EUROPEAN CHEMICALS AGENCY

Helsinki, 23 August 2018

Addressee: [redacted]

Decision number: TPE-D-2114438959-28-01/F
Substance name: Reaction mass of 2,2'-[(4-methylphenyl)imino]bisethanol and 2-[[2-(2-hydroxyethoxy)ethyl](4-methylphenyl)amino]-ethanol
EC number: 911-490-9
CAS number: -
Registration number: [redacted]
Submission number subject to follow-up evaluation: [redacted]
Submission date subject to follow-up evaluation: 11 October 2017

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

By decision TPE-D-2114310297-55-01/F of 15 October 2015 ("the original decision") ECHA requested you to submit information by 23 October 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 IX, Section 8.4., column 2; test method: 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* Comet assay (Annex IX, Section 8.4., column 2)

The original decision requested you to provide *In vivo* mammalian alkaline comet assay according to OECD 489 using the registered substance.

In the updated registration subject to this follow-up evaluation, you have provided the results of an *in vivo* comet assay with the registered substance.

In the dossier update subject to the follow-up evaluation (submission [redacted] from 11 October 2017), you report the tail intensity percentage for the vehicle/negative control to be 70.33± 3.42 % in stomach cells and 55.34± 15.40 % in duodenal cells.

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. There are not enough data at this time to make recommendations about optimum or acceptable ranges for other tissues. [...]"*

While the OECD test guideline 489 does not provide explicit values as acceptability criteria for the vehicle 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 understands that a 'low range' in vehicle treated animals is needed in order to ensure a sufficient sensitivity of the comet assay to detect a treatment related effect.

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

[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

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 updated dossiers. As also visible from the ECHA dissemination website, several independent comet assays performed from 2014 to 2017 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.

Taking into account the elements above, the mean tail intensity percentage for the vehicle control in glandular stomach cells is 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.

ECHA notes that the above-mentioned JaCVAM validation report does not establish a threshold value for duodenum. However, ECHA considers that the statement in para. 16 of EOCD TG 489 - "*the % tail DNA for vehicle treated animals [should fall] within a reproducible low range*" - is a general statement that applies to all tissues. As already stated above a 'low range' in vehicle treated animals is needed in order to ensure a sufficient sensitivity of the comet assay to detect a treatment related effect. Moreover, several dossiers already registered under REACH contain comet assay data (also available on ECHA dissemination website) with percentage tail DNA values for vehicle control in the duodenum that are all below 10%. ECHA thus considers that the negative control value for duodenum should not exceed the 30% value defined by the JaCVAM report for the stomach. ECHA therefore concludes that the negative control values for duodenum in the provided *in vivo* Comet assay are not acceptable.

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

ECHA notes that in your comments to the draft decision you agreed with the request in the draft decision and proposed together with the test laboratory to repeat the comet assay for glandular stomach and duodenum and to adapt the electrophoresis method used in the test laboratory in order to achieve tail intensity values for the negative control group that match with the reference values described above. You proposed two actions:

1) to "generate negative & positive control historical data for glandular stomach and duodenum according an optimized method".
2) to "perform the repeat of the Comet assay for Accelerator (PT 25E or PT 25E/2)", "if acceptable %tail intensity values (1-30% (preferably 1-20%)) are obtained and the difference between the negative control values and the positive control values demonstrate sensitivity of the test system".

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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

ECHA notes that you plan to use an "adapted electrophoresis method" and an "optimized method". ECHA reminds that the OECD TG 489 (2016) provides recommendations regarding some parameters of the electrophoresis: e.g. duration (at least 20 minutes), potential (0.7 V/cm), or current (300mA).

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.

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.

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.