EUROPEAN CHEMICALS AGENCY

Helsinki, 10 July 2018

Addressee: [redacted]

Decision number: CCH-D-2114436051-64-01/F
Substance name: Butanoic acid, 4-amino-4-oxo-2(or 3)-sulfo-,N-(C16-C18 (even numbered), C18 unsaturated alkyl)), disodium salts
List number: 939-691-7
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 28/05/2013
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. **Classification and labelling (Annex VI, Section 4.): Apply classification and labelling on the registered substance for reproductive toxicity (Repr. 1B or 2) *or* provide a justification for not classifying.**

2. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance;**

3. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat), oral route with the registered substance;**

4. **Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity); and**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI to the REACH Regulation. To ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective annex, and adequate and reliable documentation.
You are required to submit the requested information in an updated registration dossier by ***17 January 2022*** except for the information requested under points 1. classification and

labelling and 2. for a sub-chronic toxicity study (90-day) , which shall be submitted in an updated registration dossier by **17 July 2019**. You may only commence the extended one-generation reproductive toxicity study as requested under point 4. After **17 October 2019**, unless an indication to the contrary is communicated to you by ECHA before that date. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2 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 **Claudio Carlon**, Head of Unit, Evaluation **E2**

[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

TOXICOLOGICAL INFORMATION

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.

Your registration dossier contains for multiple endpoints adaptation arguments in the form of a grouping and read-across approach according to Annex XI, 1.5. of the REACH Regulation. ECHA has assessed first the scientific and regulatory validity of your Grouping and read-across approach in general before the individual endpoints (sections 8.6.2., 8.7.2. and 8.7.3.).

Grouping and read-across approach for toxicological information

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

- sub-chronic toxicity (90-day) study (Annex IX, Section 8.6.2.) with existing information from a proposed source substance Butanoic acid, 4-amino-4-oxosulfo-,N-tallow alkyl derivs., disodium salts (CAS no. 68988-69-2)
- pre-natal developmental toxicity (PNDT) study (Annex IX, Section 8.7.2.) with existing information from the following proposed source substances:
    - Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate (CAS no. 577-11-7)
    - calcium bis{1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate} (CAS no. 128-49-4)
- Extended One-Generation Reproductive Study (EOGRTS) (Annex IX, Section 8.7.3.) with existing information from a proposed source substance Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate (CAS no. 577-11-7)

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

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances[2].

This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into

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

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

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

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

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

You consider to achieve compliance with the REACH information requirements for the registered substance Butanoic acid, 4-amino-4-oxo-2(or 3)-sulfo-,N-(C16-C18 (even numbered), C18 unsaturated alkyl)), disodium salts (EC no. 939-691-7) using data of structurally similar substances Butanoic acid, 4-amino-4-oxosulfo-,N-tallow alkyl derivs., disodium salts (CAS no. 68988-69-2), calcium bis{1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate} (CAS no. 128-49-4) and Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate (CAS no. 577-11-7) (hereafter the 'source substances').

You have provided a read-across documentation as two separate attachments in the registration dossier.

You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group: on the basis of structural similarity, similarity in physico-chemical, ecotoxicological and toxicological (including kinetic/metabolic) properties in certain endpoints, it is possible to predict the human health properties of the registered substance for other endpoints.

As an integral part of this prediction, you propose that the source and registered substance(s) have similar properties for the above-mentioned information requirements. ECHA considers that this information is your read-across hypothesis.

ECHA's evaluation and conclusion

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

Your proposed adaptation argument is that the similarity in chemical structure and in some of the physico-chemical/ toxicological properties between the source and registered substance is a sufficient basis for predicting the properties of the registered substance for other endpoints. Structural similarity is a prerequisite for applying the grouping and read-across approach. However similarity in chemical structure and similarity of some of the physico-chemical/ ecotoxicological/ toxicological properties does not necessarily lead to predictable or similar human health properties in other endpoints. Your justification based on structural similarity, similar physico-chemical, ecotoxicological and toxicological properties has not established why the prediction is reliable for the human health end-points for which the read-across is claimed.

You have also claimed that there is kinetic/ metabolic similarity between substances. However, ECHA notes that this statement is not substantiated by sufficient data: toxicokinetic data are available only for CAS No. 577-11-7 from the di-ester subgroup but not for any other subgroup to enable a comparison, and the impact of the structural differences on metabolism was not discussed. So it is not possible to conclude whether there is kinetic/ metabolic similarity in the absence of comparable data. Therefore, it is not possible to conclude that the toxicological properties of the target could be predicted from the data obtained with this source substance on the basis of kinetic/ metabolic similarity.

Additionally, regarding the similarities in toxicological properties, ECHA notes that there is no single study providing information equivalent to the information requirements in question for the target and the N1-subgroup source substances - to which the target substance belongs to. For the di-ester subgroup source substances reproductive and developmental toxicity studies with limitations are available. However, in addition to insufficient information, you have not provided a scientific justification why and how information from the di-ester subgroup source substances could be used to predict the missing information. Therefore, the limited toxicological information available with the target substance and the N1-subgroup source substance does not allow prediction of the toxicological properties of the target substance. As a consequence, it is not possible to conclude that there is "*toxicological similarity between subgroups*". There are specific considerations for the individual endpoints which also result in a failure to meet the requirement of Annex XI, Section 1.5., and these are set out under the endpoint concerned.

Additionally, ECHA has taken into account all of your arguments together. ECHA firstly notes that you have not provided a reasoning as to why these arguments add to one another to provide sufficient basis for read-across. Secondly, the defects of each individual argument are not mitigated by the other arguments you have provided, and so ECHA considers that the arguments when taken all together do not provide a reliable basis for predicting the properties of the registered substance.

Therefore, 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 substances 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

As described above, further elements are needed to establish a reliable prediction for a toxicological or ecotoxicological property, based on recognition of the structural similarities and differences between the source and registered substances. This could be achieved (if it is possible) by a well-founded hypothesis of (bio)transformation to a common compound(s),

or that the registered and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties.

ECHA notes that in your comments on the draft decision you indicated that the read across approach will not be required any longer for the endpoints: the sub-chronic repeated dose toxicity (90-day) study and the pre-natal developmental toxicity study, since you agreed to perform these studies with the registered substance.

1. Classification and labelling (Annex VI, Section 4.): Apply classification and labelling on the registered substance for reproductive toxicity (Repr. 1B or 2) *or* provide a justification for not classifying

Pursuant to Article 10(a)(iv) of the REACH Regulation your technical dossier shall contain information on classification and labelling of the substance as specified in Annex VI, Section 4 of the REACH Regulation in conjunction with Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation).

Annex VI, section 4.1. clarifies that the hazard classification of the substance shall result from the application of Title I and II of the CLP Regulation. In addition, for each entry, the scientifically justified reasons why no classification is given for a hazard class or differentiation of a hazard class should be provided. According to Article 5(1) of Title I of the CLP Regulation, a substance shall be classified on the basis of available information.

Furthermore, the technical dossier must include the resulting hazard label for the substance in line with Title III of the CLP Regulation (Annex VI, section 4.2 of the REACH Regulation).

For reproductive toxicity, you have provided a combined repeated dose and reproduction / development screening study (OECD TG 422) in rats, with the following findings:

i. At the high dose group (300 mg/kg bw/day):
- *"The pre-implantation loss index was statistically significantly increased to 33.6% per dam"* (i.e. +19.6% when compared to the control group (14%))
- *"The post-implantation loss index was statistically significantly increased to a mean value of 68.0% per dam"* (i.e. +56% when compared to the control group (12%)).

You also report that *"Correlating, the gestation index, the birth index and the mean number of pups per dam were statistically significantly reduced in the high dose group."* However, you conclude that these reductions are *"considered secondary to the maternal/paternal toxicity at 300 mg/kg bw/day".*

You have not self-classified the substance for reproductive toxicity despite the concerns in relation with reproductive toxicity observed in the screening study and in the justification for non-classification you only claim that *"the test substance does not have to be classified and has no obligatory labelling requirement for reproductive and developmental toxicity."* According to Annex I, Section 3.7. of the CLP Regulation, classification of the substance as reproductive toxicant, Category 1B is indicated when there is clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

ECHA notes that the effects observed in the screening study indicate adverse effects on sexual function and fertility. In both the mid- and high-dose groups, body weight reduction was observed in the parental generation and their food intake was also affected. However, you have not justified why the effects on sexual function and fertility can be dismissed as "secondary to the maternal/toxicity at 300 mg/kg bw/day." ECHA notes that although statistically significant body weight reductions (up to 13 % in males to test day 36 and up 9.2% in females on GD 20 in the high dose group) were noticed, they do not automatically account for the observed fertility effects. Losses of >20% of maternal body weight were reported to have no effect on pup loss[4] . Furthermore, there were no additional gross pathological and histopathological findings in the parental animals.

In your comments on the draft decision you indicate that the "*observed effects in the F1-generation of the OECD 422 are considered to be secondary*". Moreover, with reference to the comments provided under the extended one-generation reproductive toxicity endpoint section, you claim that "*decreases in body weight and food consumption of 10% or more are considered as substantial*". As already noted above, under this section, ECHA notes that it has been shown that maternal body weights reduction by 10 to 20% did not affect reproductive function[5].

Hence, ECHA considers that there is insufficient evidence to conclude that the concerns observed are only a result of the "*maternal/paternal toxicity*".

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to classify and label the registered substance taking into account the information above, as Repr. 1B or 2. In the alternative, you are requested to provide the scientifically justified reasons why no such classification is considered.

Notes for your consideration

In section 4 of this decision ECHA has requested you to perform an extended one generation reproductive toxicity study. ECHA reminds you that according to Annex IX, Section 8.7., column 2, if the substance is "*known to have an adverse effect on fertility, meeting the criteria for classification as toxic for reproduction category 1A or 1B: May damage fertility (H360F), and the available data are adequate to support a robust risk assessment, then no further testing for fertility will be necessary. However, testing for developmental toxicity must be considered*".

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

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.

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.

In the technical dossier you have provided the following study records:

i. Key study: Combined repeated dose and reproduction / development screening

[4] Carney E., Marty S., Crissman J., Anderson P., Woolhiser M and Holsapple M. "The effects of feed restriction during in utero and postnatal development in CD rats". TOXICOLOGICAL SCIENCES 82, 237–249 (2004)

study ([redacted] 2013) with the registered substance in rats, via oral route (gavage), rel. 1, according to OECD TG 422, GLP compliant;

ii. Key study: Sub-chronic (90-day) study ([redacted] 1976) in rats, via the oral route with the analogue substance, butanoic acid, 4-amino-4-oxosulfo-,N-tallow alkylderivs., disodium salts (CAS no. 68988-69-2), equivalent to OECD TG 408, non-GLP, rel. 2;

iii. Supporting study: Sub-acute (30-day) study ([redacted] 1956) in rats, via the oral route with the analogue substance, butanoic acid, 4-amino-4-oxosulfo-,N-tallow alkylderivs., disodium salts (CAS no. 68988-69-2), equivalent to OECD TG 407, non-GLP, rel. 2;

iv. Supporting study: 14-day dose range-finding study for the combined repeated dose and reproduction / development screening study ([redacted] 2013) with the registered substance in rats, via oral route (gavage), rel. 1, equivalent to OECD TG 422, GLP compliant; and

v. Supporting study: Sub-chronic (90-day) study (Tegeris & Underwood, 1976) in dogs, via the oral route with the analogue substance, butanoic acid, 4-amino-4-oxosulfo-,N-tallow alkylderivs., disodium salts (CAS no. 68988-69-2), according to OECD TG 409, non-GLP, rel. 3.

However, these studies do not provide the information required by Annex IX, Section 8.6.2., as explained hereunder.

ECHA notes that you have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing three study records, (ii.), (iii.) and (v.), with the analogue substance butanoic acid, 4-amino-4-oxosulfo-,N-tallow alkylderivs., disodium salts (CAS no. 68988-69-2). However, as explained above, in the *Grouping and read-across approach for toxicological information* section of this decision, your adaptation of the information requirement is rejected. Moreover, ECHA notes that there are shortcomings on the individual studies with the analogue substances which have not been addressed in your technical dossier, such as poor quality (Klimisch reliability 3 for study v.) (failure to list organs subject to histopathological examination and hence a failure to produce adequate and reliable documentation for study ii.) and shorter exposure duration for study iii. (i.e. failure to cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter).

You have also provided the screening study (study i. above) and the 14-day dose range-finding study (study iv. above), with the registered substance, as key and supporting studies, respectively. However, ECHA notes that these studies do not provide the information required by Annex IX, Section 8.6.2., because the exposure duration is less than 90 days and the number of animals examined per dose group for histopathology and clinical chemistry is significantly lower than in the 90 day sub-chronic toxicity study (OECD TG 408).

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 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 6.0, July 2017) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is reported to occur as a soluble powder with no significant proportion (>1%

on weight basis) of particles of inhalable size (MMAD < 50 µm). The substance has a low vapour pressure (≤0.0037 Pa) and there are no spray applications. Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.

According to the test method EU B.26./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: EU B.26./OECD TG 408) in rats.

ECHA notes that in your comments on the draft decision you agreed to perform the requested study with the registered substance.

Notes for your consideration

ECHA notes that a revised version of OECD TG 408 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

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

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.

A "pre-natal developmental toxicity study" (test method EU B.31./OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. 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 the following study records:

i. Key study: Pre-natal developmental toxicity study in rats via the oral route (feed) (equivalent to OECD TG 414; non-GLP; rel. 2) with the analogue substance Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate (CAS no. 577-11-7); and
ii. Supporting study: Pre-natal developmental toxicity study in rats via the oral route (feed) (equivalent to OECD TG 414; non-GLP; rel. 2) with the analogue substance calcium bis{1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate} (CAS no. 128-49-4).

However, as explained above, in the *Grouping and read-across approach for toxicological information* section of this decision, your adaptation of the information requirement is rejected.

Additionally, ECHA notes that both studies (i. and ii above) fail to provide adequate and reliable documentation (as required by Annex XI, 1.5) and have important shortcomings.

More specifically, in study (i.) there is missing information on the study design, including details on the analytical verification of the doses, details on mating procedure and frequency of treatment. Moreover, only two dose levels were tested. There is also a lack of information on the results concerning the general toxicity of the maternal animals, including data on clinical signs, mortality, body weight and weight changes, ophthalmological findings (if tested), haematology, histopathology, and organ weight findings including organ / body weight ratios. As regards, study (ii.) there is missing data concerning the study design and the results on the general toxicity of the maternal animals. Hence, the data provided from these two studies cannot be considered to be equivalent to the data generated by the corresponding test methods referred to in Article 13(3) (Annex XI, section 1.1.2.(4)).

In the technical dossier, as another supporting study, you have provided a study record for a "combined repeated dose toxicity study with the reproduction/developmental toxicity screening test" (test method: OECD TG 422) with the registered substance (Hansen, 2013). However, this study does not provide the information required by Annex IX, Section 8.7.2., because it does not cover key parameters of a pre-natal developmental toxicity study like examinations of foetuses for skeletal and visceral alterations. Therefore, your adaptation of the information requirement is rejected.

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

According to the test method EU B.31./OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. Following a proposal for amendment (PfA) made by one of the Member State Competent Authorities (MSCAs) it was noted that there were effects seen in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) performed in the Crl:CD(SD) rat with the registered substance (Hansen, 2013), such as an increased incidence of post-implantation loss. These effects demonstrate a concern for developmental toxicity in the rat species. According to ECHA's guidance document[5] "*the most sensitive species and/or strain should be used as a first species*". In this case ECHA considers that the effects seen in Crl:CD(SD) rats in the OECD TG 422 study demonstrate that the rat is a sensitive species, and that in the absence of information on susceptibility to developmental toxicity of other species, the rat is the most sensitive species. ECHA therefore concludes that the testing should be performed with rats as a first species. Furthermore, ECHA considers that the Crl:CD(SD) strain of rat is known to be sensitive, and therefore ECHA recommends that the study should be performed in Crl:CD(SD) rat.

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 soluble powder, 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: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a first species (rat) by the oral route.

[5] ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6.2.3.2 (version 6.0, July 2017).

ECHA notes that in your comments on the draft decision you agreed to perform the requested study with the registered substance.

Notes for your consideration

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

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

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.

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex IX of the REACH Regulation, if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD TGs 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. If the conditions described in column 2 of Annex IX are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

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

a) The information requirement

ECHA considers that concerns in relation with reproductive toxicity are observed. More specifically, adverse effects were observed in the findings of the 'combined repeated dose and reproduction / development screening study' (OECD TG 422) conducted with the registered substance (Hansen, 2013), namely the following findings:

i. At the high dose group (300 mg/kg bw/day):
- *"The pre-implantation loss index was statistically significantly increased to 33.6% per dam"* (i.e. +19.6% when compared to the control group (14%))
- *"The post-implantation loss index was statistically significantly increased to a mean value of 68.0% per dam"* (i.e. +56% when compared to the control group (12%)).

Pursuant to Annex IX, Section 8.7.3. an extended one-generation reproductive toxicity study is thus an information requirement for registrations of the registered substance.

However, you have not provided any study record of an extended one-generation

reproductive toxicity study in the dossier that would meet the information requirement of Annex IX, Section 8.7.3.

In the technical dossier you have provided the following study records:

i. Key study: Combined repeated dose and reproduction / development screening study ([redacted] 2013) with the registered substance in rats, via oral route (gavage), rel. 1, follows OECD TG 422, GLP compliant;
ii. Key study: Three generation reproductive toxicity study ([redacted] 1986) with the analogue substance, Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate, (CAS no 577-11-7), in rats via oral route (feed), rel. 2, equivalent to OECD TG 416, GLP compliant; and
iii. Supporting study: Two-generation reproductive toxicity study report ([redacted] 1970) with the analogue substance, Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate, (CAS no 577-11-7), in rats via the oral route (feed), rel. 2, equivalent to OECD TG 416, no information on GLP.

However, these studies (i. to iii.) do not provide the information required by Annex IX, Section 8.7.3., as explained hereunder.

With reference to study (i) ECHA notes that the reproduction/developmental toxicity screening study (OECD TG 422) is a standard information requirement at REACH Annex VIII level (section 8.7.1.). As indicated above, due to the adverse effects observed in this screening study, the extended one-generation reproductive toxicity study has been triggered, according to Annex IX, Section 8.7.3. Hence, since the test substance has been registered at Annex IX, the screening study (OECD TG 422) itself does not fulfil the information requirement of Annex IX, section 8.7.3.

As regards studies (ii) and (iii) ECHA notes that you have also sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing these two studies with the analogue substance, Butanedioic acid, sulfo-, 1,4-bis (2-ethylhexyl) ester, sodium salt; sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonate, (CAS no 577-11-7). However, as explained above, in the *Grouping and read-across approach for toxicological information* section of this decision, your adaptation of the information requirement is rejected.

Moreover, ECHA notes that in these studies (ii.) and (iii.) there is no adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods of Article 13(3) (Annex XI, section 1.1.2. (2)). More specifically, the two studies fail to provide information on sperm investigation, oestrous cycle, organ weights, haematology and histopathology and extensive investigations in F1 generation. Additionally in study (iii) only two dose levels plus control were tested and there were only 16 mating pairs per dose group; according to OECD TG 443 three dose levels plus control should be tested and sufficient mating pairs to produce 20 pregnant animals should be present per dose group.

Therefore, your adaptation of the information requirement is rejected.

In the comments to the draft decision you claim that the high dose "*reached excessive with (sub)lethal toxicity*" and that the surviving animals had "*scarce but severe*" effects. You state that "*Mortality was observed both in the male and female high dose group of 300 mg/kg bw*". With regards to the effects noted, you claim that body weight loss is an

"*important signal of substantial toxicity*". You also state that other effects such as "*reduction in food consumption*", "*salivation, piloerection and breathing sounds*" are "*symptoms of bad condition*".
ECHA notes that according to the OECD TG 422 "*the highest dose level should be chosen with the aim of inducing toxic effects but not death nor obvious suffering*."

However, ECHA notes that the effects mentioned are merely indicators of general toxicity. Furthermore, you stated in the dossier for the clinical signs that there were "*slight signs of toxicity in a few animals of the high dose group in form of pilo-erection and increased salivation*". ECHA also notes that in the dossier and the original report these signs are considered "slight" and in the comments on the draft decision are considered as "severe": "*Clinical observations in surviving animals of the high dose were indeed scarce but severe, i.e. (reddish) salivation, piloerection and breathing sounds, which can be considered as symptoms of bad condition*". ECHA further notes that e.g. breathing sounds were noted in the high dose in one male (no. 66) and one female (no. 77) only for 2 days during the mating period, and piloerection was noted for one female during pre-mating period and for another female during the gestation period on one or two test days. Therefore, these effects cannot be generalized for all the high dose animals.

You also bring statistically significant reductions in body weight both in males (7-13% mean reduction) and females (9-11% mean reduction in gestation-lactation) and statistically significant reduction in food consumption up to 11.0% in males and females (during gestation) as arguments for a severe toxicity in parental animals at the highest dose. Nevertheless, it has been shown that in general, body weight differences of 10–15% by themselves in female rats were not adverse to normal reproduction (Birth Defects Res B 74:431–441, 2005). You did not demonstrate a specific mechanism confirming that the reproductive effects are due to reduced food consumption and body weights.
The statistically significant hematological changes are indicative of an inflammatory response but you did not show the association between such a response and the reproductive effects observed.

Even though you only mention the mortality in the high dose group as an indication of a steep dose-response curve, ECHA notes that in the OECD TG 422 study provided in the dossier four animals died prematurely: there were two mortalities noted in the highest dose group and additionally two in the intermediate dose group (one male and one female in each dose group). Furthermore, three of the animals, which died, had no clinical signs of toxicity prior death; only the one female from the intermediate group showed piloerection and an anus soiled with faeces one or two days before death.

ECHA also notes that you are proposing to confirm/exclude the reproductive concern by conducting a modified 90-day study, "*by repeating the OECD 422 study [...] with extended pre-mating exposure period to 90 days (13 weeks) before mating them*" and with "*complete 90-day toxicity histopathology*" examinations.

Firstly, ECHA notes that the provided screening study is a new (2013), GLP study performed in accordance with OECD TG 422 and hence, the study can be considered as being valid and a repetition of the study is not justified. The request for an extended one-generation reproductive toxicity study is based on this existing information showing concerns in relation with reproductive toxicity.

ECHA further notes that a screening study with extended premating exposure duration combined with 90-day study parameters would not fulfill the standard information

requirement of an extended one-generation reproductive toxicity study according to Annex IX section 8.7.3. More specifically the 90-day/screening study will not assess important key parameters addressed under EOGRTS, such as in-depth investigations in F1 generation: sexual maturation, haematology and clinical chemistry. Moreover there would be insufficient mating pairs since according to OECD TG 422 only 10 mating pairs per dose group are required while for OECD TG 443 sufficient mating pairs are required to produce 20 pregnant animals per dose group. ECHA also notes that the 90-day study requested in the decision may provide additional information that may trigger further investigations under the EOGRT study design.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according to Annex IX, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the required study

Information from studies to be conducted before the extended one-generation reproductive toxicity study

The sub-chronic toxicity study shall be conducted before the extended one-generation reproductive toxicity study and the results from that study shall be used, among other relevant information, to decide on the study design of the extended one-generation reproductive toxicity study following ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).The sub-chronic toxicity study may provide information on effects that is relevant for triggers (e.g. weight changes and histopathological observations of organs as indication(s) of one or more modes of action related to endocrine disruption which may meet the toxicity-trigger for extension of Cohort 1B or as evidence of specific mechanism/modes of action and/or neurotoxicity and/or immunotoxicity which may meet the particular concern criteria for developmental neurotoxicity and/or developmental immunotoxicity cohorts).

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017), the starting point for deciding on the length of the premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).
The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that

results from a range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Species and route selection

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

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

c) Outcome

Based on the available information, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

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

Currently, the extension of Cohort 1B and the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) are not requested. However, the sub-chronic toxicity study (90-day) requested in this decision and/or any other relevant information may trigger changes in the study design. Therefore, the sub-chronic toxicity study (90-day) is to be conducted first and the study results submitted to ECHA in a dossier update by the 12-month deadline indicated in this decision. If, on the basis of this update and/or other relevant information, a need for changes to the study design is identified, ECHA will inform you within three months after expiry of the 12-month deadline to provide the sub-chronic toxicity study (90-day)), as indicated in this decision, of its intention to initiate a new decision making procedure under Articles 41, 50 and 51 of the REACH Regulation to address the design of the extended one-generation reproductive toxicity study.

If you do not receive a communication from ECHA by the expiry of three months following the 12-month deadline for providing the results of the sub-chronic toxicity study (90-day), the request of the present decision for the extended one-generation reproductive toxicity study remains effective and you may commence the conduct of the study and the results will need to be submitted by the deadline given in this decision.

Notes for your consideration

When submitting the study results of the sub-chronic toxicity study (90-day) you are invited

to also include in the registration update your considerations whether changes in the study design are needed (see also ECHA Guidance on information requirements and chemical safety assessment Chapter R.7a, Section R.7.6 (version 6.0, July 2017)).

Furthermore, after having commenced the extended one-generation reproduction toxicity study in accordance with the ECHA decision, you may also expand this study to address a concern identified during the conduct of it and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the changes in the study design must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/ triggers must be documented.

Appendix 2: Procedural history

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

The compliance check was initiated on 13 September 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 amended the request(s).

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-60 written procedure and ECHA took the decision according to Article 51(6) 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.