EUROPEAN CHEMICALS AGENCY

Helsinki, 21 February 2020

Addressees
Registrants of [redacted] listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
21 July 2016

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 2-piperazin-1-ylethylamine
EC number: 205-411-0
CAS number: 140-31-8

Decision number: [Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/D)]

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***31 May 2021***.

A. Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method OECD TG 476 or TG 490) with the Substance

Conditions to comply with the requests

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.

To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa;
- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if you have registered a substance at 100-1000 tpa;
- you have to comply with the requirements of Annexes VII to X of REACH, if you have registered a substance at above 1000 tpa.

The Appendices in this decision state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH. The ECHA Guidance documents are listed in the Appendix entitled Observations and technical guidance.

The test material used to perform the required studies must be selected and reported in accordance with the specifications prescribed in the Appendix entitled Observations and technical guidance.

The data sharing obligations of REACH require registrants to ensure that the costs of sharing information are determined in a fair, transparent and non-discriminatory way. Registrants are only required to share the costs of information that they are must submit to fulfil the information requirements for their registration.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

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.

Approved[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[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 A: Reasons for the requests to comply with Annex VIII of REACH

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 10 to 100 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII and VIII to the REACH Regulation.

1. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

An *in vitro* gene mutation study in mammalian cells is a standard information requirement in Annex VIII to REACH in case of a negative result in the *in vitro* gene mutation test in bacteria and the *in vitro* cytogenicity test.

Your dossier contains negative results for Ames and a negative result in an *in vivo* micronucleus test (which addresses the missing *in vitro* cytogenicity requirement). Therefore, the information requirement is triggered.

You have provided key and supporting studies in your dossier:

i. *in vitro* salmonella/microsome test similar to OECD TG 471 ([redacted] 1991), negative with and without metabolic activation;
ii. *in vitro* salmonella/microsome test similar to OECD TG 471 ([redacted] 1987), weakly positive with metabolic activation;
iii. *in vitro* salmonella/microsome test similar to OECD TG 471 ([redacted], 1983), negative with and without metabolic activation;
iv. *in vitro* salmonella/microsome test similar to OECD TG 471 ([redacted] 1980), negative with and without metabolic activation;
v. *in vitro* salmonella/microsome test similar to OECD TG 471 ([redacted] 1978), negative with and without metabolic activation;
vi. *in vitro* CHO HGPRT assay, ([redacted] 1981), negative with and without metabolic activation;
vii. *in vitro* CHO SCE assay [redacted]1981), positive with and without metabolic activation;
viii. *in vitro* unscheduled DNA synthesis (UDS) assay ([redacted]1981), with and without metabolic activation;
ix. *in vivo* micronucleus test ([redacted], 1994), negative.

We have assessed this information and identified the following issues:

To fulfil the information requirement, the in vitro gene mutation study on mammalian cells has to meet the requirements of OECD TG 476 or OECD TG 490. Some of the key parameter(s) of these test guidelines include:

a) One positive control must be included in the study. The positive control substance must produce a statistically significant increase in the response compared with the concurrent negative control.
b) Data on the cytotoxicity and the mutation frequency for the treated and control cultures must be reported.

The draft decision indicated that the reported data in the dossier for study (vi) does not include:

a) the inclusion of one positive control that produced a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control.

b) the reporting of data on cytotoxicity and of mutation frequency for the treated and control cultures.

In your comments to the draft decision, you have provided supporting information to address the issues (a) and (b) with regards to study (vi).
Some of the information provided is relevant to the request in the draft decision and we agree with your comments on positive controls, cytotoxicity, and on mutation frequency.

More specifically, regarding issue (a), the supporting information provided in your comments confirms that a positive control was used in all conditions and the control met the appropriate criteria as described above, i.e. the positive control functioned appropriately in each experiment by inducing a revertant colony count that was statistically increased versus the concurrent negative control.

Regarding issue (b) the supporting information provided in your comments proves that the study included relevant cytotoxicity measurements and mutation frequency in all treated groups and they are expressed relative to the concurrent negative control as described above.

However, the results of the study (vi) are inconclusive. The results show the Substance was negative with and without metabolic activation system over a 32-fold range of concentrations. Statistically significant increases above the concurrent solvent control were produced at a few non-consecutive dose levels tested. Therefore, to explain these differences additional data is required to conclude the Substance's potential to induce gene mutations in mammalian cells.

In your comments to the draft decision you explain that the statistically significant increases observed are acceptable, because they are in the range of historical controls. Furthermore, you refer to the *"scientific robustness of the study for the period in which it was performed"*.

We acknowledge your comment that the statistically significant increases observed are acceptable because they are in the range of historical controls. However, we note that, based on the information provided with your comments to the draft decision, some mutation frequencies are equal to 0 (zero) at concentrations where the cytotoxicity is not high. This is usually not observed and indicates that the test has not been performed accurately and according to the current test guideline requirements. Therefore, this study, which was performed almost four decades ago, is not scientifically robust and we consider it unreliable.

Therefore, the information requirement is not fulfilled.

In your comments to the draft decision you have provided an adaptation according to Annex XI, Section 1.2 of REACH (weight of evidence).

In your comments you provided the following justification for the adaptation: *"along with the weight of evidence assessment for all in vitro and in vivo genotoxicity studies for this molecule, there is no indication of genotoxic hazard potential. This includes 4 negative Ames studies in all strains and conditions, a negative in vitro UDS study, and a negative in vivo micronucleus test, in addition to this negative in vitro mammalian cell mutagenicity assay".*

Annex XI, Section 1.2 states that there may be sufficient weight of evidence (WoE) from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance of the information for the given regulatory information requirement. Subsequently, relevance, reliability, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

For *in vitro* gene mutation in mammalian cells, you have provided only one study (vi) which is unreliable, as demonstrated above. The other studies (i-v and vii-ix) address different types of mutagenicity but not mutation in mammalian cells *in vitro*.

Accordingly, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an OECD TG 476 or OECD TG 490 study.

Therefore, your adaptation is rejected and the information requirements is not fulfilled.

To fulfil the information requirement for the Substance, both the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) and the thymidine kinase gene (OECD TG 490) are considered suitable. However, when considering which Test Guideline to follow, we recommend OECD TG 490, because it also can provide information on the chromosomal aberration potency since there is no *in vitro* data currently available on that endpoint.

Deadline in the decision

The timeline indicated in the draft decision to provide the information requested was 6 months from the date of adoption of the decision. In your comments on the draft decision, you requested an extension of the timeline to 12 months. You justified your request stating that the study itself takes 5.5 months and additional time is needed for e.g. contract negotiations with a laboratory.

Therefore, ECHA has set the deadline to 12 months.

Appendix B: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of the REACH Regulation.

The compliance check was initiated on 16/01/2019.

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 within 30 days of the notification.

ECHA took into account your comments and did not amend the request(s), but amended the deadline.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix C: Observations and technical guidance

1. This compliance check decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

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.

4. Test guidelines, GLP requirements and reporting

   Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.

   Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses shall be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

   Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries[2]'.

5. Test material

   Selection of the test material(s)

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected test material must contain that constituent/ impurity.

   Technical reporting of the test material

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values [and other parameters relevant for the property to be tested, in this case...]. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

   Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[3].

[2] https://echa.europa.eu/practical-guides

6. List of references of Guidance documents[3]

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 in this decision.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 in this decision.

Read-Across Assessment Framework (RAAF) (March 2017), available at: https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

[3] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

Appendix D: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]