EUROPEAN CHEMICALS AGENCY

Helsinki, 12 September 2018

Addressee
[redacted]

Decision number: TPE-D-2114440060-69-01/F
Substance name: 2,2-dimethyl-3-oxopropyl acetate
EC number: 811-188-6
CAS number: 16184-79-5
Registration number: [redacted]
Submission number: [redacted]
Submission date: 15/12/2017
Registered tonnage band: NA

## 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 TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum using the registered substance.**

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 ***19 September 2019***. 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 Claudio Carlon, Head of Unit, Evaluation E2

[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 an *in vitro gene mutation study in bacteria* performed according to OECD TG 471 with the registered substance that show positive results. The provided key study (according to OECD TG 471 and GLP) from 2016 with the reliability score 1 is performed with the registered substance, 2,2-dimethyl-3-oxopropyl acetate. In the study the recommended set of five strains has been used (*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvr A) and tested with and without exogenous metabolic activation (S9 Mix). A positive result was obtained in *S. typhimurium* TA100 in the absence of S9 Mix. The revertant colony number increases showed a dose-effect relationship. The dose related increases and the positive results were confirmed in subsequent experiments in the additional plate incorporation test, and in the confirmatory mutation test. The positive result indicates 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. You considered it necessary to generate information for this endpoint.

Hence, you have submitted a testing proposal for an *in vivo* Mammalian Alkaline Comet Assay to be performed with the registered substance.

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 proposed testing in rats. According to the test method OECD TG 489, the test shall be performed in rats.

You proposed testing by the oral route. 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.

In your testing proposal, the "*organs proposed to be evaluated are the forestomach and the liver, as the site of first contact after oral administration and the site of metabolism, respectively*". ECHA notes that, in relation to the site of contact tissue(s) to be collected and analysed in the comet assay, glandular stomach should be prefered over forestomach because the comet assay on glandular stomach was validated during adoption of OECD test guideline (while forestomach was not) and it is expected that both tissues are similarly exposed to the tested substance (for further discussions, see minutes of MSC-46 meeting). MSC further agreed that, in line with the test method OECD TG 489, 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

ECHA however considers that analysis of the forestomach would not use more animals, and you may thus decide to collect and analyse the forestomach, additionally to the three tissues requested in this decision.

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the proposed study under modified conditions, with the registered substance subject to the present decision:
*In vivo* mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

*Notes for your consideration*

You are 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".

Although your dossier is registered at Annex VII, 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 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.

## Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 7 June 2017.

ECHA held a third party consultation for the testing proposals from 1 September 2017 until 16 October 2017. ECHA did not receive information from third parties.

This decision does not take into account any updates after **21 February 2018**, 30 calendar days after the end of the commenting period.

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

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request.

You updated your registration on 15 December 2017. ECHA took the information in the updated registration into account, and did not amend the draft decision.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

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 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.