EUROPEAN CHEMICALS AGENCY

Helsinki, 24 May 2019

Addressee: [redacted]

Decision number: CCH-D-2114471262-54-01/F
Substance name: Reaction mass of sodium hydrogen N-(1-oxooctadecyl)-L-glutamate and stearic acid
List number: 939-201-1
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 18/12/2015
Registered tonnage band: 100-1000

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. **Skin sensitisation (Annex VII, Section 8.3.) with the registered substance:**

   (i) *in vitro/in chemico* skin sensitisation information on molecular interactions with skin proteins, inflammatory response in keratinocytes and activation of dendritic cells (Annex VII, Section 8.3.1.); and/or

   (ii) *in vivo* skin sensitisation information (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429) with the registered substance in case the *in vitro/in chemico* test methods specified under point i) are not applicable for the substance or the results obtained are not adequate for classification and risk assessment;

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

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

4. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance;**

5. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: OECD TG 408) in rats with the registered substance;**

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

7. **Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) with the registered substance;**

8. **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;**

9. **Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: CO_2 evolution test, OECD TG 301B) or**

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: MITI test (I), OECD TG 301C) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Closed bottle test, OECD TG 301D) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Manometric respirometry test, OECD TG 301F) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Ready biodegradability – CO_2 in sealed vessels (headspace test), OECD TG 310)

   with the registered substance.

You have to submit the requested information in an updated registration dossier by **30 November 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 **Wim De Coen**, Head of Unit, 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 1: Reasons

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Consideration on the uses of the substance

In accordance with your registration dossier the substance is used exclusively in cosmetic products. However, stages of manufacturing of chemical and formulation of cosmetic products are taking place in the EU and there is no indication that they are carried out under strictly controlled conditions. As potential worker exposure may exist, testing for human health endpoints is necessary to assess the risks from exposure to workers and therefore in order to fulfil the relevant REACH requirements. This is in accordance with ECHA's factsheet[2] on the interface between REACH and Cosmetics Regulations, which was developed jointly with the European Commission. It provides that registrants of substances that are exclusively used in cosmetics may not perform animal testing to meet the information requirements of the REACH human health endpoints unless such tests are needed to assess the risks from exposure to workers. In addition, animal testing for all environmental endpoints is permitted.

Such testing would not trigger the testing and marketing bans under the Cosmetics Regulation as the testing is to be performed for the purposes of meeting the requirements of the REACH Regulation; see Commission Communication of 11 March 2013 on the animal testing and marketing ban and on the state of play in relation to alternative methods in the field of cosmetics (COM(2013)135))[3].

You submitted comments to the draft decision "*disagreeing with ECHA's interpretation that the requested animal testing would not trigger the testing and marketing bans under the Cosmetics Regulation.*" You furthermore consider that you are "*not in a position to perform the requested prenatal development study on animals taking into account the marketing ban under the Cosmetics Regulation*".

Firstly, ECHA notes that contrary to your opinion ECHA does not attempt to provide binding interpretation of Cosmetics Regulation on testing and marketing bans. ECHA only refers, as noted above, to the Communication on that subject of the competent body in the field of cosmetics i.e. European Commission.

Secondly, it seems that you misinterpret the Board of Appeal decision in Case A-013-2016 which considered that ECHA should provide interpretation of the relationship between the REACH Regulation and the Cosmetics Regulation and explain how such interpretation applies in the particular case. ECHA has provided such analysis above.

Thirdly, you consider that the marketing ban under the Cosmetics Regulation would apply if ECHA requires animal testing. However, you seem to overlook the important considerations:

[2] https://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf
[3] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52013DC0135&from=EN

(i) Testing is required for the purposes of the REACH Regulation. There is a possibility of worker exposure to the substance at the stages of manufacture and formulation as you have not applied strictly controlled conditions. Therefore, there is a necessity to assess developmental hazard of the substance to ensure that the risks to workers can be adequately controlled.

(ii) Testing is not requested for the purposes of the Cosmetics Regulation. There is an important qualification in Article 18 of the Cosmetics Regulation, that you seem to overlook, that animal testing is prohibited as far as it is undertaken *'in order to meet the requirements of this [Cosmetics] Regulation'.* The importance of this qualification is confirmed by the European Commission, the body responsible in the field of cosmetics, in its Communication referred to above (COM(2013)135). In particular: *'The Commission considers that the marketing ban is triggered by the reliance on the animal data for the safety assessment under the Cosmetics Directive/Regulation, not by the testing as such'.*

(iii) The matter has already been considered by the competent body i.e. European Commission in the communication mentioned above. You do not refer to it in your comments or explain why you seem to have contrary position to the one expressed by the competent body in the field of cosmetics.

To conclude, the animal testing is required for the purposes of REACH Regulation and in accordance with its provisions.

Grouping of substances and read-across

Your registration dossier contains for multiple endpoints adaptation arguments in the form of a grouping and read-across approach according to Annex XI, Section 1.5. of the REACH Regulation. ECHA has assessed first the scientific and regulatory validity of your grouping and read-across approach for human health endpoints under the present section, before assessing the information requirements under the respective sections below (sections 1 to 6). Furthermore, ECHA has assessed the scientific and regulatory validity of your grouping and read-across approach applied for the environmental endpoint biodegradation in water (Annex VII, Section 9.2.1.1.) under the respective section 9 below.

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

- *in vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- *in vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.)
- *in vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)
- a sub-chronic toxicity (90-day) study (Annex IX, Section 8.6.2.)
- screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

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.

Description of the proposed grouping and read-across approach

You consider to achieve compliance with the REACH information requirements for the registered substance Reaction mass of sodium hydrogen N-(1-oxooctadecyl)-L-glutamate and stearic acid (EC 939-201-1) using data of structurally similar substances L-Glutamic acid, N-coco acyl derivs., monosodium salts (EC 269-087-2, CAS 68187-32-6), and L-Glutamic acid, N-coco acyl derivs., disodium salts (EC 269-085-1, CAS 68187-30-4) (hereafter the 'source substances' **[1]** and **[2]**).

You explain in the general analogue approach hypothesis that "*the target substance and the sources substance belong to the same substance class of glutamate derivatives and differ only in the carbon chain distribution of the alkyl moiety*", and that, "*in general, same structural components can be considered as predictive for a great similarity of the toxicological profile.*" Therefore, you claim that "*due to the structural similarities and consistent trend in physico-chemical, toxicological and toxicokinetic behaviour, the read-across to the selected source substances are considered an appropriate adaptation to the standard information requirements of Annex IX of the REACH Regulation for the target substance.*"

Furthermore, you explain in the endpoint specific analogue approach hypothesis for reproductive toxicity endpoint that "*It is indicated in this document that the target and the source substance will hydrolyse immediately after oral intake and degrade into glutamic acid and coconut acid/stearic acid. Stearic and coconut acid are natural constituents present in food and not associated with a hazard regarding reproductive toxicity. Both fatty acids are approved direct food additives.*"

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

Support of the proposed grouping and read-across approach

You have provided a read-across justification as a separate attachment in IUCLID.

The justification document comprises of i) OECD toolbox profiler prints of C8 Glutamate (CAS 167888-81-5, reported minor constituent of the analogue substance, [redacted]) and C18 Glutamate (CAS not reported, reported main constituent of the registered substance, [redacted]) and ii) bibliographic review of two analogue substances, L-Glutamic acid, N-coco acyl derivs., monosodium salts (CAS 68187-32-6) **[1]**, and L-Glutamic acid, N-coco acyl derivs., disodium salts (CAS 68187-30-4) **[2]**, structurally related to the registered substance.

The bibliographic review is focusing on the below key points:

i. Common functional groups

ii. Common precursors and breakdown products via biological processes

iii. Structural similarity

iv. Similar metabolic pathways

v. Similar physic-chemical properties

vi. Similar (low) mammalian toxicity and ecotoxicity profiles

In the technical dossier the following toxicological studies conducted with **[1]** and/or **[2]**, respectively, have been provided: acute toxicity (OECD 401 and 402), sub-chronic toxicity (OECD 408), skin and eye irritation (OECD 404 and 405) and skin sensitisation (OECD 406). ECHA notes that no toxicological studies conducted with the registered substance have been provided.

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

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

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

In your read-across hypothesis you state that "*the target substance and the sources substance belong to the same substance class of glutamate derivatives and differ only in the carbon chain distribution of the alkyl moiety*", and that, "*in general, same structural components can be considered as predictive for a great similarity of the toxicological profile.*"

You acknowledged the structural differences but you claim that they will not affect the chemical reactivity of the substances and therefore you expect that these substances will undergo similar metabolism in the body.

ECHA notes that the source and the target substances are multi constituent substances with different compositions and main constituents. Moreover, the source substances' main constituent (C12 Glutamate, [redacted]) is not present in the target substance while the target substance main constituent (C18 Glutamate, [redacted]) is not present in the source substances. Additionally, ECHA notes that you have identified also other non-common target and source substance constituents. Furthermore, ECHA considers that different constituents may differ in terms of chemical reactivity and in particular have a different metabolic rate in the body (see iii below).

ECHA concludes that you have not addressed the above obvious structural differences (*i.e.* the type of alkyl substitution) between the source and target substances, and have not demonstrated why those differences would not lead to differences in the toxicity profile of source and target substances. The provided explanation is not considered as valid to establish a scientific credible link between the structural similarity and the prediction.

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

Annex XI, Section 1.5. provides that "*substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances*". One

prerequisite for a prediction based on read-across therefore is that the substances involved are structural similar and are likely to have similar properties. One important aspect in this regard is the analysis of the data matrix to compare the properties of source and target substances and to establish whether indeed they are similar or follow a regular pattern.

You suggest that hazards related to the above-mentioned information requirements of the target substance can be predicted from data on the source substances.

ECHA understands that you claim that the data available for glutamate derivative substances is adequate to support the read-cross approach for mammalian toxicology in general. Furthermore, you have proposed that the source substances have similar toxicity profile as the target substance and that the properties of the target substance can be predicted from data obtained with the source substances. However, ECHA notes that no toxicological information on the registered substance has been provided. ECHA therefore considers that the available information does not support a claim of similar toxicity because it does not allow comparison of the toxic profile or hazard between the source and the target substances for the above-mentioned information requirements.

You commented the draft decision agreeing to "*deliver additional and distinct information on the target substance to make the read across justification even more robust.*" More specifically, you intend to provide the following *in vitro* information in order to "*support the similar or regular pattern of toxicological properties as a result of structural similarity*:

- *in vitro skin irritation (OECD 439)*
- *in vitro eye irritation (OECD 437 + 492)*
- *skin sensitization (in vitro sensitization test battery: OECD 442 C, E, D)*
- *in vitro gene mutation study in bacteria (Ames, OECD 471)*
- *in vitro micronucleus test (OECD 487)*
- *in vitro Mouse-Lymphoma-Test (OECD 490)"*

ECHA acknowledges your agreement to deliver the listed *in vitro* studies, as requested in the present decision. However, those *in vitro* studies will not provide relevant information to support read-across for repeated dose toxicity and reproductive toxicity.

ECHA concludes that the presented evidence does not support a similar or regular pattern of toxicity for the property under consideration as a result of structural similarity. Therefore it cannot be approved that the proposed source substance(s) can be used to predict properties of the registered substance.

(iii) Toxicokinetics

One important aspect in establishing that substances have similar effects or follow a regular pattern is the comparison of absorption, distribution, metabolism and elimination of source and target substances. This allows assessing the qualitative and quantitative internal systemic exposure of the test organism when exposed to source and target, respectively.

You claim that "*the hydrolysis represents the first chemical step in the absorption, distribution, metabolism and excretion (ADME) pathways*" and that "*the target and the source substance will hydrolyse immediately after oral intake and degrade into glutamic acid and coconut acid/stearic acid.*" To support this argument, you have provided OECD Toolbox

profiler results containing predictions of the hydrolysis rates in pH 6.5 - 7.4, and metabolism half-life (in fish) for C8 Glutamate and C18 Glutamate, respectively.

ECHA notes the following:

a) you have not supported your theoretical considerations on ADME properties and the assumed immediate hydrolysis after oral administration by any experimental data or toxicokinetic information.

   First, the provided OECD Toolbox profiler results for hydrolysis half-life are not applicable for predicting the hydrolysis after oral uptake. More specifically, the model is not applicable for predicting parent compound degradation and hydrolysis rate to glutamic acid and coconut acid/stearic acid as the predictions concern dissociation of the sodium ion(s) instead of the amide bond hydrolysis. Therefore, ECHA finds the provided QSAR predictions of hydrolysis rates not supporting your assumption of *immediate hydrolysis after oral intake*.

   Second, a model for metabolic half-life (in fish) is not applicable for predicting rate of the hydrolysis in mammalian toxicity study after oral uptake because the prediction is based on values for fish biotransformation during a bioaccumulation experiment and not related to mammalian toxicity test.

b) as explained in point (i) you have clearly identified the compositional differences between the source and target substances. However, you have not demonstrated why these compositional differences do not influence the metabolic rate and behaviour of the substances.

In addition, ECHA notes a publicly available bibliographical reference[4] that seems to contradict your claims, as it describes only slight hydrolysis of long-chain aliphatic amides by rabbit liver extract at pH 7.4 after five hours. Moreover, according to the publication, the enzymatic hydrolysis slows down with increasing chain length, which implies slower metabolic degradation rate for the target substance than for the source substance after oral intake.

In your comments to the draft decision you noted several points: "*The comparable hydrolysis is already reported in the Bray et al paper cited by ECHA. Furthermore, the breakdown products will be discussed in more detail with literature data. The registrant wishes to point out that both substances (target and source substance) are amino acid alkyl amides (glutamates). A likely metabolic pathway was presented - and agreed between the parties during the Oral Hearing at the Board of Appeal - and the resulting metabolites are part of the daily diet and consumed by humans on a gram scale. The metabolites are without any concern. This is underlined by the fact that these glutamates are used in cosmetic products and by cosmetic companies which need to perform a safety assessment of the products and had no concern to use the substance purely on theoretical considerations and expert knowledge. If there was any concern with regards to the cosmetic use of this substances, the SCCS – within its mandate given by the EU Commission – would anyway have already asked for additional data for a re-evaluation, which has not been the case.*"

[4] The Fate of Certain Organic Acids and Amides in the Rabbit; FURTHER OBSERVATIONS ON THE HYDROLYSIS OF AMIDES BY TISSUE EXTRACTS, Bray et al., 1950
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1275209/

Additionally, *you expressed an* intention to provide *in vitro* hydrolysis studies with the target and the source substances "*to strengthen the argumentation that the source and the target substance have a similar toxicokinetic behaviour.*"

Firstly, ECHA notes that the publication by Bray et al contradicts your assumption rather than supports it, as it provides, "*amides with less than 3 or more than 11 carbon atoms were only hydrolysed to a small extent.*" Therefore, your initial claim of immediate hydrolysis after oral intake seems questionable. ECHA thus concludes that systemic exposure to the parent substances cannot be excluded based on the current information. There is therefore a need to understand the toxicological property of the target substance (or both the target substance and the source, in case of intended read-across).

Secondly, ECHA acknowledges your intention to provide *in vitro* hydrolysis studies in support of your read-across arguments with respect to developmental toxicity. However, as such data is not currently provided (even though this shortcoming has already been discussed during the appeal process to which you refer to) there is no scientific information which would prove your assumption, while there is data (as discussed above) that contradicts it.

ECHA notes also that you did not provide detailed information on the Scientific Committee on Consumer Safety (SCCS) opinion. ECHA is therefore not able to take this information into account. ECHA notes in addition that REACH information requirements remiain to be fulfilled regardless of existence or non-existence of a relevant SCCS opinion especially as worker exposure is also concerned (as explained above).

ECHA concludes that you did not sufficiently address important aspects such as the transformation rate (i.e. hydrolysis) and behaviour of the source and target substances and the resulting possible difference in the metabolite profile. Therefore, currently it is not possible to verify whether the proposed source substance and the target substance are likely to have similar toxicity profiles as a result of similar metabolic profiles. In the absence of such information there is not an adequate basis for predicting the properties of the registered substance from the data obtained with the source substance.

Conclusion on the read-across approach

ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the human health effects of the registered substance may be predicted from data for reference substance(s) within the group. Hence, this approach does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation. ECHA notes that there are specific considerations for the individual endpoints which also contribute to the overall failure to meet the requirement of Annex XI, Section 1.5., and these are set out under the endpoints concerned.

1. Skin sensitisation (Annex VII, Section 8.3.)

"Skin sensitisation" is a standard information requirement as laid down in Annex VII, Section 8.3. of the REACH Regulation: "*Information allowing: - a conclusion whether the substance is a skin sensitiser and whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A) and -risk assessment, where required*". According to subsection 8.3.1 this includes information from *in vitro/in chemico*

test addressing each of the following key events of skin sensitisation: (a) molecular interactions with skin proteins, (b) inflammatory response in keratinocytes, (c) activation of dendritic cells. Provided that the *in vitro/in chemico* test methods are not applicable, or the results obtained from those studies are not adequate for classification and risk assessment according to point 8.3, also information from an *in vivo* study is required according to subsection 8.3.2.

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 a study record for a [redacted], Buhler Test (OECD 406) with the analogue substance L-Glutamic acid, N-coco acyl derivs., monosodium salts (CAS No. 68187-32-6; EC No. 269-087-2). However, as explained above in Appendix 1, section Grouping of substances and read-across of this decision, 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.

To address the skin sensitisation endpoint *in vitro/in chemico* methods have been developed. The ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017), Chapter R.7.3 describes the applicability and the limitations of the currently adopted test methods. ECHA Guidance also lists the *in vitro/in chemico* methods that have either already been validated or are under validation assessment at the time of the publication. It is your responsibility to select the test methods which are most appropriate for the registered substance.

Provided that an *in vivo* study is required, the murine local lymph node assay (LLNA; EU B.42./OECD TG 429) is the first-choice method for *in vivo* testing.

ECHA acknowledges your willingness to provide *in vitro* information on skin sensitisation (OECD TG 4211 C, E, D).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information on skin sensitisation derived with the registered substance subject to the present decision:

i. *in vitro/in chemico* information on molecular interactions with skin proteins, inflammatory response in keratinocytes and activation of dendritic cells (Annex VII, Section 8.3.1.) and/or
ii. local lymph node assay (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429) with the registered substance in case the *in vitro/in chemico* test methods specified under point i) are not applicable for the substance or the results obtained are not adequate for classification and risk assessment.

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

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid

down in Annex VII, Section 8.4.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

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 "*bacterial reverse mutation assay*" (according to OECD TG 471) with the analogue substance L-Glutamic acid, N-coco acyl derivs., monosodium salts (CAS No. 68187-32-6; EC No. 269-087-2). However, as explained above in Appendix 1, section Grouping of substances and read-across of this decision, your adaptation of the information requirement is rejected. Furthermore, there is an additional deficiency of the study itself, which makes it inadequate to fulfil the current information requirement:

You have provided a test from the year 1994 according to OECD TG 471 and GLP with an assigned reliability score of 2. The test used four different strains of S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 and it did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). However, since the test was conducted, significant changes have been made to OECD TG guideline 471 so that additionally testing with S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101) is now required. Therefore, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

According to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used: S. typhimurium TA1535; TA1537 or TA97a or TA97; TA98; TA100; S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). This includes four strains of S. typhimurium (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by *E.coli* WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site.

ECHA notes that a test using *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 has not been submitted and that the test using one of these is required to conclude on *in vitro* gene mutation in bacteria.

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

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

ECHA acknowledges your willingness to provide an *in vitro* test on gene mutation in bacteria (OECD TG 471).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the

present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471).

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

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

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 an "*in vitro mammalian chromosome aberration test*" (according to OECD TG 487) with the analogue substance L-Glutamic acid, N-coco acyl derivs., disodium salts (CAS No. 68187-30-4; EC No. 269-085-1). However, as explained above in Appendix 1, section Grouping of substances and read-across of this decision, your adaptation of the information requirement is rejected.

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

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

ECHA acknowledges your willingness to provide an *in vitro* micronucleus test (OECD TG 487).

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

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

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

Adequate information *on in vitro* gene mutation in mammalian cells will however need to be present in the technical dossier for the registered substance to meet this information requirement provided that both studies requested under 2 and 3 have negative results.

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 an "*in vitro mammalian cell gene mutation test*" (according to OECD TG 476) with the analogue substance L-Glutamic acid, N-coco acyl derivs., disodium salts (CAS No. 68187-30-4; EC No. 269-085-1). However, as

explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

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

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

ECHA acknowledges your willingness to provide an *in vitro* mouse lymphoma test (OECD TG 490).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that both studies requested under 2 and 3 have negative results.

5. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.)

A "sub-chronic toxicity study (90 day)" is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a "sub-chronic toxicity study" record with the analogue substance L-Glutamic acid, N-coco acyl derivs., disodium salts (CAS No. 68187-30-4; EC No. 269-085-1). However, as explained above in Appendix 1, section Grouping of substances and read-across of this decision, your adaptation of the information requirement is rejected. Furthermore, there is an additional deficiency of the study itself, which makes it inadequate to fulfil the current information requirement. Specifically, although the study design is reported poorly, it appears that the study deviates significantly from a sub-chronic toxicity study according to OECD 408 (e.g. only male animals included in the study, no haematological examinations, no histopathological examinations, no gross pathological examinations, only liver, kidneys, spleen, adrenals and testes were investigated, only relative organ weights are reported, and incomplete clinical chemical investigations). Hence, this study cannot, based on the present information, be used to fulfil the requirements of Annex XI, Section 1.5.

ECHA acknowledges your comments on the draft decision confirming that a sub-chronic toxicity study is a standard information requirement and that the provided study with the analogue substance "*show some weakness*". However, you have not provided any information that would address the deviations noted above. You indicated that "*the improvement of the read-across approach will allow using a 90-day oral toxicity study of the source substance, which will be proposed for the registration of CAS 68187-30-4*". However, you have not provided any information that would address the shortcomings of the read-across approach identified in the initial draft decision.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier currently does not meet the information requirement. The read-across to the source substance is rejected for the reasons explained above in Appendix 1, section Grouping of substances and read-across of this decision as well as the source study is inadequate to fulfil this endpoint, as explained above in the current section. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 5.0, December 2016) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. In addition, no adequate oral repeated dose toxicity study is available to evaluate systemic toxicity following oral administration. Hence, the test shall be performed by the oral route using the test method OECD TG 408.

According to the test method OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

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: Repeated dose 90-day oral toxicity study (test method: OECD TG 408) in rats.

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

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

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

While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex VIII, Section 8.7.1., column 2. You provided the following justification for the adaptation

"The available data do not indicate that the substance is of toxicological concern. A OECD 414 study has already been proposed to address the teratogenic potential of the substance. It is expected that no hazard will be observed. Dependent on the result of the OECD 414 study, the reproductive toxicity needs to be assessed once the study result is available. For animal welfare reasons, a study should be avoided."

ECHA acknowledges that you have submitted a testing proposal and proposed testing for developmental toxicity (OECD 414). ECHA also acknowledges your comments provided on the draft decision stating that "*the results from the Pre-natal development study (OECD TG 414) with the source substance will be available by November 2018 and will be used also for the registration of the target substance after enhancement of the read across approach.*" You conclude that "*The registrant does not agree with ECHA's request for a screening for reproductive / developmental toxicity (Annex VIII, section 8.7.1.) study. This endpoint will be waived based on the results from the sub-chronic and Pre-natal developmental toxicity study with the source substance.*" However, ECHA notes that currently your adaptation does not meet the specific rules for adaptation of Annex IX, Section 8.7.1., column 2 because no prenatal developmental toxicity study on the registered substance or on the acceptable analogue substance has currently been provided, and therefore, your adaptation of the information requirement is rejected. As explained in Appendix 1, section Grouping of substances and read-across of the testing proposal decision (TPE-D-2114471263-52-01/F), based on the currently provided information the read-across to the source substance is not acceptable. Therefore, a developmental toxicity study on the source substance, to which you refer to, will only provide information on such property of the source, but not of the target substance.

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

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

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 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.

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

Notes for your considerations

For the selection of the appropriate test, please consult ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.5 and 7.6 (version 6.0, July 2017).

The timeline of the current compliance check decision and the related testing proposal decision have been set to allow for sequential testing. You should carefully consider the order of testing of the requested screening (OECD TG 421/422) and the developmental toxicity studies (OECD TG 414) to ensure that unnecessary animal testing is avoided, paying particular attention to the endpoint specific guidance

(https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf) Section R.7.6.2.3.2., pages 484 to 485 of version 6.0 – July 2017.

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

"Growth inhibition study in aquatic plants" is a standard information requirement as laid down in Annex VII, Section 9.1.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided a study record for a study with the registered substance on species *Desmodesmus subspicatus* performed according to OECD test guideline 201 ([redacted], 2004). Nominal concentrations between 62.5 and 1000 mg/L were tested over a period of 72 hours. The 72-hour EC50 and the 72-hour EC10 reported in the dossier were based on nominal concentrations.

ECHA has analysed this study and considers it not valid and not adequate to meet the information requirement of this endpoint, as explained below.

The study record indicates that the test item could not be detected at the concentrations used for the calculations of these EC values. It is further indicated that the formation of a turbidity was observed during incubation.

If the test item could not be detected, this may indicate that the concentrations to which algae were actually exposed were much lower than the nominal concentrations. It may also indicate that the analytical method used for that study was not adequate.

ECHA notes that in the long-term study on *Daphnia* with the registered substance provided in your dossier ([redacted] 2013), significant losses of the test substance were observed. For example, recovery percentages lower than 5% were measured after 1 week for the nominal concentration of 0.75 mg/L. Therefore, the results of the long-term study on *Daphnia* suggest that nominal concentrations are inadequate to assess the aquatic toxicity of the registered substance. This study also demonstrates that the measurement of actual concentrations in the test medium is feasible provided that an adequate and sufficiently sensitive analytical method is used.

OECD test guideline 201 ("Freshwater Alga and Cyanobacteria, Growth Inhibition Test") recommends that test concentrations should be analysed at the beginning and at the end of the test if concentrations are unlikely to remain within 80-120% of nominal. For volatile, unstable or strongly adsorbing test substances, the test guideline further recommends that additional analyses should be performed at 24-hour intervals during the exposure period in order to better define loss of the test substance.

ECHA notes that the registered substance is surface active (surface tension of 36.1 mN/m at 20°C and 1 g/L). By nature, surface active substance will preferentially distribute at interfaces and consequently are generally adsorptive. Adsorption onto the test vessels is thus a possible reason for the substance losses observed in the long-term study on *Daphnia* and may have also occurred in the algae study.

Surface active substances also tend to form dispersions or emulsions. Micelle formation can result in an overestimation of the bioavailable fraction and therefore in an underestimation of the toxicity of the substance. The turbidity observed in the algae study may have actually been caused by the formation of an emulsion/dispersion.

Therefore, ECHA considers that the information provided for "growth inhibition study in aquatic plants" is not reliable and does not meet the information requirement of Annex VII, Section 9.1.2. of the REACH Regulation. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

Algae growth inhibition test (test method EU C.3. / OECD TG 201) is a validated standard international test laid down in the Test Methods Regulation (EC) No 440/2008 and therefore it meets the requirements of Article 13(3) of the REACH Regulation. According to ECHA ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017), it is the preferred test to cover the standard information requirement of Annex VII, Section 9.1.2. of the REACH Regulation.

As significant losses of the substance can be expected during the test, the results need to be based on measured concentrations. An adequate and sufficiently sensitive analytical method shall be used. ECHA notes that for example, for the long-term toxicity study on *Daphnia* ([redacted] 2013) high-performance liquid chromatography-mass spectrometry (HPLC-MS) was used, which allowed measurements of concentrations as low as 0.1 mg/L.

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: Algae growth inhibition test, EU C.3./OECD TG 201). The results shall be based on measured concentrations.

Notes for your consideration

Due to the substance being surface active you should consult 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 and for calculation and expression of the result of the test.

If based on the results of the required study algae is observed to be the most sensitive species, then the PNECs will have to be revised accordingly. Also, more severe classification and labelling of the substance may be warranted depending on the results of the algae study.

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

"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 IX, Section 9.1.6., column 2 of the REACH Regulation. You provided the following justification for the adaptation:

> *"In Annex IX, column II (9.1) of Regulation (EC) No 1907/2006, it is laid down that a long-term toxicity test shall be proposed by the registrant if the chemical safety assessment indicates the need to investigate further the effects on fish. The hazard assessment of Reaction mass of sodium hydrogen N-(1-oxooctadecyl)-L-glutamate and stearic acid reveals that fish is not the most sensitive organism. Therefore, and for reasons of animal welfare, a long-term toxicity test in fish is not provided".*

As explained below, and contrary to your own conclusion, ECHA considers that the CSA does indicate the need to investigate further long-term effects on fish

For your assessment, you have applied an assessment factor of 10 for deriving the Predicted No Effect Concentration (PNEC) for freshwater and of 100 for deriving the PNEC for marine water. According to the Guidance on information requirements and chemical safety assessment, Chapter R. 10 (ECHA, May 2008), an assessment factor of 10 for freshwater (100 for marine water) should normally be applied only when long-term toxicity results are available for at least three species across three trophic levels (e.g. typically fish, *Daphnia* and algae). It may sometimes be possible to determine with high probability that the most sensitive species has been examined, i.e. that a further long-term results from a different taxonomic group would not be lower than the data already available. In those circumstances, a factor of 10 (100 for marine water) applied to the lowest long-term result from only two species would also be appropriate. If it is not possible to make this judgment, then an assessment factor of 50 (500 for marine water) should be applied to take into account any interspecies variation in sensitivity.

ECHA notes that a long-term toxicity result for *Daphnia* is available for the registered substance ([redacted] 2013). An invalid result on algae is provided but a new study shall be performed (see section 7 of the present decision). No long-term study on fish is available. No short-term study is available on the registered substance. Short-term studies on a read-across substance indicate that the sensitivities of fish and *Daphnia* to the read-across substance are in the same order of magnitude (respectively 96h-EC50 of 62.4 mg/L for fish and 48h-EC50 of 49 mg/L for *Daphnia*). From the available information, ECHA considers that it is not possible to determine with high probability that the most sensitive species has been examined for the registered substance. Therefore, ECHA disagrees with the assessment factors applied in your dossier for deriving the PNECs for freshwater and for marine water.

ECHA notes that using instead an assessment factor of at least 50 (500 for marine water), for calculating the PNECs as recommended in Chapter R. 10 of the ECHA Guidance, risk characterisation ratios would exceed 1 for the aquatic compartment (freshwater and marine) in exposure scenario 2 ("Manufacture of cosmetic products") suggesting that risk are not adequately controlled for that use.

Therefore your adaptation does not meet the specific rules for adaptation of Annex IX, Section 9.1.6., column 2 of the REACH Regulation and is rejected. 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).
As explained in section 7 of the present decision, significant losses of the substance can be expected during the test. Therefore, the results need to be based on measured concentrations using an adequate analytical method.

In your comments following the procedure set out in Article 50(1) of the REACH Regulation you acknowledged that information on long-term toxicity on fish was needed but you disagreed that the test should be conducted according to OECD test guideline 210. You proposed instead to perform the test according to OECD test guideline 212. You claimed that animal testing was forbidden for substances exclusively used in cosmetic products and that OECD test guideline 212 was not a vertebrate animal test.

ECHA disagrees that the requested fish early-life stage (FELS) toxicity test (test method OECD TG 210) is forbidden. Recital 5 of the Cosmetics Regulation explicitly states that potential environmental concerns from the substances used in cosmetic products need to be considered through the application of the REACH Regulation. Animal testing for all environmental endpoints is permitted, as also further explained in ECHA's factsheet, (referred in section on 'Consideration on the uses of the substance'). .

With regard to your claim that an OECD 212 test is preferable for animal welfare reasons, ECHA considers this argument questionable. The OECD 212 test is conducted on larvae, but by the end of the test, the larvae do possess a nervous system. OECD test guideline 212 acknowledges that the larvae may be susceptible to stress, e.g. during water renewal operations. Similarly, the OECD fish toxicity testing framework[5] implicitly acknowledges that larvae used in an OECD 212 test could be subject to pain when it recommends that larvae with severe deformities should be terminated to avoid suffering. This test is performed without any external food supply. It should normally be stopped just before the yolk sac of any larvae has been completely absorbed or before mortality by starvation starts in the controls. However, the exact point at which this occurs may be ill defined in practice. The lack of feeding could be considered unacceptably distressful for the test organisms. As such this test, which is sometimes termed as the "fish starvation test"[6], is ethically problematic. Furthermore, an acute test with adult fish (e.g. according to OECD test guideline 203) is

[5] Fish Toxicity Testing Framework. OECD Series on Testing and Assessment, No. 171. ENV/JM/MONO(2012)16. https://www.oecd-ilibrary.org/environment/fish-toxicity-testing-framework_9789264221437-en
[6] E.g. see the OECD Fish Toxicity Testing Framework: https://www.oecd-ilibrary.org/environment/fish-toxicity-testing-framework_9789264221437-en

generally used as a range finding test. This would contradict your claim that no vertebrate animal would be used if an OECD 212 test were conducted.

ECHA further notes that OECD 212 is a short-term test during which exposure to the test substance starts from the newly fertilised egg to the end of the sac-fry stage. For OECD 210, exposure also starts from fertilised eggs but is continued until the juvenile life-stage is reached. As explained in the introduction of OECD test guideline 212, "*only tests incorporating all stages of the life-cycle of fish are generally liable to give an accurate estimate of the chronic toxicity of chemicals to fish, and* [...] *any reduced exposure with respect to life stages may reduce the sensitivity and thus underestimate the chronic toxicity".* For this reason, OECD 212 is considered insensitive relatively to OECD 210.

Finally, ECHA notes that OECD test guideline 212 allows for considerable variations in its design (e.g. number of test chambers, test concentrations, starting number of fertilised eggs). It is thus regarded as less reliable than an OECD 210 test.

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).). The results shall be based on measured concentrations.

Notes for your consideration

Due to the substance being surface active you should consult 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 and for calculation and expression of the result of the test.

If based on the results of the required study fish is observed to be the most sensitive species, then the PNECs will have to be revised accordingly. Also, more severe classification and labelling of the substance may be warranted depending on the results of the long-term study on fish.

9. Ready biodegradability (Annex VII, Section 9.2.1.1.)

"Ready biodegradability" is a standard information requirement as laid down in Annex VII, Section 9.2.1.1. 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 using a read-across approach (Annex XI, Section 1.5. of the REACH Regulation) by providing:

- a study record for a ready biodegradability, modified OECD screening test,.(OECD TG 301E) with analogue substance L-Glutamic acid, N-coco acyl derivs., monosodium salts (EC no 269-087-2) ([redacted] 1989),
- a study record for a ready biodegradability, CO_2 evolution test, (OECD TG 301B) with analogue substance stearic acid (EC no 200-313-4) ([redacted] 1984).

You provided the following justification for your read-across approach on the biodegradability of the substance:

> "[...] *Although the read across substance l-Glutamic acid, N-coco acyl derivs., monosodium salts contains components with slightly shorter carbon side chains compared to L-Glutamic acid, N-(1-oxooctadecyl)-, monosodium salt, the read across is considered valid, because i) the very rapid and almost full degradation of the analogue substance indicate that even the longer-chained components (C16) will be degraded easily and ii) the relevant physico-chemical properties (water solubility = high, log Kow < 1) are identical, which assume identical environmental behaviour. An increase of the length of the carbon chain moiety are generally considered to decrease the biodegradability of substances, because of their lower solubility. However, this is not the case for these alkyl glutamates, which are soluble even with an C18 alkyl moiety* [...]".

ECHA considers the information provided does not allow an adaptation of the information requirement of this endpoint as it does not meet the requirements of Annex XI, Section 1.5.as explained below.

Firstly, the study of [redacted] 1989 is not reliable.

In particular, ECHA notes that in this study a DOC (dissolved organic carbon) removal of 94.4% was observed after an incubation period of 28 days. However, it is worth noting that DOC removal measurement does not differentiate actual biological degradation from abiotic elimination processes such as adsorption or stripping into the air. Based on their structures (polar headgroup and hydrophobic alkyl chain), alkyl glutamates can be expected to be surface active and to adsorb onto solids (e.g. onto the inoculum and/or the test vessels).

You have reported a log Koc value of -0.423 in your dossier and you have concluded that the registered substance was not adsorptive. However, ECHA notes that this log Koc value was estimated with the KOCWIN v2.00 program from log Kow. Log Kow (predicted or measured) is a poor predictor of adsorption for surface-active substances.

ECHA further notes that using instead a model based on the Molecular Connectivity Index (MCI), also implemented in KOCWIN v2.00, a much higher log Koc value (5.062) is predicted for sodium hydrogen N-(1-oxooctadecyl)-L-glutamate, the main constituent of the registered substance. Using the MCI model as well, a log Koc value of 3.452 is predicted for N-(1-oxododecyl)-L-glutamic acid, the main constituent of the source substance, which was tested in the ready biodegradability study of [redacted] 1989.

Based on the above considerations, ECHA considers that the hypothesis that significant adsorption of the test material onto the inoculum and/or the test vessels has taken place cannot be ruled out in the study of [redacted] 1989. There is no indication whether abiotic controls were set up in this study (i.e. by sterilising the reaction mixture containing the test substance) to investigate the extent of removal from adsorption compared to biodegradation. Therefore, ECHA regards this study as unreliable to assess the actual biodegradability of the source substance, which in itself invalidates this study for your read-across approach.

Secondly, the analogue substance L-Glutamic acid, N-coco acyl derivs., monosodium salts (EC no 269-087-2) cannot be used to predict the biodegradability of the registered

substance. In your read-across justification document, you claim that the physico-chemical properties of the target and source substances are either similar or follow a regular pattern. The patterns observed then depend on the length of the alkyl chain. ECHA notes that your justification document does not provide evidence that the adsorption/desorption properties of the target and source substances are similar. On the contrary, QSAR predictions based on the MCI methodology suggest that adsorption increases with the length of the alkyl chain. Indeed, prediction for the registered substance is significantly higher than the predicted adsorption for source substance L-Glutamic acid, N-coco acyl derivs., monosodium salts (EC no 269-087-2). This suggests that the bioavailability and therefore the biodegradation rate for the registered substance might be less than for the source substance. This may indicate that the bioavailability and therefore the biodegradability decrease with the length of the alkyl chain. This is in contradiction with your claim that the biodegradability of the target substance will not be affected by the length of its alkyl chain (C18). Additionally, in your justification, you state that the water solubility is high and log Kow is low for both the target and source substances. However, water solubility and log Kow are not regarded as reliable predictors of adsorption for surface-active substances[7], and therefore should not be invoked to compare the bioavailability of the source and target substances.

Finally, ECHA notes that the study of [redacted] 1984 suggests rapid biodegradation of stearic acid. In the registered substance, stearic acid, constitutes the C18 alkyl chain (hydrophobic tail); it is covalently bound to the glutamate group (the latter constitutes the hydrophilic headgroup). ECHA considers stearic acid inadequate to assess the biodegradability of the registered substance. Indeed, the biodegradation of stearic acid considered individually does not automatically imply it will be biodegraded when covalently bound to the glutamate group. The literature on surfactants suggests that it is the cleavage of the bond between the hydrophilic headgroup and the hydrophobic tail, which very often drives the ultimate biodegradability of a surface active substance.

Therefore, it is crucial to assess the biodegradability of the substance as a whole, i.e. when the C18 alkyl chain is covalently bound to the glutamate group.

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.

Regarding the test method, depending on the substance profile, you may conclude on ready biodegradability, by applying the most appropriate and suitable test guideline among those listed in the ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) and in the paragraph below. The test guidelines include the description of their applicability domain.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to perform one of the following tests with the registered substance subject to the present decision:

[7] The critical micelle concentration (CMC) seems to be more appropriate to predict the bioavailability of surface-active substances, but this was not provided in your dossier.

- Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: CO_2 evolution test, OECD TG 301B), or

- Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: MITI test (I), OECD TG 301C), or
- Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Closed bottle test, OECD TG 301D), or

- Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Manometric respirometry test, OECD TG 301F), or

- Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Ready biodegradability – CO_2 in sealed vessels (headspace test), OECD TG 310) with the registered substance.

Notes for your consideration

ECHA notes that more severe classification and labelling of the substance may be warranted depending on the results of the requested ready biodegradability test.

Also, as explained in section A.16-3.2.2 in ECHA Guidance on information requirements and Chemical Safety Assessment, Chapter R.16: Environmental exposure assessment (Version 3.0, February 2016), the results of ready biodegradability tests should be used by default to extrapolate degradation rates in the environment and in sewage treatment plant (STP) if no more specific information is available.

Deadline to submit the requested information in this decision

The deadline to submit the requested information in this decision is set to allow for sequential testing together with the information requests made under the testing proposal decision which was simultaneously notified to you.

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 20 June 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 proposals for amendment.

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

Appendix 3: Further information, observations and technical guidance

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

2. Failure to comply with the 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