EUROPEAN CHEMICALS AGENCY

Helsinki, 18 May 2018

Addressee: [redacted]

Decision number: CCH-D-2114408192-60-01/F
Substance name: 2-amino-2-methylpropanol
EC number: 204-709-8
CAS number: 124-68-5
Registration number: [redacted]
Submission number: [redacted]
Submission date: 27/09/2012
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

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

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

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

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

4. **Classification and labelling (Annex VI, Section 4.):**
   - apply classification and labelling on the registered substance as STOT RE 2 (target organ liver) for repeated dose toxicity or provide a justification for not classifying.

You have to submit the requested information in an updated registration dossier by **25 November 2020**. 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 **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 more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Your registration dossier contains for the endpoints pre-natal developmental toxicity and reproductive toxicity *(Annex IX, 8.7.2, Annex X, 8.7.2. and 8.7.3.)* adaptation arguments in form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual endpoints (sections reproductive toxicity and developmental toxicity).

Grouping of substances and read-across approach for toxicological information

You have sought to adapt the information requirements for reproductive toxicity and pre-natal developmental toxicity *(Annex X, 8.7.2. and 8.7.3.)* by applying a read-across approach in accordance with Annex XI, Section 1.5. 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 aspects the chemical structures have in common and the differences between the structures of 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 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.

[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.

Thus physicochemical properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical 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) and (2) Different compounds have the same type of effect(s).

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

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

You seek to adapt the human health information requirements for reproductive toxicity and pre-natal developmental toxicity *(Annex IX, 8.7.2, Annex X, 8.7.2. and 8.7.3.)* by applying a read-across approach according to Annex XI, Section 1.5.

You propose read-across between the structurally similar substance, 4,4-dimethyl-1,3-oxazolidine (CAS: 51200-87-4 EC:257-048-2) as source substance and the substance subject to this decision, 2-amino-2-methylpropanol (AMP, EC 257-048-2, CAS No 124-68-5) as target substance.

You use the following arguments to support the prediction of properties of the target substance from data for reference substance(s) within the group by interpolation to other substances in the group: "

[redacted] *2007).* [redacted] ."

"

[redacted] (

2007). [redacted] ."

According to you the source and target substances have similar properties for the above-mentioned information requirements.

ECHA considers that this information is your read-across hypothesis, which provides the basis whereby you predict the properties of the target substance from the source substance.

B. ECHA analysis of the grouping and read-across approach

You have proposed that the properties of the target substance can be predicted from the properties of the source substance, on the basis that the source substance degrades to the target substance by arguing the following: "*oral dosing leads to systemic exposure to AMP*

[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).

(and formaldehyde to a lesser extent)" and that "hydrolysis is almost immediate and results in systemic exposure to AMP predominantly".

While ECHA considers it plausible that 4,4-dimethyl-1,3-oxazolidine hydrolyses to the target substance and formaldehyde , especially in acidic conditions, you have not provided toxicokinetic or other evidence from which it would be possible to deduce the speed of the hydrolysis and whether there is systemic exposure to the source substance. That is, you have not provided any experimental information, or other adequate and reliable information about the speed of hydrolysis of the source substance *in vivo*, and information about the bioavailability of AMP after dosing of 4,4-dimethyl-1,3-oxazolidine for the purpose of showing that there is quantitatively similar bioavailability of AMP.

ECHA notes that you have provided summary information in the 'Executive summary' of the key study for toxicokinetics, but that you have not provided an endpoint study record for this information, and ECHA is accordingly unable to independently evaluate this information. Without the study record addressing this issue, it is not possible to reliably and quantitatively predict the formation of the common product (the registered substance) and its bioavailability. Therefore, ECHA is unable to evaluate whether only the target substance will be present in the blood or if there will be systemic exposure also to the source substance (the read-across substance). Consequently, your read-across hypothesis does not provide a basis for predicting the properties of the target substance.

In addition, your read-across hypothesis does not take into account the potential effects of exposure to the source substance (as its parent compound) or the other break down product, formaldehyde, and whether these effects could confound the prediction for the target substance. For this reason also, your read-across hypothesis does not provide a basis for predicting the properties of the target substance.

Moreover, there appears to be substantial differences in the potency and toxicity arising from exposure to the target and source substances. For example, in the prenatal developmental toxicity range finding study ([redacted], 1989) where SD-rats were given 0, 250, 500, 750, 1000 and 1500 mg/kg of the source substance from GD 6-15 by oral gavage "*All animals given 750 mg/kg/day or higher died between GD 6 and GD 9. One animal given 500 mg/kg/day died on GD 14.*" whereas with the target substance in a 5-day oral gavage study on Long-Evans rat, 2/5 females died after 1000 mg/kg/day (all males and females died at higher doses), with no females dying at 500 mg/kg ([redacted], 1977).

This data shows that there are differences in potency and toxicity of the substances, and does not demonstrate that the human health properties of the target substance can be predicted from the properties of the source substance.

Moreover, the dose levels (and exposure to AMP) that are acheivable in rats are markedly different for the target and source substances. For example, the OECD 421 study in CD rats ([redacted], 2005) uses dietary administration of up to 1000 mg/kg/day of AMP. After adjusting the pH to 7, AMP can be dosed by oral gavage in rat at doses over 1g/kg without mortality in a 90-day study ([redacted], 1977). By contrast, the two-generation study with source substance in CD rats by oral gavage ([redacted], 2008) uses a maximum dose of 200 mg/kg/day (consistent with the results of the developmental toxicity range-finding study), and the availability of AMP will be correspondingly lower based on the molecular formula. Therefore, the proposed read across source substance 4,4-dimethyl-1,3-oxazolidine is more toxic in repeated dosing, and cannot be administered at equivalent doses to the target substance. In view of the lower systemic doses of AMP achieved after dosing with the

source substance, it would not be possible to predict the properties of AMP which could be dosed at a higher dose levels. Both these issues mean that it is not possible to predict the properties of the target substance from the source subtance.

Therefore, ECHA considers that there is not a reliable basis for predicting the properties of the target substance "from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach)".

In your comments on the draft decision you provided a copy of the toxicokinetics study by [redacted] (2007), and ECHA notes that that indeed the source substance is rapidly hydrolysed to the target substance and formaldehyde. You have also provided a descriptive comparison of the toxicity data for the human health endpoints for the source and target substances. However, ECHA notes that you did not consider how the substantial differences in the potency and toxicity, observed with the two substances (as highlighted above in draft decision) can affect the read-across to the target substance. As regards the issue of different systemic dose levels achieved for the target and source susbtance, you state that both substances are "*severe irritants*" which limit the amount of the systemic concentrations that can be "*humanely administered to test animals*". However, you do not provide any additional justification how to use the more toxic source substance to predict the properties of the target substance, which could be dosed at higher dose levels. Moreover, you do not provide any information on whether the toxicity profile of the target substance could be influenced by the presence of formaldehyde (i.e. the other metabolite formed from the breakdown of the source substance).

C. Conclusion on the grouping and read-across approach

For the reasons as set out above, ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the human health effects of the target 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 result in a failure to meet the requirement of Annex XI, 1.5, and these are set out under the endpoint concerned.

As described above, further elements are needed to establish a reliable prediction for toxicological properties, based on recognition of the structural aspects the chemical structures have in common and the differences between the structures of 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 target and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties.

1. 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 more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

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.

In the technical dossier you have provided a study record for an OECD 414 in rats by the dermal route ([redacted] 2006), together with the range-finding study. However, this study does not provide the information required by Annex IX, Section 8.7.2. for the following reasons.

You have provided toxicokinetics studies from which you conclude: "[redacted]."

In the study you have provided, rats were dosed with 0, 30, 100, 300 mg/kg/day dermally. You arrived at this dosing through a dose-range finding study, and you noted that dermal dosing was limited by the occurrence of local dermal toxicity which precluded higher doses. However, it is evident that only local (dermal) effects occur and that there are no systemic effects after dermal dosing. By contrast, in the oral 90-day study in rats (1977), doses of 1100 mg/kg/day could be dosed by oral gavage. In view of the higher systemic bioavailability of registered substance after oral administration, in combination with the higher doses possible by the oral route, it is possible to achieve much higher systemic concentrations and exposure of the registered substance after oral administration, as compared to dermal administration.

As specified in ECHA's Guidance (Chapter R.7a: Endpoint specific guidance Version 6.0 – July 2017), Section R.7.6.2.3.2, Stage 4.1 iv., "*REACH specifies that the reproductive toxicity studies should be conducted via the "most appropriate route of administration, having regard to the likely route of human exposure". "Likely routes of human exposure" within REACH are oral, inhalation and dermal. The selection of the "most appropriate route of administration" focuses on identification of hazards (see the Introduction to this Guidance, R7a and sub-section "Selection of the appropriate route of administration for toxicity testing", under R.7.2 Human health properties or hazards) and depends on the most appropriate route for identification of the intrinsic properties of the substance for reproductive hazard.*"

Further, "*According to the test methods for reproductive toxicity which focus on the detection of reproductive hazards, the oral route (gavage, in diet, or in drinking water) is the "default" route, except for gases.*"

Additionally, "*Case-specific deviations from the default approach must be justified, such as in the case of available information on route-specific toxicity or toxicokinetics indicating that the use of oral administration of substance would not be relevant for assessing the human health hazards via inhalation, which would be the main route of exposure.*"

In view of the much higher systemic concentrations and exposure of the registered substance after oral exposure, as compared with dermal exposure, ECHA considers that the dermal route is not the most appropriate route of administration, and that the two dermal developmental toxicity studies with the registered substance do not fulfil the information requirement.

The dossier also has an oral OECD 414 study using read across from 4,4-dimethyl-1,3-oxazolidine (CAS: 51200-87-4, EC:257-048-2), and the corresponding range-finding study. However, as explained in Annex I, "Grouping of substances and read-across approach for toxicological information" above, the read across is not accepted and therefore these studies do not fulfil the information requirement.

In your comments on the draft decision you indicate that the developmental toxicity endpoint in rats has been adequately addressed by means of: (i) the dermal OECD TG 414 with the registered substance and (ii) the oral OECD TG 414 with the source substance. As regards the issue of the route of exposure you claim that both the target and the source substance are "*severe irritants*" which limit the amount of the systemic concentrations that can be evaluated in the studies. You indicate that the test material used in the OECD TG 414 study performed via the dermal route of exposure represents an equilibrium between the HCl salt of the target substance and the non-ionized base (AMP-base). Moreover, you state that this material results in maximal exposure to human from dermal contact with a product containing the target substance. You also refer to the blood sample results analysed during the dermal PNDT study where the results indicate dermal absorption of the registered substance.

With reference to (i) ECHA notes that the results indicate that there is a low systemic exposure via the dermal route and the fact that no effects were seen in post-implantation loss or developmental toxicity does not necessarily mean that the registered substance does not pose a developmental toxicity hazard. As already indicated above (in this section), according to the toxicokinetics data the total dermal absorption of the target substance was "*~43% of the dose*" and to reach the maximum concentration in blood it took longer (~4 hours) than the oral dose (0.3 hour) application, hence this indicates a "*slower dermal penetration*" of the target substance. In your comments you indicate that the potential systemic dose would only have been between 100 and 150 mg/kg/bw. ECHA considers that by using the oral exposure route higher systemic doses can be reached which would be necessary in order to conclude whether there are developmental toxicity effects on rats arising from the registered substance.

Regarding point (ii); as explained under the *Grouping of substances and read-across approach for toxicological information* section, currently ECHA cannot accept the read-across approach. Hence, the study cannot be taken into consideration for the evaluation of this endpoint.

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. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits as a first species.

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

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 or rabbit) by the oral route.

Notes for your consideration

ECHA notes that a revised version of OECD TG 414 may be adopted later on this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. After the adoption of the revised version of the OECD TG 408 you should test in accordance with that 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.

Even if you start testing before the guideline is published, it is appropriate to consider including these endocrine-sensitive parameters in your testing protocol in accordance with the proposed revised version of the draft guideline (see http://www.oecd.org/env/ehs/testing/section4-health-effects.htm).

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

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

In the technical dossier you have provided a study record for an OECD 414 in rats by the dermal route ([redacted] 2006), together with the range-finding study. However, this study does not provide the information required by Annex IX, Section 8.7.2. for the reasons as set out in section 3 (Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species) above.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study record for a OECD Guideline 414 study in rabbit (Prenatal Developmental Toxicity Study) with the analogue substance 4,4-dimethyl-1,3-oxazolidine (CAS: 51200-87-4 / EC: 257-048-2). However, as explained in Annex I, "Grouping of substances and read-across approach for toxicological information" above, the read across is not accepted and therefore these studies do not fulfil the information requirement.

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. On the basis of this default assumption, ECHA considers that the test should be performed with rat or rabbit as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 5.0, December 2016) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route. See also ECHA's considerations on the most appropriate route of exposure as set out in section 3 (Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species) above.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species (rat or rabbit) by the oral route.

In your comments on the draft decision you agreed to perform the request with the registered substance.

Notes for your consideration

ECHA notes that a revised version of OECD TG 414 may be adopted later on this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. After the adoption of the revised version of the OECD TG 408 you should test in accordance with that 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.

Even if you start testing before the guideline is published, it is appropriate to consider including these endocrine-sensitive parameters in your testing protocol in accordance with the proposed revised version of the draft guideline (see http://www.oecd.org/env/ehs/testing/section4-health-effects.htm).

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

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

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

a) The information provided

In your dossier, you have provided the results of and OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test) conducted with the registered substance ([redacted]

2005). However, this study does not provide the information required by Annex X, Section 8.7.3. because because it does not cover key elements, such as exposure duration, life stages and statistical power of an extended one-generation reproductive toxicity study. More specifically, the main missing key elements are: 10 weeks pre-mating exposure duration, at least 20 pregnant females per group, and an extensive postnatal evaluation of the F1 generation. Therefore, your adaptation of the information requirement is rejected.

Additionally, you have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing an OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) conducted with a read across substance 4,4-dimethyl-1,3-oxazolidine (CAS: 51200-87-4 EC:257-048-2). However, as explained above your adaptation of the information requirement is rejected.

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

b) The specifications for the study design

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 5.0, December 2016).

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 conducted 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 5.0, December 2016) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

c) Outcome

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

In your comments on the draft decision you agreed to perform the request with the registered substance.

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity)] were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the new information shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 5.0, December 2016).

You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

4. Classification and labelling (Annex VI, Section 4.)

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).

You have provided for the repeated dose toxicity several studies, including in rats and dogs, showing species differences. From the rat studies, the 90-day study of [redacted] 1981 a

NOAEL of 25 mg /kg bw/d was established on the basis of the liver effects observed in the highest dose (250 mg/kg bw).

You provided a 90-day study in dogs also by [redacted] 1981. In this study severe effects were seen at 62.5 mg/kg bw/ day (highest dose), such as high increases in the liver enzymes (magnitude of the increases ranged from 2-4 fold for serum glutamic oxaloacetic transaminase, 12-28 fold for serum glutamic pyruvic transaminase, and 5-6 fold for alkaline phosphatase) correlated with histopathological findings, including vacuolization, periportal cirrhosis characterised by hepatocellular necrosis and fibrosis, and bile duct hyperplasia.

You stated with regard to the clinical chemistry findings that "*The above effects observed in the high dose animals suggest a high degree of hepatocellular damage in the high dose animals with no significant changes in the low or mid dose groups. The combination of increased serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels, both of which are present in high concentrations in hepatocytes, are indicative of hepatocellular necrosis. The increased alkaline phosphatase and direct bilirubin levels are suggestive of cholestasis and/or bile duct epithelial necrosis. It appears, therefore, that the liver is the promary target organ, based on the clinical chemistry data, when the test substance is administered via the diet.*"

A NOAEL of 0.63 mg/kg bw/d was determined in this study and it was based on:

- Organ weight changes in the high dose group (liver and kidney),
- Liver histopathology in the high dose group,
- Clinical chemistry parameters (plasma liver enzyme levels) in the high dose group.
- Increased pro-thrombin time for males in mid and high dose group

You also provided 28-day and one- year studies in dog. The one year study uses a maximum dose of approximately 2.8 mg/kg/day, and you conclude that this is a No Effect Level. The 28-day study has one male and one female dog per dose level, and this is too few animals to draw any definitive conclusions. Nonetheless, the results appear to be consistent with the 90-day study results, with evidence of toxic liver injury at dose levels below 300 mg/kg/day (the STOT-RE Category 2 classification threshold of 300 mg/kg/day is provided in sections 3.9.2.9.5 and 3.9.2.9.7., as well as table 3.9.3. of Annex I to the CLP Regulation and also given in table 3.9.2.2 of the CLP Guidance on the Application of the CLP Criteria, Version 5.0. July 2017 for oral 28-day studies).

Based on the severe effects observed at the highest dose of 62.5 mg/kg bw/ day seen in the 90-day study in dogs, ECHA considers that a classification as specific target organ toxicity - repeated exposure, Category 2 (STOT RE 2) for target organ liver would be warranted. This will be explained in the following.

In the endpoint summary you state: "*No classification for Chronic toxicity or Target Organ toxicity is proposed. According to the GHS criteria for "specific target organ toxicity", severe fatty change in the liver is considered to be an effect of relevance for classification. However, in all the available studies, the fatty change observed at doses below 100 mg/kg bw in the rat were minor changes and could not be classed as severe. Thus the effect does not meet the criteria for classification.*"

ECHA observes that indeed in the rat studies no severe effects at doses below 100 mg/kg bw were seen. However, this was not the case for the dog studies.

ECHA further notes that both studies, in rat and in dog are equivalent or similar to OECD 408 and OECD 409 respectively, have been assigned the same reliability (Klimisch 2), and are performed in the same laboratory. Furthermore the Registrant acknowledge that "*the effects observed in rats and dogs are likely relevant to man*" and considers rat and dogs as sensitive "*There is also a difference in species sensitivity, dogs = rats > mice.*" However, there is no justification why the findings from the study of [redacted] 1981 in dogs are not considered for a classification as STOT RE2 for liver toxicity of the registered substance subject to this decision.

The Guidance on the Application of the CLP criteria, specifies in Section 3.9.2.4. that "*Reliable evidence associating repeated exposure to the substance with a consistent and identifiable toxic effect demonstrates support for the classification.*" Further, the Guidance states "*Where a number of studies are available these should be assessed using a weight of evidence approach to determine the most appropriate classification.*" ECHA considers this is the appropriate way to evaluate the evidence.

As explained above, the registered substance causes significant toxic effects at repeated dose levels below the values for classification for STOT-RE set out in the CLP Regulation and the ECHA Guidance and there is not sufficient justification for the non-classification.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to apply classification and labelling on the registered substance as STOT RE 2 (target organ liver) for repeated dose toxicity. In the alternative, you are required to provide the scientifically justified reasons why no such classification is given. You are reminded that also for a differentiation of a hazard class, scientifically justified reasons need to be provided.

In your comments on the draft decision you acknowledge the fact that dogs and rats show similar sensitivity while mice are relatively insensitive to AMP-induced liver steatosis, and that it is still unknown for humans. To determine whether the effects seen are relevant for humans and, if as a consequence, the registered substance should be classified as STOT RE 2 (target organ liver) for repeated dose toxicity, you indicated that further *in vitro* data are required to test the potency in human cells and that this data will be available by June 2018.

ECHA reminds you that all new information in the later update(s) of the registration dossier will be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation (after ECHA has sent the final decision).

Deadline to submit the requested information in this decision

In the draft decision communicated to you, the time indicated to provide the requested information was 30 months. In your comments on the draft decision, you expressed your concerns on the possibility to comply with this deadline, as on the grounds of limited laboratory capacity and financial constraints you intend to undertake the requested tests in a sequential manner. ECHA would like to point out that the deadline of 30 months already

allows you to undertake sequential testing. Consequently, ECHA did not invite you to provide a justification on the grounds of limited laboratory capacity, ECHA did however communicate with you on this matter. Therefore, the deadline set in the draft decision was not amended. Regarding your concern on financial constraints, this aspect is not within the remit of ECHA and so is not a factor that can be taken into consideration when determining an appropriate deadline.

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 16 March 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 requests.

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.