Decision number: TPE-D-2114329763-45-01/F Helsinki, 21 April 2016

DECISION ON TESTING PROPOSALS SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For 2,2,6,6-tetramethyl-4-oxopiperidinooxy, EC No 220-778-7 (CAS No 2896-70-0), registration number: [redacted]

Addressee: [redacted]

The European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 51 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

I. Procedure

Pursuant to Article 40(1) of the REACH Regulation, ECHA has examined the following testing proposal submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for 2,2,6,6-tetramethyl-4-oxopiperidinooxy, EC No 220-778-7 (CAS No 2896-70-0), submitted by [redacted] (Registrant).

- Developmental toxicity / teratogenicity study (OECD 414) using the analogue substance 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (OH Tempo) (CAS No 2226-96-2).

This decision is based on the registration as submitted with submission number [redacted], for the tonnage band of 100 to 1000 tonnes per year.

This decision does not take into account any updates after 21 August 2015, i.e. 30 calendar days after the end of the commenting period.

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.

ECHA received the registration dossier containing the above-mentioned testing proposals for further examination pursuant to Article 40(1) on 5 February 2014.

ECHA held a third party consultation for the testing proposals from 14 August 2014 until 28 September 2014. ECHA did not receive information from third parties.

On 15 June 2015 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision. That draft decision was based on submission number [redacted].

On 20 July 2015 ECHA received comments from the Registrant on the draft decision.
On 28 July 2015 the Registrant updated his registration dossier with the submission number [redacted]

The ECHA Secretariat considered the Registrant's comments and update. In their update the registrant has removed the testing proposal for a repeated dose toxicity 90-day study via oral route. The Registrant provided a valid Annex IX, Section 8.6.2. column 2 adaptation (1st indent) with a self-classification as STOT Rep. Exp. 2.

On basis of this information, Section II was amended. The Statement of Reasons (Section III) was changed accordingly. The ECHA Secretariat in particular removed the request for a 90-day repeated dose toxicity study from the draft decision.

On 3 March 2016 ECHA notified the Competent Authorities of the Member States of its draft decision and invited them pursuant to Article 51(1) of the REACH Regulation to submit proposals for amendment of the draft decision within 30 days of the receipt of the notification.

As no proposal for amendment was submitted, ECHA took the decision pursuant to Article 51(3) of the REACH Regulation.

II. Testing required

A. Test required pursuant to Article 40(3)

The Registrant shall carry out the following modified test pursuant to Article 40(3)(c) and 13(4) of the REACH Regulation using the indicated test method and the registered substance subject to the present decision:

> Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414) in rats or rabbits, oral route.

while the originally proposed test(s) for a Sub-chronic toxicity study (90-day) (OECD 408) and Pre-natal developmental toxicity study (OECD 414) proposed to be carried out using the analogue substance 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (CAS No 2226-96-2) are rejected pursuant to Article 40(3)(d) of the REACH Regulation.

Note for consideration by the Registrant:

The Registrant 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 to and conforming with the appropriate rules in the respective Annex, and an adequate and reliable documentation.

B. Deadline for submitting the required information

Pursuant to Articles 40(4) and 22(2) of the REACH Regulation, the Registrant shall submit to ECHA by **28 April 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report.

Failure to comply with the request(s) in this decision by the deadline set, 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.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposals submitted by the Registrant for the registered substance.

A. Test required pursuant to Article 40(3) REACH

The decision of ECHA is based on the examination of the testing proposal submitted by the Registrant proposed to be performed with the analogue substance 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (CAS No 2226-96-2). ECHA in the following explains its conclusions on the scientific validity of the read-across hypothesis and in the section thereafter on the assessment of the testing proposed.

1. Read-across approach

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".

According to Annex XI, Section 1.5 of the REACH Regulation there needs to be structural similarity among the substances within a group or category and, moreover, 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 by interpolation to other substances in the group (read-across approach). Furthermore, Annex XI, Section 1.5 lists several additional requirements, including that adequate and reliable documentation of the applied method be provided.

a. Introduction of the grouping approach and read-across hypothesis proposed by the Registrant and information submitted by the Registrant to support the grouping and read-across hypothesis

The Registrant has proposed to cover the standard information requirement for a pre-natal developmental toxicity study (Annex IX, 8.7.2.) by performing the test with an analogue substance, 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (OH Tempo) (CAS No 2226-96-2).

The Registrant has provided the following justification: "*The two compounds, Oxo Tempo and OH Tempo, are structurally similar and based on existing available data, exhibit similar properties with respect to toxicity, environmental fate and ecotoxicity. Furthermore, literature data demonstrates that OH Tempo is a metabolite of Oxo Tempo in the mammalian environment (Kroll, Borchert 1998), (Kroll et al, 1998)*". In addition, three studies to support the metabolism of the registered substance have been provided.

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

ECHA understands that the read-across approach proposed by the Registrant is based on the (i) similar structures, (ii) similar toxicity, environmental fate and exotoxicity properties, and (iii) the metabolism of the registered substance to the analogue substance.

(i) ECHA notes that the registered and analogue substances have similar core structures. However, the Registrant has not addressed the impact of different functional groups in the substances on e.g. toxicokinetics and toxicological profiles of the substances.

(ii) ECHA notes that no experimental toxicological data on the analogue substance has been provided. ECHA therefore concludes that toxicological profiles of the substances cannot be evaluated.

(iii) ECHA notes that the Registrant has provided three experimental studies to support the metabolism of the registered substance to the analogue substance.

- In the study conducted with isolated perfused rat liver, two main metabolites of the registered substance were identified: TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl = the analogue substance) and 1-hydroxy-TEMPOL (1,4-dihydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl). In addition, these metabolites were further metabolised, and two additional unknown peaks "*as the secondary amines of the TEMPONE and TEMPOL hydroxylamines*" were also detected. The $T_{1/2}$ was reported to be 47–70 minutes. The Registrant states that the results of the 28-day study with a 14-day recovery period indicate that the registered substance is quantitatively excreted within 24 hours or that the metabolites have negligible toxicity. ECHA notes that the impact of metabolites cannot be concluded based on the recovery period.

  ECHA notes that based on the study report, the concentration of the registered substance was 4-2 fold higher than the ones of the metabolites at one hour, and 41.3% of the registered substance was detected at three hours.

  ECHA concludes that for a read-across approach, the metabolism should be rapid and complete. However, based on the data provided by the Registrant, metabolism is relatively slow (47–70 min) and in addition to the analogue substance, several other metabolites are formed. The Registrant has not addressed the impact of the parent compound and other metabolites on toxicity.

- In an *in vitro* study with human keratinocytes cell line HaCaT, the same metabolites were detected as in the isolated perfused rat liver study. However, in this study the quantitative analysis of the metabolite formation and rate of metabolism was not reported/studied.

- In the third study, mice were given an intravenous injection of the registered substance and analogue substance. $T_{1/2}$ values were 1.5 min and 3.0 min for the registered substance and analogue substance, respectively. The metabolites were not identified in this study. The Registrant concludes that the three studies confirm that metabolism occurs also in other organs than in the liver and therefore, the half-life obtained in the *in vivo* mouse study "*may be a more accurate reflection of the half life*".

  ECHA notes that since the study designs in the mouse (single intravenous dose *in vivo*) and rat (continuous exposure of isolated perfused liver) studies are different a definite conclusion about the half-life cannot be made. In addition, the metabolites were not identified and a metabolism study with a single dose might not accurately reflect the metabolism processes after repeated exposure of the registered substance.

ECHA concludes that based on the data provided by the Registrant, several metabolites besides the analogue substance are formed from the registered substance, the impact of these metabolites on the toxicity of the registered substance has not been addressed by the Registrant, metabolism is not considered rapid, due to relatively slow metabolism the parent substance is systematically available, and the impact of the parent substance on the toxicity was not addressed by the Registrant. In addition, no experimental toxicological data on the analogue substance has been provided

In the comments on the draft decision the Registrant has provided a read-across justification document and the following reports:

- Comparison of the toxicology of of 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxyl (= Hydoxy-Tempo, the analogue substance, CAS No. 2226-96-2) and 2,2,6,6-tetramethyl-4-oxopiperidinooxy (= Oxy-Tempo, the registered substance, CAS No. 2896-70-0)
- Comparative QSAR analysis of 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxyl (CAS No. 2226-96-2) and 2,2,6,6-tetramethyl-4-oxopiperidinooxy (CAS No. 2896-70-0) and

In the comments (read-across justification) document the Registrant proposes to predict the properties of Oxo-Tempo from the data of Hydroxy-Tempo, which the Registrant considers as the worst case. According to the Registrant, the worst case approach is based on

1. *the structures and alerts present on each material (as analysed by OECD toolbox),*
2. *unpublished acute toxicity studies, genotoxicity studies and 28-day repeat dose gavage studies available for each material, in turn supported by*
3. *information on the metabolism Oxo-Tempo from published studies*

ECHA analysis of the updated read-across justification:

1. ECHA agrees with the Registrant that based on the structural alert comparison, the profiles of the registered and analogue substances are similar. However, the comparative analysis using the OECD QSAR toolbox alone cannot be considered sufficient to conclude on the similar toxicity profile of the substances regarding pre-natal developmental toxicity.

2. ECHA observes that the toxicity profile of the registered and analogue substances appears to be similar regarding lower tier endpoints. Further, based on the NOAEL and LOAEL values and the severity of the effects observed in the 28-day studies, Oxo-Tempo seems to be less potent at producing haematological effects than Hydroxy-Tempo.

   ECHA notes that based on the results of the 28-day studies, Hydroxy-Tempo may be used as a worst case approach regarding repeated dose toxicity.

3. ECHA analysis of the metabolism of Oxo-Tempo is presented above. In its comments the Registrant states that "*intermediates produced from Oxo-Tempo (oxo-metabolites that would not occur with Hydroxy-Tempo) are short-lived*" and that "*these intermediates do not appear to contribute to the toxicology of Oxo-Tempo in a way that is measurable over that of Hydroxy-Tempo*".

   The Registrant claims that oxo-metabolites are short-lived and do not impact the toxicity of Oxo-Tempo. ECHA outlines that based on the data provided (Kroll and Borchert, 1999), 1-hydroxy-Tempone (i.e. one of the oxo-metabolites) was detected

at 3 hours in isolated perfused rat liver study, which does not support the Registrant's claim about "*short-lived*" metabolites. Further, it is not clear what the half-life of the amine of 1-hydroxy-Tempone (the other oxo-metabolite) is.

ECHA notes that the Registrant has not provided sufficient evidence to conclude that the two metabolites formed are short-lived. In addition, as explained above the metabolism of Oxo-Tempo is not rapid (41.3% of the registered substance was detected still at three hours) and therefore the impact of the parent compound on toxicity cannot be excluded.

ECHA acknowledges that indeed the oxo-metabolites do not appear to impact the repeated dose toxicity of the substances. However, ECHA notes that the results of the 28-day studies do not provide any indication of the potential (mechanism of) pre-natal developmental toxicity, which may mediate via different mechanisms of action than repeated dose toxicity.

Therefore, in the absence of information on the impact of parent compound Oxo-Tempo and of the two oxo-metabolites not formed from Hydroxy-Tempo on the developmental toxicity of Oxo-Tempo, ECHA is of the opinion that the Registrant has not demonstrated that the properties of Oxo-Tempo for the endpoint under consideration can be predicted from data generated with Hydroxy-Tempo, as required by the provisions of Annex XI, section 1.5 of the REACH Regulation. Therefore, the proposed read-across approach cannot be accepted and it is necessary to generate data using the registered substance.

c. Conclusions on the read-across approach

The Registrant has not provided adequate and reliable information to demonstrate that the read-across approach is plausible for the endpoint in consideration.

Regarding the claimed structural similarity, the Registrant has not addressed the impact of different functional groups in the substances on e.g. toxicokinetics and toxicological profiles of the substances. The metabolism of the registered substance is not rapid and complete, and thus the impact of the parent substance (Oxo-Tempo) may impact the pre-natal developmental toxicity. In addition to the suggested analogue substance, other metabolites are formed, as specified above.

Therefore, based on the data submitted by the Registrant, ECHA concludes that it is not possible to predict the properties of the registered substance from the data obtained from the suggested analogue substance 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (CAS No 2226-96-2).

ECHA therefore concludes that the criteria of Annex XI, 1.5. are not met, and the read-across approach, as presented by the Registrant, cannot be considered plausible to meet the information requirements.

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

a) 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 pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. 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.
The Registrant has submitted a testing proposal for a pre-natal developmental toxicity study according to EU B.31/OECD 414 to be performed with the analogue substance 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (CAS No 2226-96-2).

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation. However, ECHA does not consider the read-across approach plausible, as explained above in section III.1, and therefore considers that the testing should be performed on the registered substance.

The Registrant did not specify the species to be used for testing. He did not specify the route for testing. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat or the rabbit as a first species to be used.

b) Outcome

For the reasons explained above the proposed test for a pre-natal developmental toxicity (test method: OECD 414) via the oral route using the analogue substance 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (CAS No 2226-96-2) is rejected pursuant to Article 40(3)(d) as the provided information on the suggested read-across did not meet the requirements of Annex XI, 1.5. of the REACH Regulation.

Pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant is requested to carry out the following study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in rats or rabbits, oral route (test method: EU B.31/OECD 414).

B. Deadline for submitting the required information

In the draft decision communicated to the Registrant the time indicated to provide the requested information was 24 months from the date of adoption of the decision. This period of time took into account the fact that the draft decision also contained a 90 day repeated dose toxicity study (Annex IX, Section 8.6.2.). As the 90 day study is not addressed in the present decision, the ECHA Secretariat considers that a reasonable time period for providing the required information in the form of an updated IUCLID dossier is 12 months from the date of the adoption of the decision. The decision was therefore modified accordingly.

Adequate identification of the composition of the tested material

The process of examination of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the new studies meet real information needs. Within this context, the Registrant's dossier was sufficient to confirm the identity of the substance to the extent necessary for examination of the testing proposal. The Registrant must note, however, that this information has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In addition, it is important to ensure that the particular sample of substance tested in the new studies 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. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Finally, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the studies to be assessed.

IV. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Article 51(8) of the REACH Regulation. Such appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[1] by Guilhem de Seze, Head of Unit, Evaluation, E1.

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