EUROPEAN CHEMICALS AGENCY

Helsinki, 10 March 2020

Addressees
Registrants of JS_294-785-9 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
28/06/2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Fatty acids, tall-oil, reaction products with boric acid (H3BO3) and diethanolamine
EC number: 294-785-9
CAS number: 91770-03-5

Decision number: [Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/D)]

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***15 June 2021.***

A. Requirements applicable to all the Registrants subject to Annex VII of REACH

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

2. The long-term toxicity testing on aquatic invertebrates also requested at C.2. below (triggered by Annex VII, Section 9.1.5., column 2) with the Substance;

3. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method EU C.3./OECD TG 201) with the Substance;

B. Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. Only if a negative result in Annex VII, Section 8.4.1. is obtained, In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method OECD TG 476 or TG 490) with the Substance;

C. Requirements applicable to all the Registrants subject to Annex IX of REACH

1. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method OECD TG 414) in a first species (rat or rabbit), oral route, with the Substance;

2. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method EU C.20./OECD TG 211) with the Substance;

3. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method OECD TG 210) with the Substance;

Conditions to comply with the requests

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.
To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annex VII of REACH, if you have registered a substance at 1-10 tonnes per annum (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if you have registered a substance at 100-1000 tpa;

When a study is required under several Annexes of REACH, the reasons are provided in the corresponding appendices of this decision. The registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants in accordance with Article 53 of REACH.

Registrants are only required to share the costs of information that they must submit to fulfil the information requirements for their registration.

The Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

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] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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

Appendix A: Reasons for the requests to comply with Annex VII of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 1 to 10 tonnes or more per year must contain, as a minimum, the information specified in Annex VII to REACH.

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

An *In vitro* gene mutation study in bacteria is a standard information requirement in Annex VII to REACH.

You have provided supporting studies in your dossier:

i. *In vitro* gene mutation study in bacteria (1983) similar to OECD TG 471 with the Substance.
ii. *In vitro* gene mutation study in bacteria (1984) similar to OECD TG 471 with the Substance.

Furthermore, in your comments on the draft decision, you have provided an adaptation for this information requirement, providing the following information in support:

iii. an *in vitro* gene mutation studies in mammalian cells - OECD TG 476 study (1983)
iv. an *in vitro* gene mutation studies in mammalian cells - OECD TG 476 study (1984)
v. an *in vitro* chromosomal aberrations study - OECD 473 (1999)

You argue that the available data give sufficient information to conclude on the genotoxicity hazard of the Substance in bacterial and mammalian cells.

You finally provide explanations of why you believe that repetition of a bacterial mutagenicity study including S. typhimurium strain TA102 or E.coli WP2 uvrA or E.coli WP2 uvrA (pKM101) would not change the WoE conclusion.

While the legal basis for your adaptation was not specifically indicated by you, ECHA has evaluated the provided information according to Annex XI, Section 1.2 (Weight of Evidence), and identified the following issues:

1) Inadequate *in vitro* gene mutation studies in bacteria

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471 (1997). The key parameters of this test guideline include:

a) The test must be performed with 5 strains: four strains of S. typhimurium (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).
b) Triplicate plating must be used at each dose level.

The reported data for the *in vitro* gene mutation studies in bacteria you have provided (i)-(ii) however does not include:

a) the appropriate 5 strains, as the information provided does not include results in the required fifth strain, S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). At least the five strains of bacteria recommended in OECD TG 471 should be used to cover different mechanisms of genotoxic action. ECHA acknowledges that there is some uncertainty regarding the likely mechanism(s) of genotoxic action of the Substance. However, you have not provided any evidence to exclude the possibility of a genotoxic effect detectable by the use of S. typhimurium TA102 or E. coli WP2 uvrA

or E.coli WP2 uvrA (pKM101) strains. Therefore, all the potential mechanisms of genotoxic action covered by OECD TG 471 should be investigated.

b) triplicate plating at each dose level and for each strain tested. This is to ensure an adequate estimate of variation. The use of duplicate plating is only acceptable when scientifically justified. However, no such justification was provided for the use of duplicates in the studies provided. The individual plate counts, the mean number of revertant colonies per plate and the standard deviation for the Substance and positive and negative (untreated and/or solvent) controls were not reported either.

Therefore, the information provided does not cover key parameters required by OECD TG 471.

Additionally, following current best practice, negative results in an OECD TG 471 study obtained following a plate incorporation protocol would need to be confirmed for each strain in a repeat experiment using a preincubation protocol, which was not the case in the studies provided.

2) Inappropriate weight of evidence adaptation

Annex XI, Section 1.2 states that 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 (hazardous) property, while information from a single source alone is insufficient to support this notion.

In order to allow concluding on no genotoxicity in bacterial cells for the Substance in a weight of evidence adaptation, the justification must cover the key elements (parameters) foreseen to be investigated in an OECD TG 471 study.

ECHA has assessed to what extent the information submitted enables a conclusion of hazardous properties for genotoxicity in bacterial cells and identified the following deficiencies:

a) the *in vitro* gene mutation studies in bacteria you have provided (i)-(ii) are inadequate for the reasons detailed above.

b) none of the additional studies referred to in your comments investigates gene mutation in bacteria. The OECD TG 473 study (v) investigates chromosomal aberrations and not gene mutation, and the OECD TG 476 studies (iii)-(iv) investigate gene mutation mechanisms in mammalian cells, which differ from those investigated in bacteria.

Therefore the above studies (i)-(v), considered alone or together, do not allow to conclude on whether your Substance has or has not hazardous properties related to gene mutation in bacteria. Therefore, your adaptation according to Annex XI, Section 1.2. is rejected and the information requirement is not fulfilled.

2. The long-term toxicity testing on aquatic invertebrates also requested at C.2. below (triggered by Annex VII, Section 9.1.5., column 2)

Short-term toxicity testing on aquatic invertebrates is a standard information requirement in Annex VII to REACH. However, according to Annex VII, section 9.1.1, column 2, for poorly water soluble substances (e.g. water solubility below 1 mg/L) long-term toxicity study on aquatic invertebrates (Annex IX, Section 9.1.5) must be considered instead of an acute test.

You have provided a key study in your dossier:

i. An OECD TG 202 study (2000) with the Substance.

We have assessed this information and identified the following issues:

For poorly water soluble substances (e.g. water solubility below 1 mg/L or below the detection limit of the analytical method of the test substance) long-term toxicity study on aquatic invertebrates must be considered instead of an acute test (REACH Annex VII, Section 9.1.1, Column 2). Poorly water soluble substances require longer time to reach steady-state conditions and the short-term tests may not give a true measure of toxicity for this type of substances.

In the technical dossier you have indicated that the Substance is poorly water soluble (Water solubility (WS) <4.93 mg/L at 20 ºC).

Your comments to the draft decision are addressed in section C.2., Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.), below.

Therefore, long-term toxicity testing is needed to accurately define the hazard of the Substance.

Therefore, the information provided does not meet the information requirement.

3. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

Growth inhibition study aquatic plants is a standard information requirement in Annex VII to REACH.

You have provided:

i. Key study: An OECD TG 201 study (1999) with the Substance.

We have assessed this information and identified the following issues:

Tests on substances must be conducted in accordance with the OECD test guidelines or other internationally recognised test method (Article 13(3) of REACH).

OECD TG 202 in combination with the revised OECD Guidance 23, ENV/JM/MONO(2000)6/REV1 require that the following conditions are met (among others):

- fulfilment of validity criteria as set up in the test guideline: The mean coefficient of variation for section-by-section specific growth rate in the control cultures must not exceed 35%.
- effect concentrations based on the measured values rather than nominal values unless the test concentrations are maintained within 20% of the measured initial concentrations throughout testing. (OECD TG 201 and ECHA Guidance R7B).
- For difficult to test substances, a sufficiently sensitive analytical method is particularly necessary due to the likelihood of losses of the Substance from the test medium. The possibility of losses during sampling, sample treatment and analysis must be considered and documented.

In the technical dossier you have indicated that the Substance is poorly water soluble (WS <4.93 mg/L at 20ºC).

In the key study (1999), algae were exposed to water accommodated fractions (WAF), which is covered by OECD GD 23.

Chemical analysis was performed which showed that significant losses occurred:
- You reported that measured concentrations of the Substance at time 0 and 72 hours were in the range of 1-34 % of the nominal;
- results are reported as nominal loading rate (72 hour $ErLR_{50}$ of 16 mg/L);
- you did not report raw data on the analytical monitoring (i.e. exact measured concentration at each test concentration and sampling time) and recovery rates in the study summary;
- you reported that all validity criteria were fulfilled.

Your mean coefficient of variation for section-by-section specific growth rate in the control cultures exceeded 35%, based on a recalculation in the control cultures using the raw data on the cell density you have provided. In your comments to the draft decision, you acknowledged that the mean coefficient of variation for section-by-section growth rate in the control cultures falls outside the required maximum of 35%, and as a result you agree with the limitations of the key study (1999).

Furthermore, you reported effect concentrations in normal values while the measured concentration range provided indicated that the recovery rates were significantly below 80%. The reported effect concentrations are not compliant with the requirements of OECD TG 201. As indicated there you need to report the effect concentrations based on measured concentrations especially when the test concentrations are not maintained within the required 20 % of the measured initial concentrations throughout testing (ECHA Guidance R7B).

Regarding your remaining comments to the draft decision, please see our responses in section C.2., Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.), below.

The aforementioned conditions are not met, therefore the information provided does not fulfil the information requirement.

Appendix B: Reasons for the requests to comply with Annex VIII of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 10 to 100 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII and VIII to REACH.

1. Only if a negative result in Annex VII, Section 8.4.1. is obtained, In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

An *in vitro* gene mutation study in mammalian cells is a standard information requirement in Annex VIII to REACH in case of a negative result in the *in vitro* gene mutation test in bacteria and the *in vitro* cytogenicity test.

You have provided supporting studies in your dossier with the Substance:

i. an OECD TG 476 study (1983)
ii. an OECD TG 476 study (1984)
iii. an *in vitro* gene mutation study in bacteria (1983) similar to OECD TG 471
iv. an *in vitro* gene mutation study in bacteria (1984) similar to OECD TG 471
v. an OECD TG 473 study (1999)

In your comments to the draft decision, you further provide explanations of why you believe that:

- The point values higher than the acceptable spontaneous mutant frequency for the negative and/or vehicle controls in both studies provided can be explained by spontaneous fluctuations.
- The negative results provided in (i) can be supported by the fact that statistical analysis of the data showed no significant differences between mutation frequencies of the test concentrations and the negative controls.
- The elevated mutant frequency point values obtained in (ii) for single concentrations can be explained by spontaneous fluctuations.
- The absence of concentration-related response in both studies show a consistent lack of mutagenic activity of the Substance in mammalian cells.
- These studies adequately address the potential of the substance to induce gene mutations in mammalian cells for the purpose of hazard evaluation.

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

1) Inadequate *in vitro* gene mutation studies in bacteria

As stated above, information on an *in vitro* gene mutation study in mammalian cells is only needed if a valid *in vitro* gene mutation test in bacteria as well as an *in vitro* cytogenicity test show negative results.

While the OECD TG 473 study you provided (v) shows negative results, the results of the *in vitro* gene mutation studies in bacteria (iii)-(iv) mentioned above) are rejected for the reasons provided in section A.1.

The information requested under point A.1 of this decision will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered. Consequently, you are required to provide information for this endpoint, if the *in vitro* gene mutation study in bacteria provides a negative result. The deadline set by this decision allows for the sequential testing.

2) Inadequate in *vitro* gene mutation studies in mammalian cells

To fulfil the information requirement, the *in vitro* gene mutation study on mammalian cells must meet the requirements of OECD TG 476 or OECD TG 490. The key parameters of these test guidelines include:

a) The response for the concurrent negative control must be inside the historical control range of the laboratory;
b) The spontaneous mutant frequency should be between 5 and $20x10^{-6}$.

However, the *in vitro* gene mutation studies on mammalian cells (i)-(ii) you have provided :

a) do not include a historical control range of the laboratory for the negative control. In your comments to the draft decision, you acknowledge the absence of reported historical control range values for the laboratory that performed the *in vitro* gene mutation studies in mammalian cells provided (i)-(ii). Without the confirmation of a concurrent negative control response within the historical control range of the laboratory, the validity of the negative controls, and by way of consequence of all the test results, in both studies cannot be confirmed.
b) include point values higher than $20x10^{-6}$ for both the negative and vehicle controls:
    - $34.1x10^{-6}$ for one of the replicates of the negative control and $39.8x10^{-6}$ for one of the replicates of the vehicle control, with metabolic activation in the first study (i)
    - $34.7x10^{-6}$ for one of the replicates of the negative control, with metabolic activation in the second study (ii)
    - $57.6x10^{-6}$ for one of the replicates of the vehicle control with metabolic activation and $46.1x10^{-6}$ for one of the replicates of the vehicle control without metabolic activation in the second study (ii).

ECHA agrees that the outlying values reported for the negative and vehicle controls were generally only point values and not confirmed in the corresponding replicates. However, because of the fact that mutant frequency values higher than the acceptable value of $20x10^{-6}$ were repeatedly reported in both studies provided (i)-(ii), under different experimental conditions (with or without metabolic activation), and in different negative (solvent or untreated) controls, ECHA cannot accept considering them only as spontaneous fluctuations. This also raises some concerns regarding the quality and validity of the studies.

Furthermore, regarding data interpretation, no detailed results or statistical analysis were reported in the dossier for any of the studies provided, which did not allow ECHA to evaluate these.

ECHA also considers that the uncertainty regarding the validity of the negative controls in both studies precludes any firm conclusion on the mutagenic potential of the Substance. In particular, the fact that single points with mutant frequency values higher than the negative controls were reported in several test sample concentrations, in both studies, despite sometimes high background values, raises some concerns:

- For instance, it is not clear how the high negative control values highlighted above were considered in the statistical analysis and to which extent this had an impact on the fact that there were no significant differences between the mutation frequency values of the test concentrations and the negative controls in (i).
- In your comments to the draft decision, you only mention one duplicate test sample with a high mutant frequency value ($89.8x10^{-6}$, i.e. more than four times higher than the acceptable spontaneous mutant frequency) at a single concentration of 0.07 mg/ml and without metabolic activation in (ii). However, in the same study, ECHA notes that

high values (64.1 and 84.4x10^{-6}) were also reported at two test sample concentrations (0.14 and 0.18 mg/ml) with metabolic activation. In (i), it is also indicated that several test sample concentrations had single points with mutant frequencies higher than the negative controls but no further details were provided.

- You consider these high test values as the results of spontaneous fluctuations and highlighted the fact that no concentration-response was observed in any of the studies provided. However, according to OECD TG 476, the absence of a concentration-related increase in mutant frequency, as evaluated with an appropriate trend test, is only one of the three criteria that a test substance should meet in any of the experimental conditions examined to be considered clearly negative. The two other criteria are: none of the test concentrations exhibits a statistically significant increase in mutant frequency compared with the concurrent negative control and all results are inside the distribution of the historical negative control data. OECD TG 476 states that if only one or two of these criteria are fulfilled, the test substance is not clearly negative, and expert judgement and/or further investigations are recommended to assist in establishing the biological relevance of a result.

Based on the above, ECHA considers that the validity of the *in vitro* gene mutation studies in mammalian cells provided (i)-(ii) is questionable in view of the negative and vehicle control values. Additionally, the high mutant frequency values reported in these studies for the test samples are unlikely to be due to spontaneous fluctuation only. Therefore the information provided does not fulfil the information requirement and adequate *in vitro* gene mutation data in mammalian cells are needed in case the *in vitro* gene mutation studies in bacteria requested in Appendix A.1 provide negative results..

To fulfil the information requirement for the Substance, both the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) and the thymidine kinase gene (OECD TG 490) are considered suitable.

Appendix C: Reasons for the requests to comply with Annex IX of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier registered at 100 to 1000 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII to IX to REACH.

1. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species**

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

You have provided in your dossier an OECD TG 415 study (1999).

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

In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, information provided has to meet the requirements of OECD TG 414 in one species.

You have not provided information following OECD TG 414. Instead, you have provided an OECD TG 415 study. In this study, structural malformations and variations are not investigated as required in the PNDT study (OECD TG 414).

Therefore, this study does not fulfil the information requirement.

In your comments to the draft decision you accept that a detailed assessment of structural malformations and variations in offspring, as those undertaken in an OECD TG 414 pre-natal developmental toxicity study, has not been performed with the Substance. Although you agree that there is a data gap, you state that you find it unlikely that a new study will lead to additional risk management measures. However, in the absence of the REACH standard information on pre-natal developmental toxicity ECHA cannot accept such conclusions, as there is at present no basis for such a conclusion. Therefore a study is needed.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[2] administration of the Substance.

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

Long-term toxicity testing on aquatic invertebrates is a standard information requirement at Annex IX.

You have provided an OECD 211 study (2000).

We have assessed this information and identified the following deficiencies: Under Articles 3(28) and 10(a)(vii) and Annex I, Section 3.1.5. of REACH, a robust study summary must be provided for the study/ies giving rise to the highest concern. A robust study summary must cover critical information and allow an assessment of the validity and reliability of the study. For a study conducted according to OECD 211, information necessary for the

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

assessment of its validity and reliability includes:

- the results of all analyses to determine the concentration of the test substance in the test vessels (see example data sheets in Annex 5, OECD TG 211);

- effect concentrations based on the measured values rather than nominal values (see Annex 6, OECD TG 211).

In the technical dossier you have indicated that the Substance is poorly water soluble (WS <4.93 mg/L at 20ºC).

In the key OECD TG 211 study (2000), daphnids were exposed to water accommodated fractions (WAF), which is covered by OECD GD 23.

Chemical analysis were performed which showed that significant losses occurred:
- analysis of the freshly prepared test media at 0 hour showed measured concentrations in a range of 0.106 to 0.826 mg/L;
- analysis of the old media showed measured concentrations in a range of below the limit of quantification (0.021 mg/L) to 0.863 mg/L;

However, results are reported as nominal loading rate (21 day EL50 of 27-48 mg/L); you did not report raw data on the analytical monitoring (i.e. exact measured concentration at each test concentration and sampling time) and recovery rates in the study summary.

For the same reasons explained above for request A.3., the reported effect concentrations are not compliant with the requirements of OECD TG 211. As indicated there you need to report the measured concentrations and calculate effect concentrations based on measured concentrations especially when the test concentrations are not maintained within the required 20 % of the measured initial concentrations throughout testing (see Annex 6 of OECD TG 211 and ECHA Guidance R7B).

In your general comments to the draft decision you outline that WAF is an appropriate testing approach for this type of Substance and it can be appliable to classification.

In your specific comments to the draft decision you outline that the effects concentrations should be based on nominal loading rates rather than the measured concentrations for all the aquatic toxicity endpoints and that the reporting of the existing acute and chronic Daphnia studies are sufficient to meet testing requirements and criteria for hazard assessment.

Regarding your general comments on WAF as an appropriate testing approach for this type of Substance and its applicablility to classification, you outline the following:

- Unlike mono-constituent substances in which clear dose-effect relationships can be shown, complex substances (UVCB) do not exhibit specific and exact properties. They are characterized by a range for physical-chemical, toxicological and environment values. For aquatic toxicity, aqueous solubility is the most critical factor (ECETOC, 1996) and for complex substances it is difficult to validate the toxicity result with measured concentrations. Data indicate that this substance is poorly water soluble overall (<4.93mg/L at 20°C). In an aqueous mixture, the water solubility of each constituent is not the same as that of the pure constituent. The solubility in water of liquid mixtures is affected by each component of the mixture. Moreover, the interaction of components in the mixture can cause complex and substantial changes in the solubility of the constituents ([redacted], 1973).

- In aquatic toxicity testing, a two-phase system is generated when adding petroleum-like compounds to water to produce the test solution ([redacted], 2000). The water accommodated fraction (WAF) method is a technique developed for poorly water-soluble complex mixtures which are only partially soluble in water ([redacted], 1994; [redacted], 1998). It is recommended as an effective approach for aquatic toxicity test of UCVBs by OECD (OECD, 2000). Test methods based upon varying the loading concentration of the test substance to determine the toxicity produce data more applicable to classification.

- It is concluded that it is not appropriate to express the aquatic toxicity in relation to specific components of this complex substance because as toxicity relates to the total substance.

- Also, the water accommodated fraction technique is an appropriate approach to evaluate the aquatic toxicity of complex multi-component substances and, because the exposure of the mixture is neither wholly dissolved nor complete for a stable dispersion, the loading rate (defined as the mass to volume ratio of the mixture to medium used in the preparation of a WAF) is an applicable end point to express the aquatic toxicity of this complex substance

ECHA acknowledges that measuring test concentrations is difficult for UVCB (complex) test substances. ECHA agrees to your above general comments concerning a WAF approach, except for the following two statements:

1. Test methods based upon varying the loading concentration of the test substance to determine the toxicity produce data more applicable to classification.

2. The loading rate is an applicable endpoint to express the aquatic toxicity of this complex substance

Regarding your comment 1., ECHA notes a WAF approach is an applicable approach to generate data for classification but it is not considered to be more applicable than any other available method for example the block approach.

Regarding your comment 2., on the loading rate being an applicable endpoint to express the aquatic toxicity of this complex substance, ECHA agrees based on certain conditions that loading rate is an applicable endpoint to express the aquatic toxicity of complex test substances. ECHA's conditions are as follows:

- Where it is not technically possible to perform the analysis, results based on nominal loading rates may then indeed be the only available option.

- If it is technically possible to perform the analysis and the measured concentrations are within 20% of the nominal concentrations, the effect concentrations can also be based on the nominal loading rates.

- When it is technically possible to peform the analysis and the measured concentrations are actually outside of 20% of the nominal concentrations, the effect concentrations must be based on the measured concentrations.

Results based on measured concentrations are more accurate for assessing the intrinsic toxicity of the test substance as they establish a direct link between the effects observed (taking into account any losses) and actual exposure test substance concentrations in the test media during the duration test.

In your specific comments to the draft decision you outline that:

(1) The effects concentrations should be based on nominal loading rates rather than the measured concentrations for all the aquatic toxicity end-points and that

(2) The reporting of the existing acute and chronic Daphnia studies are sufficient to meet standard information requirements of REACH and criteria for hazard assessment.

Regarding point (1) above in your specific comments to the draft decision that the effects concentrations should be based on nominal loading rates rather than the measured concentrations for all the aquatic toxicity endpoints, you state the following:

a. In the algae study, "clear evidence of effects of the test substance on both growth and biomass was observed at higher loading rate Water Soluble Fractions (WSF)" and that the effects observed were better described by the loading rates than the concentrations reported based on the HPLC analysis.

b. No single analytical method is likely to be able to measure all components of the Substance. It is not appropriate to express the aquatic toxicity in relation to specific component of the Substance, because the observed toxicity is related to the total Substance and not to any particular constituent.

Regarding point a. above, you have provided a statement regarding the reporting of effect concentrations, in the algae study (OECD TG 201, 1999). However, currently ECHA cannot validate this statement as you have not provided any data to support it and there is no such information in the current robust study summary. In addition as explained in the reasons for the request A.3. above, the algae study (OECD TG 201, 1999) is still not valid.

Regarding point b. above, ECHA agrees with you that for a complex test substance, like your Substance, there is no single analytical method applicable to measure all components of the Substance. In your comments you indicated that you observed two peaks in your HPLC analysis. However, currently ECHA cannot establish the relevance of these two peaks in relation to the oberved effect. Furthermore, ECHA notes that in Section 7.9.2.5 of the OECD GD 23 states that "the measured concentrations of dissolved and/or emulsified key or major components would greatly improve interpretation and also assist in evaluating acceptable loading values for a UVCB, thus, underscoring the importance of chemical-specific analytical determinations". Thus, it is important to provide evidence that all reasonable efforts have been taken to minimise losses and to ensure that the dissolved concentration of the UVCB has been equilibrated and maximised with minimal observed emulsion for the duration of the test.

Regarding point (2) above in your specific comments to the draft decision you outline that the reporting of the existing acute and chronic Daphnia studies are sufficient to meet testing requirements and criteria for hazard assessment, and you state the following:

I. For acute and chronic daphnia studies, the method of analysis used was for total organic carbon, thus non-carbon containing components such as free-boric acid, were not included in the analysis.

II. Compositions of dissolved fractions were loading rate dependent.

Regarding point I. above, you state that non-carbon containing components such as free-boric acid, were not included in the analysis. However, boric acid is water soluble thus the toxicity of such a substance is reflected by the acute test data whereas chronic test reflect toxicity of less soluble constituents (ECHA Guidance R7b. Table 7.8-3). Thus, analysis of free-boric acid in a chronic study is not needed. In addition, ECHA notes that total organic carbon analysis is not sensitive enough to identify gross changes in the composition of WAFs over time. Thus it cannot demonstate the attainment of equilibrium nor the stability of individual UVCB components over time.

ECHA considers acute tests are not adequate for poorly water soluble substances or any of its consituents which are poorly water soluble such as your Substance as acute studies may not give a true measure of toxicity for such test substances. Thus, statement that the existing acute studies (Daphnia and fish) are sufficient to meet the testing requirements and criteria for hazard assessment, cannot be supported.

Regarding point II. above, on compositions of dissolved fractions being loading rate dependent, you provide the following information:

- the peak at 9.599 mins was a major component in the mid and high loading rates of 100 to 1000 mg/l but was not identified in the low loading rates of 10 and 32 mg/l. In contrast, the peak at 13.1 mins was identified in the low loading rate but was only a minor component in the high loading rate. These data support the conclusion that the concentration of different components of the substance in the exposure solutions were loading rate dependent.
- measured concentrations at the high loading rate of 150mg/L showed concentrations >1mg/L which remained >1mg/L for the 48hr stability evaluation both in light and dark conditions showing that at high loading rates, concentrations >1mg/L were achievable.

You outline your high and low loading rates, your observed peaks and your observed media stability at your high loading rate over a 48 hr period and you observed instablilty at your low loading rate. You mention observing different components at both high and low loading rates. You have not associated your high or low loading rates with any measured concentrations and/or sampling time over the duration of the test. You do not identify any of your components. You indicate your could not maintain the stability at your low loading rate with a semi-static test design of 48 hrs. In your comments you mention that the test media was renewed every 2 or 3 days over the duration of the test. It is unclear to ECHA if the actual renewal was 48 hrs or 72 hrs. The information in the robust study summary relates to measured concentrations over a range of fresh and old test media over the duration of the test, only. Thus, it is currently difficult for ECHA to align your information in your comments on the loading rates and observed peaks with the information currently in the robust study summary on the range of measured concentrations. Thus, there is still insufficient information for us to determine the concentration of the test substance in the test vessels or the information to calculate the effect concentrations based on the measured concentrations over the duration of the test.

In your comments on the draft decision, you further indicate your intention to update the dossier to include all the analytical data for chronic Daphnia.

The present decision is the result of ECHA's evaluation of the registration dossier as submitted at the time indicated in the header of this decision. ECHA will evaluate later updates of the information in the follow-up evaluation under Article 42 of the REACH Regulation.

Following the review of your comments there remains still incorrect reporting and the missing analytical monitoring data. Therefore the data provided still do not allow an independent assessment of the validity and reliability of this study and its results for use in hazard assessment.

Currently as long as the missing elements are not provided and the incorrect reporting is not corrected, the information provided does not fulfil the information requirement.

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

Long-term toxicity testing on fish is a standard information requirement in Annex IX to the REACH Regulation.

You have adapted this information requirement by stating that long-term toxicity study with fish does not need to be conducted as "the chemical safety assessment (i.e. PEC/PNEC ratio) based on the acute aquatic toxicity data and the appropriate safety factors do not indicate a concern".

In order to adapt the information requirement for long-term toxicity to fish based on Annex IX, Section 9.1.6, Column 2, the Chemical Safety Assessment (CSA) needs to demonstrate that risks towards the aquatic compartment arising from the use of the Substance are controlled (as per Annex I, section 0.1). The Chemical Safety Assessment (CSA) needs to assess and document that risks arising from the Substance are controlled and demonstrate that there is no need to conduct further testing (Annex I, Section 0.1; Annex IX, Section 9.1.6, Column 2).

In particular, you need to take into account of the following elements in your justification:
- all relevant hazard information from your registration dossier,
- the outcome of the exposure assessment in relation to the uses of the Substance,
- the outcome of the PBT/vPvB assessment including information on relevant degradation products and constituents present in concentration at or above 0.1% (w/w).

You rely on the availability of acute aquatic toxicity data as well as PNEC derived from these studies to demonstrate that risks towards the aquatic compartment arising from the use of the Substance are controlled.

As specified in the reasons for requests A.2.-3. and C.1. the data on aquatic toxicity is not compliant with the REACH requirements. Hence your dossier currently does not include adequate information to characterize the hazard property of the Substance.

In your comments on the draft decision, you state that you believe both the acute daphnia and acute fish are reliable and sufficient for hazard assessment.

However, as already explained in the reasoning for request section C.2., Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.), above, in case the Substance or any of its consituents have been proven to be poorly water soluble (*i.e.* water solubility is not above 1 mg/L) then long-term toxicity study on aquatic invertebrates instead of acute test is required (Annex VII, section 9.1.1., column 2; for substances registered for tonnages >100 t/a this is a standard information requirement under Annex IX, Section 9.1.5.).

Therefore your Chemical Safety Assessment does not demonstrate that the risks of the Substance are adequately controlled. As a consequence, your adaptation is rejected as it does not meet the specific rules for adaptation of Annex IX, Section 9.1.6., Column 2.

You have adapted this information requirement by claiming that this information is not needed considering the available short-term studies and the long-term study in daphnia.

Therefore, your adaptation is rejected and long-term toxicity study on fish is needed.

Appendix D: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The compliance check was initiated on 22 February 2019.

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

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 proposals for amendment.

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

Appendix E: Observations and technical guidance

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

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 the Member States.

3. Test guidelines, GLP requirements and reporting

   Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.

   Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses shall be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

   Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries'[3].

4. Test material

   Selection of the test material(s) for UVCB substances

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/impurity on the test results for the endpoint to be assessed. For example, if a constituent/impurity of the Substance is known to have an impact on (eco)toxicity, the selected test material must contain that constituent/impurity. Any constituents that have harmonised classification and labelling according to the CLP Regulation (Regulation (EC) No 1272/2008) must be identified and quantified using the appropriate analytical methods.

   The OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 11 [ENV/MC/CHEM(98)16] requires a careful identification of the test material and description of its characteristics. In addition, the Test Methods Regulation (EU) 440/2008, as amended by Regulation (EU) 2016/266, requires that "*if the test method is used for the testing of a* [...] *UVCB* [...] *sufficient information on its composition should be made available, as far as possible, e.g. by the chemical identity of its constituents, their quantitative occurrence, and relevant properties of the constituents*".

[3] https://echa.europa.eu/practical-guides

In order to meet this requirement, all the constituents or groups of constituents of the test material used for each test must be identified as far as possible. For each constituent or group of constituents the concentration value in the test material must be reported in the Test material section of the endpoint study record.

Technical Reporting of the test material for UVCB substances

The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents or groups of constituents of the test material and their concentration values. Considering the specific characteristics of the registered substance, the composition should be reported at least to the same level of detail as the current boundary composition of the Substance, including at least constituents or group of constituents such as 'Boric acid, tris-diethanolamine', 'Boric acid, tris-diethanolamine, mono-adduct with Fatty acids, tall-oil', 'Boric acid, tris-diethanolamine, di-adduct with Fatty acids, tall-oil', and 'Boric acid, tris-diethanolamine, tri- and higher adducts with Fatty acids, tall-oil', 'Fatty acids, tall oil', 2,2'-iminodiethanol, and boric acid. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers" on the ECHA website[4].

5. List of references of the ECHA Guidance and other guidance/ reference documents[5]

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 in this decision.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)[6]

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

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

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

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

[4] https://echa.europa.eu/manuals
[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

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

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

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

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

OECD Guidance documents[7]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.
Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

[7] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix F: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.