Decision number: TPE-D-2114312640-64-01/F Helsinki, 05 January 2016

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

For carbonohydrazide, CAS No 497-18-7 (EC No 207-837-2), 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 proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for carbonohydrazide, CAS No 497-18-7 (EC No 207-837-2), submitted by [redacted] (Registrant):

- Genetic toxicity *in vivo* (OECD 474);
- Developmental toxicity / teratogenicity study (OECD 414).

This decision is based on the registration dossier 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 8 April 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.

On 9 April 2013, pursuant to Article 40(1) of the REACH Regulation, ECHA initiated the examination of the testing proposals set out by the Registrant in the registration dossier for the substance mentioned above.

ECHA held a third party consultation for the testing proposals from 18 February 2014 until 5 April 2014. ECHA received information from third parties (see section III below).

On 30 January 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].

By 9 March 2015 the Registrant did not provide any comments on the draft decision to ECHA.

On 23 July 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, proposal for amendment to the draft decision was submitted.

On 28 August 2015 ECHA notified the Registrant of the proposal for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposal for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposal for amendment received and has not amended the draft decision.

On 7 September 2015 ECHA referred the draft decision to the Member State Committee.

By 28 September 2015 the Registrant did not provide any comments on the proposals for amendment.

A unanimous agreement of the Member State Committee on the draft decision was reached on 13 October 2015 in a written procedure launched on 1 October 2015.

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

II. Testing required

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

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

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

2. *In vivo* mammalian alkaline comet Assay (Annex IX, Section 8.4., column 2; in accordance with the OECD test guideline 489, in rats, oral gavage administration, with examination of liver and either glandular stomach or duodenum/jejunum,

while the originally proposed test for an *in vivo* mammalian micronucleus test (Annex IX, Section 8.4., column 2; test method OECD 474) proposed to be carried out using the registered substance is 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.
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.

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 **12 January 2018** an update of the registration dossier containing the information required by this decision. The timeline has been set to allow for sequential testing as appropriate.

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 and scientific information submitted by third parties.

1. Pre-natal developmental toxicity study

a) Examination of the testing proposal

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

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 with the following justification:

A further developmental study is considered to be valid to conduct due to the following:
- The effects seen on reproductive performance and offspring size/viability observed in the OECD 422 screening study, which were not sufficient for definitive classification.
- A toxicokinetic assessment indicates limited evidence of absorption of the test substance.
- Results for mutagenicity testing: Positive result in an Ames study (mutagenic under the conditions of this test) and ambiguous result without metabolic activation in a chromosome aberration study (weakly clastogenic to human lymphocytes in vitro), which may potentially have an influence on developmental toxicity.

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

The Registrant did not specify the species to be used for testing. He neither specified 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) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. The third party recommended in its comments that the decision allow the Registrant to follow a testing strategy, i.e. that the genotoxicity test is conducted before the prenatal developmental toxicity study. Based on the third party comment, this strategy could make conducting the prenatal developmental toxicity study unnecessary if a positive result will be obtained in the proposed test on genetic toxicity *in vivo*, because the substance could be self-classified as a germ cell mutagen and appropriate risk management measures be implemented. While ECHA takes no position on the potential effects of the genotoxicity test results on the risk management measures, the possibility to follow a sequential approach has been accommodated by extending the deadline of the decision.

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed 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).

2. *In vivo* mammalian alkaline comet Assay (test method: OECD test guideline 489)

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.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "[i]f there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant."

The Registrant has submitted a testing proposal for an *in vivo* mammalian erythrocyte micronucleus test according to EU B.12/OECD 474 with the following justification:

"Based on the results of *in vitro* testing it is considered necessary to perform an appropriate *in vivo* somatic cell genotoxicity study based on the fact that the following results were observed in the *in vitro* testing:

- Bacterial reverse mutation assay (Ames test): Positive - Carbohydrazide was considered to be mutagenic under the conditions of this test.
- Chromosome Aberration Test in Human Lymphocytes in vitro: The test item was considered to be weakly clastogenic to human lymphocytes *in vitro*."

ECHA notes that the proposed test is in principle an appropriate test to investigate further concern for chromosomal aberrations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment, chapter R.7.7.1. and figure R.7.7-1 (August 2014).

However, as the Registrant has pointed out in its IUCLID dossier chapter 7.6.1, the results of the *in vitro* chromosomal aberration study (Lacey, 2012) were described as "ambiguous". Furthermore it was stated that only a "very modest but statistically significant increase in the frequency of cells with aberrations in the 24 h group was noted but occurred at a single dose level which exhibited optimum levels of toxicity and may be due to a cytotoxic mode of action." Moreover, in the relatively recent and good quality (GLP) Salmonella assay (Bowles, 2012) "there were dose related and statistically significant increases in the frequency of TA100 and TA1535 revertant colonies at sub-toxic test item dose levels, in both the absence and presence of S9-mix. The increases observed for TA1535 were particularly large and in excess of the in-house historical control ranges with increases greater than 12 fold over the concurrent vehicle control."

The data available in the technical dossier show two concerns for the registered substance: gene mutation, which appears to be the main potential concern (although not confirmed in the assay on mammalian cells); chromosomal aberration, which seems to be a lower concern. The micronucleus test proposed by the registrant is a suitable in vivo test to investigate the concern for chromosomal aberration but does not address the gene mutation endpoint. According to ECHA guidance (Chapter R.7a: Endpoint specific guidance Version 3.0 – August 2014, pages 343 Table R.7.7-3, 357, 358), the comet assay is the only assay considered appropriate to follow-up both chromosomal aberration *and* gene mutation concerns. The comet assay recognises primary DNA damage which can lead to gene mutations or chromosome aberration. Moreover, in principle, every tissue from which single cell or nuclei suspensions can be prepared can be studied using the comet assay, including specific site of contact tissues, whereas the *in vivo* micronucleus test is restricted to bone marrow cells.

Therefore, a comet assay is considered as a choice for an *in vivo* follow-up assay to address the main concern identified *in vitro*, i.e., gene mutation..

As regards the route of administration, paragraph 39 of the OECD test guideline states "*The anticipated route of human exposure should be considered when designing an assay*" and "*In any case the route should be chosen to ensure adequate exposure of the target tissue(s)*". In light of the physicochemical properties of the substance, ECHA considers that testing by the oral route is appropriate.

Regarding the tissues on which the comet assay shall be performed, ECHA notes that paragraph 42 of the OECD test guideline states "*The liver has been the tissue most frequently studied and for which there are the most data. Therefore, in the absence of any background information, and if no specific tissues of interest are identified, sampling the liver would be justified as this is a primary site of xenobiotic metabolism and is often highly exposed to both parent substance(s) and metabolite(s). 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.*" Therefore ECHA considers that the comet assay should be performed in liver and either glandular stomach or duodenum/jejunum.

According to this test method, the rat is the default species. ECHA considers this species as being appropriate and testing should be performed with the rat.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. The third party has proposed that for *in vivo* genotoxicity test a comet assay be conducted instead of the *in vivo* mammalian erythrocyte micronucleus test. As outlined above, ECHA indeed considers that in the present case the comet assay is the better study to follow-up positive results obtained *in vitro* (see above).

c) Outcome

Therefore, 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: *In vivo* mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; in accordance with the OECD test guideline 489, in rats, oral gavage administration, with examination of liver and either glandular stomach or duodenum/jejunum.

whereas the proposed study is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

Note for consideration by the Registrant

The Registrant is reminded that according to the column 2 of section 8.4 of Annex IX 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". ECHA notes that in order to optimize the use of animals, gonadal cells may be sampled at the same time as the somatic tissues and analysed in the comet assay. Such analysis may provide a proof that the tested substance and/or its metabolites have reached the gonads and caused genotoxic effects. The registrant should however be aware that: i) the standard alkaline comet assay as described in the OECD guideline 489 is not considered appropriate to measure DNA strand breaks in mature germ cells and ii) gonadal cells contain a mixture of somatic and germ cells. Therefore, positive results in gonadal cells are not necessarily reflective of germ cell damage but they demonstrate exposure and genotoxic effect in the gonads.

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 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, or the information submitted by other registrants of the same substance, has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In relation to the proposed tests, the sample of substance used for the new studies must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is within the specifications of the substance composition that are given by the joint registrants. It is the responsibility of all joint registrants of the same substance to agree to the tests proposed (as applicable to their tonnage level) and to document the necessary information on their substance composition.

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 by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new studies must be suitable to assess these grades.

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.

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.