EUROPEAN CHEMICALS AGENCY

Helsinki, 19 October 2016

Addressee: [redacted]

Decision number: TPE-D-2114346827-38-01/F
Substance name: N,N,N-triethylethanaminium hydroxide
EC number: 201-073-3
CAS number: 77-98-5
Registration number: [redacted]
Submission number: [redacted]
Submission date: 3 February 2016

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA has taken the following decision.

You are requested to perform the following test:

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

while your originally proposed test for Sub-chronic toxicity study (90-day), oral route (EU B.26./OECD TG 408) in rats with the analogue substance tetramethylammonium chloride, EC No. 200-880-8 is rejected.

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 of the REACH Regulation. In order 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 an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **26 April 2018**. You shall also update the chemical safety report, where relevant.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. 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, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Ofelia Bercaru, Head of Unit, Evaluation E3

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

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposal(s) submitted by you.

0. Grouping of substances and read-across

Article 13(1) of the REACH Regulation provides that information on intrinsic properties of substances may be generated by means other than tests. Such other means include the use of information from structurally related substances (grouping of substances and read-across), "provided that the conditions set out in Annex XI are met".

In the registration, you have proposed to adapt the standard information requirements for a Sub-chronic toxicity study (Annex IX, Section 8.6.2.) by applying a read-across adaptation following REACH Annex XI, Section 1.5. You have submitted a testing proposal proposing to perform the test using the analogue substance trimethylammonium chloride, CAS No 75-57-0 (EC No 200-880-8).

Annex XI, 1.5. requires a structural similarity among the substances within a group or category such that relevant properties of a substance within the group can be predicted from the data on reference substance(s) within the group by interpolation. The following analysis presents your justification for the proposed grouping approach and read-across hypothesis, together with ECHA's analysis concerning the justification.

a. Description of the grouping and read-across approach

You propose to use grouping and read-across to adapt the standard information requirement for a sub-chronic toxicity study (90-day) for the registered substance (tetraethylammonium hydroxide, TEAH, target substance). Study results of a sub-chronic toxicity study (90-day), according to the OECD TG 408 with tetramethylammonium chloride (TMAC, source substance) shall be used to predict the sub-chronic toxicity of the registered substance. You stated that TEAH and TMAC are quaternary ammonium salts well soluble in water, which will readily dissociate in biological fluids into their respective cations and anions: tetraethylammonium and tetramethylammonium ions, hydroxide and chloride ions, respectively. You claimed that it is not fully known how the substances metabolize, however, mammalian metabolism is predicted to be similar. As a conclusion, you expect the source and target substances to have similar toxicity based on similar structure, physico-chemical properties and predicted metabolism.

ECHA understands this as the hypothesis under which you propose to perform the sub-chronic repeated dose toxicity with the source substance to read-across to the registered substance.

b. Support of the grouping and read-across approach

You have provided a read-across justification as a separate "*Read-across report*" in IUCLID, Section 13. In summary you provide the following arguments to support the read-across approach:

- *"... the substances account for the structural similarity in terms of a quaternary ammonium ion core with short alkyl chains and additionally they would address an incremental and constant change of chain-length."*

- *"... ideally the source chemical for read across should contain a quaternary ammonium ion core with short alkyl chains, and preferably also the hydroxide ion."*

- *"Counter ion variation is considered to be less relevant for biological effects, with hydroxide being the worst case for local/contact site toxicity evaluation in view of their high alkalinity..."*

- *"Since the tetraethylammonium cation is expected to be responsible for biological, systemic type of actions, the identity of the counter anion appears to be of less importance and may be chloride or bromide. Hydroxides are considered to be worst case substances for local/contact site toxicity evaluation in view of their high alkalinity."*

- *"It is not fully known how the various substances metabolize" and "TOXTREE v 2.5.0 predicts similar CYP450 metabolism for primary and secondary sites of tetraethylammonium (TEAH) and tetramethylammonium (TMAH and TMAC); only the third most likely metabolite differs. As N-oxidation is considered to yield the most reactive metabolites, effects which may be caused as a result of this metabolism route can be predicted to be similar for TEAH, TMAH and TMAC."*

- *"A toxicokinetic assessment based on physico-chemical properties predicted absorption via the oral, inhalation and dermal route to be 50, 100 and 100%, respectively."*

- Data matrix presenting physico-chemical properties of the registered substance (TEAH), source substance (TMAC), and another source substance tetramethylammonium hydroxide (TMAH) showing that the target substance TEAH exists only as aqueous solution, whereas the source substance TMAC and source substance TMAH form colourless crystalline solid. Further, you provided information on the partition coefficient n-octanol/water, water solubility and pH of the resulting solutions.

- Data matrix with information on toxicological properties of the registered and source substances with the following information:
    a. LD50 values for oral and dermal toxicity of all the three substances
    b. Skin irritation/corrosion data for the source TMAC and analogue TMAH
    c. Eye irritation and skin sensitization data for the source TMAC
    d. In vitro genotoxicity studies with the analogue TMAH and in vitro gene mutation in bacteria with the source TMAC
    e. 28-day with the analogue TMAH
    f. Screening reproductive/developmental toxicity with the analogue TMAH
    g. Pre-natal developmental toxicity with the analogue TMAH

- It is noted that for the property under consideration, i.e. repeated dose toxicity (90-days), TMAC is proposed to be used as source substance.

c. Analysis of the grouping and read-across approach in the light of the requirements of Annex XI, 1.5

With regard to the proposed predictions ECHA has the following observations:

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

In order to meet the provisions in Annex XI 1.5 to predict physicochemical, toxicological and ecotoxicological properties from data for a reference substance within the group by interpolation to other substances in the group, ECHA considers that structural similarity alone is not sufficient. It has to be justified why such prediction is possible in view of the identified structural differences and the provided evidence has to support such explanation.

In particular, the structural similarities must be linked to a scientific explanation of how and why a prediction is possible.

You have identified the structural basis for the prediction, *i.e.* that the substances are salts containing a quaternary ammonium core with of incremental change of alkyl chains length. You expect the tetraethylammonium cation to be responsible for biological systemic type of effects, whilst you consider the difference in the counter ion to be less relevant for biological effects of the substances with hydroxide being the worst case for local/contact site toxicity evaluation in view of the high alkalinity.

In this regard ECHA notes that you stated that the registered and source substances would address an incremental and constant change of chain-length. However, you did not explain how the different length of the alkyl chains bound to the nitrogen influences the properties of the registered and source substances and what this would mean in terms of the possibility to predict the outcome of a sub-chronic toxicity study conducted with the registered substance by read-across from the proposed source substance.

Thus ECHA concludes that you have not addressed obvious structural differences between the registered substance and the source substance. Accordingly, you have not provided sufficient evidence that the differences in the structure of the registered and source substance would not lead to differences in the presence of the substance in biological fluids in dissociated and non-dissociated form, mode of action and toxicity profile. The provided explanation is not considered as valid to establish the link between the structural similarity and the prediction. ECHA therefore considers that there is not an adequate basis for predicting the properties of the registered substance from the source substance.

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

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances. One prerequisite for a prediction based on read-across therefore is that the substances involved are structural similar and are likely to have similar properties. One important aspect in this regard is the analysis of the data matrix to compare the properties of source and target substances and to establish whether indeed they are similar or follow a regular pattern.

You provided limited data on the registered and source substances with respect to their toxicological properties in the data matrix:

a) The only data available in the data matrix for the registered substance TEAH is an acute dermal toxicity study in rat. Further, you provided results of acute dermal toxicity studies for the source substance TMAC in rabbit and from another source substance tetramethylammonium hydroxide (TMAH). You claimed that the data indicated a lower acute dermal toxicity of the tetraethylammonium ion.

b) Regarding studies investigating repeated dose toxicity, you provided two sub-acute studies (oral and dermal) and a screening reproductive/developmental toxicity study with the source substance TMAH. You have not provided any studies with repeated dose exposure for the registered substance TEAH and proposed source substance TMAC.

Since for the registered substance almost no toxicity information is available, a similar or regular pattern of toxicity which results from structural similarity cannot be verified, in particular not for repeated dose toxicity. The only toxicity information available for the registered substance is obtained from an acute dermal toxicity study. A comparison of the

dermal acute toxicity profiles does not contribute to the the comparison of the systemic toxicity profiles after repeated dose administrations.

ECHA concludes that you have not provided data that would enable comparison of the repeated dose toxicity properties of the registered and source substances. ECHA concludes that the presented evidence in the data matrix does not support a similar or regular pattern of toxicity as a result of structural similarity. Therefore it cannot be verified that the analogue substance can be used to predict the repeated dose toxicity (90-days) of the registered substance.

(iii) Toxicokinetics

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

ECHA notes the following:

- The dissociation of the registered substance in biological fluids to its cation and anion appears to be plausible as TEAH exists predominantly in the form of an aqueous solution. However, regarding dissociation of the source substance TMAC, you have not provided data on the speed of this process under different pH conditions in the registration dossier. No evidence was provided that the source substance cannot be taken-up in non-dissociated form. It is therefore unclear what differences in absorption may exist between source and target substances.

- You have explained why the default values for oral, inhalation and dermal absorption should be applied. The Guidance recommends certain default values for absorption based on the knowledge of relevant physico-chemical properties, e.g. dissociation constant, partition coefficient and molecular mass. ECHA observes that you have used an adaptation instead of providing the dissociation constant and that the partition coefficients of the registered substance (log P -3.4) and the source substance (log P -1.6) are outside of the Guidance range of -1 to 4 which would justify the default values of absorption. Moreover, ECHA observes that you have not addressed the impact of the different length of the alkyl substituents bound to the nitrogen not only on absorption, but also on distribution and elimination of the registered and source substance.

- The used metabolites prediction model TOXTREE v 2.5.0 uses SMARTCyp Plugin which may predict the sites in molecules most liable to cytochrome P450 mediated metabolism. Therefore, metabolic pathways mediated by other than cytochrome P450 biotransformation enzymes, such as flavin-containing monooxygenases, aldo-keto reductases and phase II biotransformation enzymes are not taken in account in the TOXTREE predictions. ECHA further notes that while N-dealkylation is a commonly observed metabolic reaction for secondary and tertiary amines, it may not be the case for small quaternary ammonium compounds. Even more important is that the fact that a common metabolic pathway is predicted to occur does not prove that the resulting metabolites from this pathway have a similar pattern of toxicity. In this regard, you have not addressed the impact of the different aldehydes that would arise from the alkyl chains via the N-dealkylation, as well as of the different metabolites created via N-oxidation and the hydroxylation of the alkyl chains.

ECHA notes that small differences in the chemical structure of small molecules and their metabolites can have a major impact on the toxicokinetic profiles. Consequently, ECHA concludes that you did not sufficiently address important aspects such as the toxicokinetics of the registered substance TEAH and the source substance TMAC. As consequence, it is not possible to assess the qualitative and quantitative internal systemic exposure of the test organism to the parent substances and their metabolites when exposed to the source and target substance, respectively. Thus, it is not possible verify that the registered and source substances would show a similar or regular pattern of toxicity as a result of structural similarity. Therefore, ECHA considers that there is no adequate basis for predicting properties of the registered substance from the source substance.

(iv) Bias in the selection of source substances and source study

Annex I section 1.1.4 requires *"...that the study or studies giving rise to the highest concern shall be used to establish the DNELs.;"* In the context of a read-across approach this has two aspects: the selection of the source substance and the selection of the source study.

ECHA notes that in the *"Read-across report"* on the analogue approach, you presented the following source substances to be suitable to fill standard information requirements for the registered substance TEAH based on their chemical nature and similar physico-chemical properties:
1. tetramethylammonium hydroxide (TMAH) (EC no 200-882-9)
2. tetramethylammonium chloride (TMAC) (EC no 200-880-8)
3. tetraethylammonium chloride (TEAC) (EC no. 200-267-5; surrogate)

ECHA observes you did not explain why TMAC was proposed as the most appropriate source substance to fulfil the standard information requirement for the sub-chronic toxicity of the registered substance. Thus, ECHA concludes that it is not possible to verify that you have selected the source substance which is the most appropriate as required in Annex I, section 1.1.4.

d. Conclusion on the read-across approach

The proposal to adapt the standard information requirements for the sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.) in the technical dossier is based on the read-across approach examined above. ECHA does not consider the read-across justification to be a reliable basis to predict the properties of the registered substance for the reasons set out above. Thus, the adaptation does not comply with the general rules of adaptation as set out in Annex XI, 1.5. Therefore, ECHA rejects the proposed adaptation that is based on Annex XI, 1.5.

1. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.)

Examination of the testing proposal

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other tests in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

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. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) by the oral route according to OECD TG 408 with the analogue substance tetramethylammonium chloride (EC No 200-880-8). As explained above in the section "Grouping of substances and read-across", the adaptation does not comply with the general rules of adaptation as set out in Annex XI, 1.5. and the proposed adaption is rejected.

You proposed testing by the oral route. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA agrees that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is a liquid of very low vapour pressure and no uses with spray application are reported that could potentially lead to aerosols of inhalable size. 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. You stated that the registered substance contains a high percentage of water (typical concentration [redacted]%, concentration range [redacted]%). ECHA considers that the concentration of the registered substance and the water content has to be taken into account in the calculation of the dose levels during the testing.

Outcome

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the study with the registered substance subject to the present decision: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD TG 408), while your originally proposed test with the analogue substance tetramethylammonium chloride (EC No 200-880-8) is rejected according to Article 40(3)(d) of the REACH Regulation.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal(s) for examination pursuant to Article 40(1) on 29 May 2015.

ECHA held a third party consultation for the testing proposal(s) from 30 September 2015 until 16 November 2015. ECHA did not receive information from third parties.

This decision does not take into account any updates after 8 August 2016, 30 calendar days after the end of the commenting period.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA did not receive any comments by the end of the commenting period.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation:

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 decision does not imply that the information provided by the Registrant in his registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the Enforcement Authorities of the Member States.

3. In carrying out the test(s) required by the present decision it is important to ensure that the particular sample of substance tested 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. If the registration of the substance covers different grades, the sample used for the new test(s) must be suitable to assess these. Furthermore, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.