Helsinki, 13 December 2018

Addressee: [redacted]

Decision number: TPE-D-2114453848-34-01/F
Substance name: [redacted]
EC number: 483-940-8
CAS number: NS
Registration number: [redacted]
Submission number: [redacted]
Submission date: 27 October 2017
Registered tonnage band: 1-10

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

Your testing proposal is modified and you are requested to carry out:

1. ***In vivo*** **mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD 489) in rats, oral route; which may be combined with either an *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method: OECD 474) or a mammalian bone marrow chromosome aberration test (Annex VIII, Section 8.4., column 2; test method: OECD 475); with oral administration.**
   For the comet assay, the following tissues shall be analysed: liver, glandular stomach and duodenum. If you opt for a combination with the micronucleus test or a mammalian bone marrow chromosome aberration test, the bone marrow shall be analysed.

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 to the REACH Regulation. 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 have to submit the requested information in an updated registration dossier by ***22 June 2020***. You also have to update the chemical safety report, where relevant.

The reasons for 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 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 proposals submitted by you.

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

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 VII, Section 8.4. of the REACH Regulation. Column 2 of Annex VII, Section 8.4. provides that "Further mutagenicity studies shall be considered in case of a positive result".

The technical dossier contains two *in vitro* studies with the registered substance that show positive results.

- Bacterial reverse mutation assay ([redacted] *2008*) conducted according to the OECD 471 test guideline: positive in *S. typhimurium* TA 100 in presence of metabolic activation;
- *In vitro* mammalian cell gene mutation test ([redacted] *2015*) conducted according to the OECD 476 test guideline (1997): positive in presence of metabolic activation, with a predominant increase in the mutant frequency of small colonies.

The positive results indicate that the substance is inducing gene mutations under the conditions of the tests.

Appropriate *in vivo* genotoxicity studies to follow up the concern on gene mutations are not available for the registered substance. You considered it necessary to generate information for this endpoint.

Hence, you have submitted a testing proposal for *"a combined alkaline in vivo comet assay in liver and glandular stomach (OECD 489) and micronucleus test in bone marrow (OECD 475)".*

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA considers that you have adequately demonstrated the need to perform the proposed test. ECHA considers that the proposed test is appropriate to investigate effects on gene mutation *in vivo* as described in the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017), Chapter R.7a, Section R.7.7.1. and figure R.7.7-1.

You have proposed to combine the *in vivo* mammalian alkaline comet assay with *"micronucleus test in bone marrow (OECD 475)"*. ECHA notes an inconsistency in the reference to this additional test in the technical dossier: the test guideline for a mammalian erythrocyte micronucleus test is the OECD test guideline 474 whereas the OECD test

guideline 475 refers to the mammalian bone marrow chromosome aberration test. Since the positive results observed *in vitro* were detected in gene mutation assays, ECHA is of the opinion that these results should be followed up by an *in vivo* gene mutation test. The micronucleus test and the mammalian bone marrow chromosome aberration test are suitable to investigate other potential mechanisms of genotoxicity such as chromosomal aberration (i.e. clastogenicity and/or aneuploidy). Integrating one of these tests with the comet assay can help you reduce the number of tests performed and the number of animals used while providing useful information on the potential of the substance to induce clastogenicity as may be suggested by the increase in the mutant frequency of small colonies observed in the *in vitro* mammalian cell gene mutation test. You should take into account the combination aspects such as dosing and sampling following the principles described in the literature (see OECD test guideline 489, para. 33 & para. 34 (2016); and e.g. Bowen et al. 2011[2]). Hence, ECHA concludes that in view of the optimal use of animals you may consider to perform a comet assay combined with a micronucleus test or with a mammalian bone marrow chromosome aberration test.

You proposed performing the test in rats. You proposed testing by the oral route. According to the test method OECD TG 489, the test shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

You have proposed that the *in vivo* mammalian alkaline comet assay is conducted by analysing tissues from the liver and the glandular stomach. In line with the test method OECD TG 489, ECHA considers that the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, and glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

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

In vivo mammalian alkaline comet assay (Annex VII, Section 8.4., column; test method: OECD 489) in rats, oral route; which may be combined with an *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method: EU B.12./OECD 474) or a mammalian bone marrow chromosome aberration test (Annex VIII, Section 8.4., column 2; test method: EU B.12./OECD 475); with oral administration. For the comet assay, the following tissues shall be analysed: liver, glandular stomach and duodenum. If you opt for a combination with the micronucleus test or a mammalian bone marrow chromosome aberration test, the bone marrow shall be analysed.

Notes for your consideration
When performing the comet assay, you 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

[2] Bowen D.E. et al. 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the Comet assay and the flow-cytometric peripheral blood micronucleus test. Mutation Research, 722, 7-19.

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.

As explained above, you may consider to combine the assays as long as this will not impair the validity of and the results from each individual study. Regarding the test to be combined with the comet assay, it is reminded that the micronucleus test has the advantage of detecting both structural and numerical chromosomal aberrations (i.e. clastogenicity and aneuploidy, respectively) (ECHA Guidance on information requirements and chemical safety assessment, version 6.0, July 2017, Chapter R.7a, Section R.7.7.6.3).

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 27 October 2017.

ECHA held a third party consultation for the testing proposals from 31 January 2018 until 19 March 2018. ECHA did not receive information from third parties.

This decision does not take into account any updates after **30 May 2018**, 30 calendar days after the end of the commenting period.

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

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) for amendment.

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

In addition, you provided comments on the draft decision. These comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-62 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your 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 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 the Member States.

3. In carrying out the tests 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 tests must be suitable to assess these.

   Furthermore, 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.