EUROPEAN CHEMICALS AGENCY

Helsinki, 6 February 2020

Addressees
Registrants of RMTAD_JS listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
28 November 2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Reaction mass of N,N,N',N'-tetrabutylmethylenediamine and dibutylamine
EC number: 948-040-6
CAS number: NS

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

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***14 May 2021***.

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

1. *In vivo* mammalian alkaline comet assay (Annex VIII, 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 Substance.

Appendix A states the reasons for the requests for information.

Appendix C (Observations and technical guidance) addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

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.

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 requirements applicable to all the Registrants subject to Annex VIII of REACH

This decision is based on the examination of the testing proposals you submitted.

1. ***In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2);**

Mutagenicity is a standard information requirement in Annex VIII to the REACH Regulation. Annex VIII, Section 8.4., Column 2 provides that appropriate *in vivo* mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII.

The technical dossier contains two *in vitro* studies performed with the Substance that show positive results:

- *In vitro* gene mutation study in bacteria, performed according to OECD TG 471,
- *In vitro* mammalian chromosome aberration test, performed according to OECD TG 473.

The positive results indicate that the Substance is inducing gene mutations and chromosomal aberrations under the conditions of the tests, and appropriate *in vivo* mutagenicity studies must be considered.

ECHA acknowledges that you provided an *in vivo* Mammalian Erythrocyte Micronucleus study performed according to OECD 474 with the Substance in order to follow up the concern for chromosomal aberration raised by the *in vitro* results. However, the key parameters of this test guideline are not met, because the reported data for the study do not include a maximum studied dose that is a maximum tolerated dose (MTD), or induces toxicity.

Moreover, an appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but must be considered. Therefore, there is an information gap and 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 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 for which testing is proposed. ECHA has taken these considerations into account.

ECHA notes that the proposed test is an appropriate test to follow-up *in vivo* the gene mutations and chromosomal aberration concerns identified *in vitro* as described in ECHA Guidance R.7a, section R.7.7.1. and figure R.7.7-1.

You did not specify the species to be used for proposed testing as well as you did not specify the route for the proposed testing.

The test must be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissues, performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, 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 analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Therefore, under Article 40(3)(a) of REACH, you are requested to carry out the proposed test.

Germ cells

You may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[2]) in addition to the other aforementioned tissues, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells, in accordance to Annex IX/X, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells. 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.

[2] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

Appendix B: Procedural history

ECHA received your registration containing the testing proposal for examination on 7 December 2018.

ECHA held a third party consultation for the testing proposals from 24 January 2019 until 11 March 2019. ECHA did not receive information from third parties.

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.

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 did not receive any comments within the 30 days.

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.

ECHA
EUROPEAN CHEMICALS AGENCY

Appendix C: Observations and technical guidance

1. This testing proposal examination decision does not prevent ECHA from initiating 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 your Member State(s).

3. 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 must 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'[3].

4. 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 is known to have or could have 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. 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"[4].

[3] https://echa.europa.eu/practical-guides
[4] https://echa.europa.eu/manuals

5. List of references of the ECHA Guidance documents[5]

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.

[5] 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 fulfilled
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]