aquatic toxicity for **C12 AAPB**, where C12 is present at typical concentrations of [redacted]% ([redacted]%), as reported in its technical dossier.

In conclusion, although the C12 constituent is the one present at the highest concentration in all four registered substances, there is currently no evidence in the technical dossier that the differences in composition (C-chain length distribution) would not influence the predictions of aquatic toxicity for the registered substance. Due to the variable results of the algae studies among the analogues and due to the lack of aquatic studies with C12 on fish and *Daphnia*, the claim that the analogues have similar ecotoxicity is not supported by experimental data currently in the dossier.

In your comments on the draft decision, you indicate that the variable results of the algae studies "*seems to result from different test settings (with and without substance specific analytical monitoring) which in turn results from the time tests were made (after 2005 / bevore 2000).*" To support this argument, you refer to the results of the only two algae studies performed with substance specific analytical monitoring "*for **C8-18 and C18 unsatd. AAPB**: 72h-ErC50: 9,86 mg /L, 72h-NOErC 3.86 mg/l*" ([redacted] 2006) and for "***C12 AAPB**: 72h-ErC50: 3,15 mg/L, 72h-NOErC 0.3 mg/l*" ([redacted] 2007). You indicate that these two studies with analytical monitoring have quite similar results (72h-ErC within a factor of 3) for "*test substances being difficult to handle (micelle forming)*" and hence you consider that they "*serve very well as bridging study to justify*

read-across between the single constituent C12 AAPB and the UVCB C8-18 C18 unsatd. (..)". Furthermore, in your comments you acknowledge the poor description of the identity/composition of the test materials and you indicate that you will improve it in a future dossier update.

ECHA acknowledges that target and source substances are difficult test substances due to the surface active properties. However, ECHA considers that presence or absence of analytical monitoring alone cannot explain the large variation in the results of the algae studies (e.g. 72h-ErC50 = 0.78-334 mg/L for **C8-18 and C18 unsatd. AAPB**) due to the following. In the RSSs of the only two algae studies performed with analytical monitoring (LC-MS/MS), although you do not specify the measured concentrations at all sampling points, you indicate that the concentrations measured at test start (0h) and at test end (72h) were respectively [redacted]% and [redacted]% for **C8-18 and C18 unsatd. AAPB** and [redacted]% and [redacted]% for **C12 AAPB**. ECHA notes that from these two studies with analytical monitoring it is clear that at least [redacted]% of the substance is present in the test solutions after 72h, hence for these substances studies may be considered reliable also without analytical monitoring since great losses of test material are not expected. Therefore, since all the algae studies provided in the technical dossier (all with Klimisch 1 and 2 scores) are valid, ECHA considers that your argument that only the results of the two studies with analytical monitoring should be used to support the read-across is not justified. In addition, in this particular case, ECHA considers that in the absence of information on the composition of the test material, no meaningful comparison can be done among the results of the algae studies.

Furthermore, ECHA notes that in your comments you have not addressed if and how your claim that the analogues have similar ecotoxicity is justified in the absence of aquatic studies with C12 on fish and *Daphnia*. Therefore, ECHA considers that the presented evidence in the data matrix does not support sufficiently a similar or regular pattern of aquatic toxicity as a result of structural similarity.

ECHA concludes that for the reasons explained above the available data provided in the technical dossier and read-across justification document do not support a similar or regular pattern of aquatic toxicity regarding the environmental endpoints in consideration. Therefore ECHA considers that there is no adequate basis for predicting properties of the registered substance from the source substances.

Conclusion on the grouping and read-across approach for environmental endpoints

ECHA concludes that in the light of the deficiencies as described above the read-across approach does not comply with the general rules of adaptation as set out in Annex XI, 1.5. of the REACH Regulation. Therefore, this adaptation is not accepted and there is a data gap for the environmental endpoints covered by this read-across approach.

1. **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 analogue substance **C8-18 AAPB** (CAS no 97862-59-4, EC no 931-296-8) as test material.

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

The technical dossier does not contain an adaptation in accordance with column 2 of Annex X, Section 8.7.2. or with the general rules of Annex XI for this standard information requirement.

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 notes that the technical dossier did not contain any data nor any adaptations for the pre-natal developmental toxicity (second species). 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 registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species (rabbit) by the oral route.

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).

2. 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 the information 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 a two-generation reproductive toxicity study 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", and

"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 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 2-generation reproductive toxicity study, using 2600 animals is unjustified".

ECHA understands that your adaptation is based on Annex X, column 2, 8.7.: *"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.

a) Low toxicological activity:

ECHA notes that no experimental data has been provided with the registered substance apart from a toxicokinetic study. However, 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 read-across substances 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 **C8-18 AAPB** (CAS no 97862-59-4, EC no 931-296-8) 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 **C8-18 AAPB** (CAS no 97862-59-4, EC no 931-296-8) 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 the registered substance shows 3.5 – 6% (females) and 2 – 3.5 % (males) absorption. Further, based on the *in vivo* toxicokinetic study the registered substance 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 by stating that: *"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 **C8-18 AAPB** (CAS no 97862-59-4, EC no 931-296-8) 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.

Therefore, ECHA notes that your adaptation neither meets the specific rules for adaptation of Annex IX, Section 8.7., column 2 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. 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 r*oute*.

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), and

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 is not in line with the conclusion of the 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 registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);

- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;

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.

3. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)

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.

"Short-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex VII, Section 9.1.1. 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.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing the following 5 study records with the analogue substances **C8-18 AAPB** (EC no 931-296-8) and **C8-18 and C18 unsatd. AAPB** (EC no 931-333-8):

1. [redacted] 1992, with **C8-18 and C18 unsatd. AAPB**, key study, reliability 2, GLP, test method: OECD TG 202;
2. [redacted] 1991 with **C8-18 AAPB**, key study, reliability 2, GLP, test method: OECD TG 202/EU Method C.2;
3. [redacted] 2008, with **C8-18 and C18 unsatd. AAPB**, key study, reliability 2, GLP, test method: ISO 14669 (1999);
4. [redacted] 1993, with **C8-18 and C18 unsatd. AAPB**, supporting study, reliability 2, GLP, test method: OECD TG 202/EU Method C.2;
5. [redacted] 1996, with **C8-18 and C18 unsatd. AAPB**, supporting study, reliability 2, GLP, test method: EU Method C.2.

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement cannot be accepted.

Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) Daphnia sp. acute immobilisation test (test method EU C.2. / OECD TG 202) is the preferred test to cover the standard information requirement of Annex VII, Section 9.1.1.

In your comments to the draft decision you agree to perform the aquatic toxicity tests requested in this decision in a consecutive order, as detailed in the *Notes for your consideration for requests 3-6* below. ECHA acknowledges that you agree to consider the need for long-term aquatic testing following the completion of the information requirements for short-term aquatic toxicity testing and the subsequent update of the CSA. Furthermore, ECHA notes that your comments on the read-across approach have been addressed above in section 0. Grouping and read-across approach for (eco)toxicological information.

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: *Daphnia* sp. Acute immobilisation test, EU C.2./OECD TG 202).

4. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)

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.

"Short-term toxicity testing on fish" is a standard information requirement as laid down in Annex VIII, Section 9.1.3. 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.

Column 2 of Annex VIII, Section 9.1.3 specifies that long-term aquatic toxicity testing as described in Annex IX shall be considered if the chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms. The choice of the appropriate test(s) will depend on the results of the chemical safety assessment.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing the following 6 study records with the analogue substances **C8-18 AAPB** (EC no 931-296-8) and **C8-18 and C18 unsatd. AAPB** (EC no 931-333-8):

1. [redacted] 1993, with **C8-18 and C18 unsatd. AAPB**, key study, reliability 2, GLP not specified, test method: OECD TG 203/EU Method C.1;
2. [redacted] 1995, with **C8-18 AAPB**, key study, reliability 2, GLP, test method: Fish Prolonged Toxicity Test: 14-day Study (OECD TG 204) and OECD Guideline 215 Draft 'Juvenile growth test: 28 d' (1992);
3. [redacted] 2008, with **C8-18 and C18 unsatd. AAPB**, key study, reliability 2, GLP, test method: OECD TG 203;
4. [redacted] 2001, with **C8-18 and C18 unsatd. AAPB**, supporting study, reliability 2, non GLP, test method: ISO 7346/1-3 "*which conforms to*" OECD TG 203;
5. [redacted] 1996, with **C8-18 and C18 unsatd. AAPB**, supporting study, reliability 2, GLP,

test method: EU Method C.1;
6. [redacted] 1995, with **C8-18 AAPB**, supporting study, reliability 2, non GLP, test method: OECD TG 203 and ISO 7346-1.

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement cannot be accepted.

Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) fish acute toxicity test (test method EU C.1. / OECD TG 203) is the preferred test to cover the standard information requirement of Annex VIII, Section 9.1.3.

In your comments to the draft decision you agree to perform the aquatic toxicity tests requested in this decision in a consecutive order, as detailed in the *Notes for your consideration for requests 3-6* below. ECHA acknowledges that you agree to consider the need for long-term aquatic testing following the completion of the information requirements for short-term aquatic toxicity testing and the subsequent update of the CSA. Furthermore, ECHA notes that your comments on the read-across approach have been addressed above in section 0. Grouping of substances and read-across approach.

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: Fish, acute toxicity test (test method: EU C.1./OECD TG 203).

5. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)

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.

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing the following 4 study records with the analogue substances **C8-18 AAPB** (EC no 931-296-8) and **C8-18 and C18 unsatd. AAPB** (EC no 931-333-8):

1. [redacted] 1995, with **C8-18 AAPB**, weight of evidence, reliability 2, GLP, test method: OECD TG 211;
2. [redacted] 1990, with **C8-18 and C18 unsatd. AAPB**, weight of evidence, reliability 2, GLP, test method: OECD TG 211;
3. [redacted] 1991, with **C8-18 AAPB**, weight of evidence, reliability 2, GLP, test method: OECD TG 211;
4. [redacted] 2006, with **C8-18 AAPB**, weight of evidence, reliability 2, GLP, test method: OECD TG 211.

ECHA notes that while you have indicated that a read-across approach has been submitted for this endpoint, in the technical dossier you have flagged these four studies on the source substances as *Weight-of-Evidence*. ECHA understands that your indication of *Weight-of-Evidence* is in fact describing the read-across approach discussed in section 0 of this decision. Therefore, ECHA has assessed the information for this endpoint presented in the technical dossier according to Annex XI, Section 1.5. grouping of substances and read-across approach, only.

However, as explained in Ssection 0 above, your read-across adaptation of the information requirements for aquatic toxicity endpoints cannot be accepted.

Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) *Daphnia magna* reproduction test (test method EU C.20. / OECD TG 211) is the preferred test to cover the standard information requirement of Annex IX, Section 9.1.5.

In your comments to the draft decision you agree to perform the aquatic toxicity tests requested in this decision in a consecutive order, as detailed in the *Notes for your consideration for requests 3-6* below. ECHA acknowledges that you agree to consider the need for long-term aquatic testing following the completion of the information requirements for short-term aquatic toxicity testing and the subsequent update of the CSA. Furthermore, ECHA notes that your comments on the read-across approach have been addressed above in section 0. Grouping of substances and read-across approach.

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: Daphnia magna reproduction test (test method: EU C.20./OECD TG 211).

6. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

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.

"Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. Adequate information on Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.), or Fish, short-term toxicity test on embryo and sac-fry stages (Annex IX, 9.1.6.2.), or Fish, juvenile growth test (Annex IX, 9.1.6.3.) needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a Fish, Early-Life Stage Toxicity Test ([redacted] 2008, key study, reliability 2, GLP, test method: OECD TG 201 and EPA OPPTS 850.1400) with the analogue substance **C8-18 AAPB** (EC no 931-296-8).

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement cannot be accepted.

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

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) fish early-life stage (FELS) toxicity test (test method OECD TG 210), fish short-term toxicity test on embryo and sac-fry stages (test method EU C.15. / OECD TG 212) and fish juvenile growth test (test method EU C.14. / OECD TG 215) can be performed to cover the standard information requirement of Annex IX, Section 9.1.6.

However, the FELS toxicity test according to OECD TG 210 is more sensitive than the fish, short-term toxicity test on embryo and sac-fry stages (test method EU C.15 / OECD TG 212), or the fish, juvenile growth test (test method EU C.14. / OECD TG 215), as it covers several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (see ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), *Chapter R7b, Section R.7.8.4.1.*

Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are expected to cause effects over a longer exposure period, or which require a longer exposure period of time to reach steady state (ECHA *Guidance Chapter R7b,* version 4.0, June 2017).

In your comments to the draft decision you agree to perform the aquatic toxicity tests requested in this decision in a consecutive order, as detailed in the *Notes for your consideration for requests 3-6* below. ECHA acknowledges that you agree to consider the need for long-term aquatic testing following the completion of the information requirements for short-term aquatic toxicity testing and the subsequent update of the CSA. Furthermore, ECHA notes that your comments on the read-across approach have been addressed above in section 0. Grouping of substances and read-across approach.

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: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

Notes for your consideration for requests 3-6

Before conducting any of the tests mentioned above in points 5 and 6 you shall consult the ECHA *Guidance on information requirements and chemical safety assessment (*version 4.0, June 2017*)*, Chapter R7b, Section R.7.8.5 to determine the necessity to conduct the long-term toxicity testing on aquatic invertebrates and on fish.

Concerning the order of studies to be conducted, you may first fulfil the information requests made for short-term aquatic studies under points 3. and 4. above and subsequently update the CSA according to Annex I of the REACH Regulation.

If you come to the conclusion that no further investigation of chronic effects on aquatic organisms is required, you shall update your technical dossier by clearly stating the reasons for adapting the standard information requirement of Annex IX, 9.1.5 and 9.1.6. taking into account the new information submitted for the short-term aquatic studies as requested by the present decision and the exposure assessment and risk characterisation.

On the other hand, if after the update of the CSA you come to the conclusion that the long-term toxicity tests are still required to refine the risk assessment, you should consider the Integrated Testing Strategy (ITS) for aquatic toxicity as described in ECHA Guidance on information requirements and chemical safety assessment (version 3.0, February 2016), Chapter R7b (Section R.7.8.5., including Figure R.7.8-4). According to the ITS, if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially less sensitive than other trophic levels (i.e. fish, invertebrates, algae), long-term studies may be required on both fish and invertebrates. In such case, according to the ITS, the long-term *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study and the application of a relevant assessment factor, no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted. However, if a risk is indicated, the long-term fish study needs to be conducted.

Due to the surface active properties of the substance you should consult OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6 and ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R7b, Table R.7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested ecotoxicity test(s) and for calculation and expression of the result of the test(s).

If you however consider adapting some of the requested environmental tests by addressing the deficiencies identified in your read-across approach, you should then also consider to revise accordingly the chemical safety assessment, including the classification, as per Annex I and Annex XI 1.5 of the REACH Regulation.

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 25 January 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 did not amend the request(s).

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.