ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 22 August 2018

Addressee: [redacted]

Decision number: TPE-D-2114439151-59-01/F
Substance name: Reaction mass of 2-(1,1-dimethylpropyl)anthraquinone and 2-(1,2-dimethylpropyl)anthraquinone
EC number: 915-623-1
CAS number: NS
Registration number: [redacted]
Submission number subject to follow-up evaluation: [redacted]
Submission date subject to follow-up evaluation: 26 July 2017

## DECISION TAKEN UNDER ARTICLE 42(1) OF THE REACH REGULATION

By decision TPE-D-2114300120-80-01/F of 27 May 2015 ("the original decision") ECHA requested you to submit information by 5 June 2017 in an update of your registration dossier.

Based on Article 42(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA examined the information you submitted with the registration update specified in the header above, and concludes that

**Your registration still does not comply with the following information requirement:**

**In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; OECD 489).**

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

The respective Member State competent authority (MSCA) and National enforcement authority (NEA) will be informed of this decision.[1] They may consider enforcement actions to secure the implementation of the original decision.

[1] Only the final decision will be sent to the National enforcement authority so they can consider enforcement actions.

## 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, shall 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[2] by Kevin Pollard, Head of Unit 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.

**Appendix 1: Reasons**

**Mutagenicity – *in vivo* Mammalian Erythrocyte Micronucleus Test and *in vivo* Comet assay (Annex IX, Section 8.4., column 2)**

The original decision requested you to provide *In vivo* mammalian erythrocyte micronucleus test according to OECD 474 test guideline and *In vivo* mammalian alkaline comet assay according to OECD 489, both tests using the registered substance.

The original decision provided an opportunity for you to adapt the standard information requirement addressed in the original decision, and to perform the requested tests in combination in order to minimise vertebrate testing.

In the updated registration subject to this follow-up evaluation, you have provided the results of a combined study including an *in vivo* mammalian erythrocyte micronucleus test and an *in vivo* comet assay with the registered substance.

In the dossier update subject to the follow-up evaluation (submission [redacted] from 26 July 2017), you report the tail intensity percentage in stomach cells to be 36.82 ± 9.88 for the solvent/negative control.

The OECD 489 test guideline, adopted in 2014, indicates the following in relation to negative control [emphasis added]:

i. para 58, the first acceptability criteria is defined as *"a. The concurrent negative control is considered **acceptable for addition to the laboratory historical negative control database** as described in paragraph 16"*

ii. para 30: *"The % tail DNA in negative control animals should be **within the pre-established laboratory background range** for each individual tissue and sampling time for that species (see paragraph 16)."*

iii. para 16: *"Each laboratory should establish experimental competency in the comet assay by demonstrating the ability to obtain single cell or nuclei suspensions of sufficient quality for each target tissue(s) for each species used. The quality of the preparations will be evaluated firstly by the % tail DNA for vehicle treated animals falling **within a reproducible low range**. Current data suggest that the group mean % tail DNA [...] in the rat liver should be preferably not exceed 6%, which would be consistent with the values in the JaCVAM* [Japanese Center for the Validation of Alternative Methods] *validation trial (12) and from other published and proprietary data. [...]"*

While the OECD test guideline 489 does not provide explicit values as acceptability criteria for the solvent control in stomach, it is necessary to fulfil acceptability criteria for this parameter and the % tail DNA for vehicle treated animals should be within a 'low range'.

ECHA is guided by the acceptability criteria set out in the JaCVAM international validation

study of the in vivo comet assay (OECD 2014[3], Uno et al., 2015[4]). The JaCVAM validation studies for comet assay focused on two tissues, the liver and the (glandular) stomach, and gathered data from 14 different laboratories. In the JaCVAM report, it is stated [emphasis added]: "*Means of %DNA in tail should be 1-8% in the liver and* ***1-30% (preferably 1-20%) in the stomach***".

ECHA notes that these criteria have also been confirmed by the data in comet assays that ECHA received in the years 2014 to 2016. As also visible from the ECHA dissemination website, several independent comet assays performed from 2014 to 2016 by different test laboratories, following ECHA decisions, generated values of vehicle control percentage tail DNA in glandular stomach within the historical range reported by the respective test laboratory. These values were all well below 30%, i.e. the threshold value proposed for stomach in the JaCVAM report. This confirms the reliability of the standards of the JaCVAM validation studies.

In your comments on the draft decision, you provided a reply from the study director at [redacted], the test laboratory that performed the comet assay. The reference of this 8-page report is: [redacted].

In summary of the main points raised by this 8-page report, ECHA notes the following:

1. in your comments, you proposed and discussed a new criterion ('sensitivity/distinctive capacity'), but you did not mention or address the key argument and concern pointed out by ECHA in the draft decision, i.e. the negative control value for the comet assay in stomach that is considered too high.
2. You state that 'adaptation of the electrophoresis parameters does not necessarily increase the sensitivity/distinctive capacity of the assay'. However, ECHA considers that the elements you provide tend to show that the proposed changes in the electrophoresis parameters did increase the sensitivity of the comet assay.
3. You stated that 'Genotoxicity is not an organ-specific property and therefore all data should be taken into account in a weight-of-evidence approach when concluding on potential genotoxic properties of a test substance'.
    a. ECHA acknowledges that Genotoxicity is not an organ-specific property. However, ECHA notes that a genotoxic effect observed can be organ specific because of the different exposure of two organs (e.g. site of first contact organ vs. systemically exposed organ).
    b. Regarding weight-of-evidence (WoE), ECHA considers it is not possible to follow a WoE approach to determine the genotoxic effect on a site of contact tissue if there is no valid data available for genotoxicity assessed in a site of contact tissue.

ECHA considers that your comments did not address the argument and concern pointed out by ECHA in the draft decision.

[3] OECD 2014. ENV/JM/MONO(2014)10 Report of the JACVAM initiative International validation studies of the in vivo rodent alkaline comet assay for the detection of genotoxic carcinogens. Series on Testing and Assessment No. 196. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)10&doclanguage=en

[4] Uno Y, Kojima H, Omori T, Corvi R, Honma M, Schechtman LM, Tice RR, Beevers C, De Boeck M, Burlinson B, Hobbs CA, Kitamoto S, Kraynak AR, McNamee J, Nakagawa Y, Pant K, Plappert-Helbig U, Priestley C, Takasawa H, Wada K, Wirnitzer U, Asano N, Escobar PA, Lovell D, Morita T, Nakajima M, Ohno Y, Hayashi M. 2015. JaCVAM-organized international validation study of the in vivo rodent alkaline comet assay for detection of genotoxic carcinogens: II. Summary of definitive validation study results. Mutat Res Genet Toxicol Environ Mutagen, 786-788, 45-76. doi: 10.1016/j.mrgentox.2015.04.010

Taking into account the elements above, the mean tail intensity percentage for the solvent control in glandular stomach cells is still considered to be over the acceptable limit. Therefore, the reported comet assay study failed to comply with the JaCVAM (and hence OECD guideline 489) acceptability criteria for the comet assay for the glandular stomach tissue, and no adequate justification for the deviation was provided.

Therefore, ECHA concludes that the information provided from the comet assay for glandular stomach tissue is not acceptable and the request in the decision has not been fulfilled in this regard.

## Appendix 2: Procedural history

This decision is necessary after the follow-up evaluation according to Article 42(1) of the REACH Regulation, because in your updated registration you have provided new experimental information, which was not available to you or ECHA at the time when your registration was examined for the original decision.

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft of this decision was notified to the Member States Competent Authorities according to Article 51(1) of the REACH Regulation.

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 took into account your comments and did not amend the request(s).

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 prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. The Article 42(2) notification for the original decision is on hold until all information requested in the original decision has been received.