ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114314144-64-01/F Helsinki, 18 February 2016

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

For Copper (2+), bis [N-{amino (imino-KN) methyl} urea-KO]-, nitrate (1:2), EC No 800-038-5 (CAS No 1071838-81-7), 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 jointly submitted registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for Copper (2+), bis [N-{amino (imino-KN) methyl} urea-KO]-, nitrate (1:2), EC No 800-038-5 (CAS No 1071838-81-7), submitted by [redacted] (Registrant).

- *In vivo* mammalian alkaline comet assay (OECD 489), in rats, oral route, with examination of liver and forestomach

This decision is based on the registration as submitted with submission number [redacted], for the tonnage band of 10 to 100 tonnes per year.

This decision does not take into account any updates after 5 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.

The examination of the testing proposal was initiated upon the date when receipt of the complete registration dossier was confirmed on 12 December 2014.

ECHA held a third party consultation for the testing proposals from 23 January until 10 March 2015. ECHA did not receive information from third parties.

On 28 May 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.

By 5 July 2015 the Registrant did not provide any comments on the draft decision to ECHA.

On 3 September 2015 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.

Subsequently, proposals for amendment to the draft decision were submitted.

On 9 October 2015 ECHA notified the Registrant of the proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposals for amendment received and amended the draft decision.

On 19 October 2015 ECHA referred the draft decision to the Member State Committee.

By 9 November 2015, in accordance to Article 51(5), the Registrant provided comments on the proposals for amendment. The Member State Committee took the comments of the Registrant on the proposals for amendment into account.

After discussion in the Member State Committee meeting on 7–11 December 2015, a unanimous agreement of the Member State Committee on the draft decision was reached on 9 December 2015.

ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

A. Tests required pursuant to Article 40(3)

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

1. *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, and either glandular stomach or duodenum/jejunum

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.

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

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 **27 February 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report.

III. Statement of reasons

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

A. Tests required pursuant to Article 40(3)

1. *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2)

a) Examination of the testing proposal

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "Appropriate in vivo mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII."

The technical dossier contains several *in vitro* studies, one of which is an *in vitro* Mammalian Cell Gene Mutation Test, performed according to OECD guidelines 476/EU method B.17 with mouse lymphoma L5178Y cells (with and without metabolic activation) on the registered substance, that shows positive results.

In the absence of a rat metabolizing system, the results were inconclusive and moderate to severe toxicity was induced. In the presence of a rat metabolizing system the substance showed mutagenic activity. The positive results indicate that the substance is inducing gene mutations under the conditions of the test.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but shall be considered. Consequently, there is an information gap and the Registrant considered it necessary to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for an i*n vivo* mammalian alkaline comet assay (OECD 489), in rats, oral route, with examination of liver and forestomach with the following justification: *"CuGun revealed positive results with metabolic activation in an in vitro mammalian cell gene mutation test (OECD 476). Negative results were observed in an in vitro bacterial reverse mutation assay (OECD 471) and in vitro mammalian cell micronucleus test (OECD 487)."* and *"Therefore, according to Annex VIII, Column 2, Section 8.4.3 and Annex IX, Column 2, Section 8.4 of the REACh regulation, appropriate in vivo mutagenicity study on somatic cell shall be considered. As recommended by the ECHA in the "Guidance on information requirements and chemical safety assessment Chapter R. 7a: Endpoint specific guidance, R.7.7. Mutagenicity and carcinogenicity (Version 3.0, August 2014)" for substance that appear preferentially to induce gene mutation, an in vivo mammalian alkaline comet assay (OECD 489) is proposed."*

ECHA notes that the proposed test is an appropriate test to investigate effects on gene mutations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.1. and figure R.7.7-1 (August 2014).

As regards the species to be used, the OECD test guideline 489 states that the choice of rodent species should be based on the species used in other toxicity studies. Rats are routinely used for this test and also for other toxicity studies so ECHA considers that testing in the rat is appropriate. As regards the route of administration, the test shall be performed by the oral route to ensure adequate exposure of the target tissue(s).

As regards the tissues to the analysed the Registrant states that the "*Organs proposed to be evaluated are the forestomach and the liver. The forestomach is considered because it is the site of first contact after oral exposure and toxicological effects were observed in the repeated dose toxicity study (e.g. minimal atrophy of the glandular portion, hyperplasia at the level of the limiting ridge). Concerning the second organ, the liver is proposed to be evaluated as it is the site of metabolism and also frequently a target organ for carcinogenicity. Furthermore, toxicological effects were also observed in the repeated dose toxicity study (e.g. single cell necrosis with inflammatory cell foci)*".

ECHA considers that the effects noted in the repeated dose toxicty study, namely "*minimal atrophy of the glandular portion, hyperplasia at the level of the limiting ridge*" do not justify the selection of the forestomach but rather indicate that analysis of the glandular stomach is appropriate. Proliferative lesions in the rodent forestomach may result from a combination of factors related to route-specific tissue irritation and/or unnatural dosing regimens and are less likely to be relevant in humans. Furthermore ECHA notes that while the OECD test guideline 489 does not make reference to the forestomach it does refer to the glandular stomach (e.g. in para 42 '*In some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant*').

Consequently, the test shall be performed by using tissues from the liver as the primary site of xenobiotic metabolism and from either the glandular stomach or duodenum/jejunum as sites of direct contact.

b) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, the Registrant is requested to carry out the following study with the registered substance subject to the present decision:

In vivo mammalian alkaline comet assay (test method: OECD 489) in rats, oral route, with analysis of the following tissues: liver, and either glandular stomach or duodenum/jejunum.

Note for consideration by the Registrant

The Registrant is reminded that according to Annex IX, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "*the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered*".

The Registrant may consider examining gonadal cells, as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

IV. 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 study 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 study 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 study 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 study to be assessed.

V. 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 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.