EUROPEAN CHEMICALS AGENCY

Helsinki, 17 November 2017

Addressee: [redacted]

Decision number: TPE-D-2114376203-54-01/F
Substance name: 2,5-bis-isocyanatomethyl-bicyclo[2.2.1]heptane
EC number: 411-280-2
CAS number: 74091-64-8
Registration number: [redacted]
Submission number subject to follow-up evaluation: [redacted]
Submission date subject to follow-up evaluation: 5 December 2014

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

Based on Article 42(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA has examined the information you submitted as a response to decision TPE-D-0000003665-69-04/F ("the original decision").

ECHA concludes that after the expiry of the deadline set in the original decision, your registration does not comply with the information requirements in Annex IX, 8.4.

The original decision set a deadline to provide the requested information. Whilst you updated your registration dossier by that deadline, some of the study results were found to be invalid. Therefore this decision[1] is sent to the respective Member State competent authority (MSCA) and national enforcement authority (NEA). They may consider enforcement actions to secure the implementation of the original decision.

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.

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 Ofelia Bercaru, Head of Unit E3

[1] Only the final decision will be sent to the Member State competent authority and the national enforcement authority so they can consider enforcement actions.
[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

This [draft] decision is necessary after the follow-up evaluation according to Article 42(1) of the REACH Regulation, because in your updated registration as a response to the decision TPE-D-0000003665-69-04/F ("the original decision"), you have provided substantial new experimental data which ECHA has assessed for compliance with the information requirements of the REACH Regulation and the outcome is that your registration still does not comply with the information requirements addressed in the original decision.

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* Comet assay in accordance with the protocol provided by you in your registration dossier (submission [redacted]); 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, that was performed with the registered substance and according to a test protocol provided by you in your registration dossier subject to decision TPE-D-0000003665-69-04/F.

The final decision was based on the registration dossier as submitted with submission number [redacted] from 24 January 2013. In that submission, you provided a protocol in which the first criterium to be met for the *in vivo* comet assay to be considered acceptable was '*The tail moment* [tail intensity x tail length] *observed of the solvent control should be less than 6'.*

In the dossier update subject to the follow-up evaluation (submission [redacted] from 5 December 2014), the evaluation criteria are different and are specific for each tissue: for the stomach, you consider that the *in vivo* comet assay is considered acceptable if '*the mean percentage tail intensity of the solvent control would be less than 35 '* and if '*the positive control ethyl methanesulfonate should produce at least a 2-fold statistically significant increase (p<0.01) in the tail intensity percentage compared to the vehicle treated animals*'. It is also indicated in the IUCLID dossier of submission [redacted] that these criteria are not absolute and other modifying factors may be taken into account in the final evaluation decision.

The updated registration dossier states that the tail intensity percentage in stomach cells was 47.20 ± 3.97 for the solvent/negative control and 89.39 ± 4.11 for the positive control (ECHA understands that you report 'the mean tail intensity percentage'). Therefore, the mean tail intensity percentage is significantly over the acceptable limit and the positive control did not reach the 2-fold limit.
You did not mention any "other modifying factors [that] may enter into the final evaluation decision" but only stated the following in "Overall remarks, attachments" of the updated registration dossier: "*The variation in the DNA damage of the vehicle treated stomach cells (with 47.20% higher than the acceptance criterion of 35%) was very low, which made it*

possible to clearly detect compound effects in the present study in stomach. This was confirmed by the effect that was seen with EMS. Although the induction factor with 1.9-fold was just below the acceptance criterion of 2-fold, a clear induction of the Tail Intensity with the positive control EMS was observed. The study integrity was not adversely affected by the deviation."

The OECD 489 test guideline 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 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 report (http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)10&doclanguage=en) and current practice. The JaCVAM validation studies for comet assay focused on two tissues, the liver and the stomach. In the JaCVAM report, section 6-4 Data-acceptance criteria, sub section 6-4-1 Negative control, 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**"*.

On 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 is an indication of how the quality standard of the OECD test guidelines should be interpreted. Taking this into account, it is not clear how the values in this case could qualify as low range and the information provided ("very low variation") does not provide a scientific justification for the deviation with the 35%.

Taking into account the elements above, ECHA is of the opinion that the reported comet assay study failed to comply with the acceptability criteria defined in the robust study summary of this comet assay and with the JaCVAM (and hence guideline) acceptability criteria for the comet assay, and no adequate justification is provided.

In your comments on the present decision, you indicated that once the decision is final you will initiate re-testing of glandular stomach cells without undue delay and update the dossier as soon as possible with new data in order to be compliant with the regulation, which is your highest priority. You also mentioned the re-evaluation document that was communicated to ECHA in September 2016 (see below for details). On the basis of this re-evaluation document, you make the following claims in your comments to the present decision:

- it is demonstrated that the outcome of the assay performed with glandular stomach cells is reliable in spite of the relatively high background values,
- the high background values are resulting from non-optimal test conditions /electrophoresis conditions optimized for liver cells, not for stomach cells.
- arguments are provided for acceptance of the validity of the assay, in spite the control values in the study deviate from the recommendations in the test guidelines.
- the provided historical background control data [...] are all in the same range.
- the sensitivity of the protocol used in the present study is considered acceptable, as demonstrated by the fact that positive results were obtained with different test substances under the same experimental conditions.
- the protocol as applied in the lab is sensitive enough to detect potential genotoxicity.
- by demonstrating reproducible negative and positive test conditions, the validity of the study protocol is warranted and the data are considered to be valid.

However, ECHA does not agree with your claim that this re-evaluation document demonstrated the reliability and validity of the outcome of the comet assay on glandular stomach. ECHA remains of the opinion that the background/negative control value obtained for the stomach is too high (even compared to the threshold values that you defined in the robust study summary) and is not acceptable. The explanation you provided for the deviation is that the test/electrophoresis conditions were optimised for liver cells and not for stomach cells. ECHA is of the opinion that it may also be beneficial to optimise the methods used to collect glandular stomach cells and to prepare cell suspensions. Non-optimal test conditions and electrophoresis conditions are not adequate reasons for considering that the study is valid. Moreover, the fact that positive results were obtained with different test substances under the same experimental conditions does not hide the fact that the negative control value for glandular stomach is judged too high (when comparing it with the acceptance criterion for the glandular stomach mentioned in the robust study summary or in the JaCVAM report). This thus does not allow to conclude that the sensitivity of the present study meets the OECD test guidelines.

Furthermore, ECHA understands that the historical background control data obtained in your laboratory are all in the same range, but ECHA also considers that this range is not acceptable (because reproducibility does not mean that the test protocol is sufficiently sensitive) and cannot warrant reliable results.

Therefore, as detailed above, the request in the original decision was not fully met, and you are still required to provide valid comet assay data for glandular stomach tissue.

During the follow-up evaluation, a telephone conference was organised between ECHA and the registrant, and as a result of the teleconference, you provided a document entitled [redacted], dated 13 September 2016. This re-evaluation of the test data, sent to ECHA on 16 September 2016, considered an extended historical data set. However, it did not change the outcome of the follow-up evaluation performed on the updated IUCLID dossier with submission number [redacted] dated 5 December 2014.

Appendix 2: Procedural history

The original decision was issued on 31 March 2014. You were required to update the registration with the requested information by 31 March 2016.

You updated your registration on 5 December 2014.

The follow-up evaluation was initiated on 2 November 2016.

ECHA notified you of the draft decision taken under Article 42(1) of the REACH Regulation on 17 January 2017, and invited you to provide comments.

ECHA took into account your comments and did not amend the decision.

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-56 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 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.