EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 18 January 2021

Addressee
Registrant of JS_218-451-9 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
29/08/2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Dibutyl itaconate
EC number: 218-451-9
CAS number: 2155-60-4

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 ***25 July 2022***.

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

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method OECD TG 408) in rats combined with a screening test for reproductive/developmental toxicity (Annex VIII, Section 8.6.1; test method: OECD TG 421);

Your originally proposed test using the Substance is rejected, according to Article 40(3)(d):

2. In vivo alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay) (OECD TG 489), oral route, in the following tissues: liver and blood

Conditions to comply with the requests

The 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 to IX of REACH, if you have registered a substance at 100-1000 tpa;

Registrants are only required to share the costs of information they are required to submit to fulfil the information requirements for their registration.

The Appendix on general considerations addresses issues relevant for several requests while the other Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

The Appendix entitled 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 IX of REACH

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2);

Examination of the testing proposal

A sub-chronic toxicity study (90 day) is a standard information requirement in Annex IX, Section 8.6.2. to REACH.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats by the oral route according to OECD TG 408, combined with a screening study OECD TG 421 with the Substance.

ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

You proposed testing by the oral route, in rats. ECHA agrees with your proposal.

According to OECD TG 408, the rat is the preferred species and the most appropriate route of administration is the oral route since the Substance is a liquid of very low vapour pressure and no uses with spray application that could potentially lead to aerosols of inhalable size, are reported.

You proposed to extend the sub-chronic toxicity study (90 day) by including additional examinations/parameters: reproductive and developmental parameters by combining the 90 day study with a screening study OECD TG 421. ECHA accepts the combination and requests you to have a separate groups for the 90-day and fertility parts for the females. It is your discretion to perform the intended additional examinations, as long as those additional examinations do not interfere with the examinations prescribed by the OECD TG 408.

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

Under Article 40(3)(a) of REACH, you are requested to carry out the proposed test, with the Substance.

2. *In vivo* mammalian alkaline comet assay

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

Under Annex IX Section 8.4., column 2 of REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

Your dossier contains negative results for the *in vitro* gene mutation study in bacteria. Moreover, your dossier does not contain any study or adaptation in accordance with column 2 of Annex VIII, Section 8.4.2. Instead, you provided the following justification: "Since a false positive outcome of an in vitro genotoxicity test is expected based on cytotoxicity of DBI, it is technically not feasible to perform a valid in vitro genotoxicity test with mammalian cells". Therefore, you claim that "*As no reliable outcome is expected from the required in vitro tests with mammalian cells, it is proposed to address the potential genotoxic properties of DBI in an in vivo COMET assay. As this assay addresses all potential mode-of-actions (the COMET*

assay recognises primary DNA damage that would lead to gene mutations and/or chromosome aberrations, but will also detect DNA damage that may be effectively repaired or lead to cell death), performance of this in vivo study is considered to be sufficient to conclude on this endpoint."

Based on the above, you submitted a testing proposal for an *in vivo* mammalian alkaline comet assay to be performed with the Substance. You propose to perform the test by the oral route, in a single species, in the blood and liver.

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(s) for which testing is proposed. ECHA has taken these considerations into account.

As notified to you in a separate decision on a compliance check (CCH) on this Substance (communication no. CCH-D-2114538624-46-01/F), you are first requested to perform the *in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study. As explained in that decision, Annex XI Section 2 of REACH states that testing for a specific endpoint may be omitted, if it is technically not possible to conduct the study as a consequence of the properties of the substance. According to the ECHA Guidance R.52 such properties include solubility, high volatility, colour, reactivity with water, mixing of substances that may present a danger of fire or explosion, high reactivity and impossibility of radio-labelling of substances required in certain studies.

In your justification you claim that the misleading effect of cytotoxicity on the outcome of an *in vitro* genotoxicity study with mammalian cells has been described by several authors. You refer to publications by Armstrong *et al.* (1992), Kirkland *et al.* (2007), Fowler *et al.* (2012) and Honda et al. (2018). Based on these publications you conclude that "As a consequence, it is scientifically not justified to perform in vitro testing, as a positive outcome is expected related to cytotoxicity and not related to intrinsic genotoxic properties of the test item ("false positive" result)".

However, ECHA notes that cytotoxicity is not considered as a physico-chemical characteristic of the substance that may render the conduct of the study technically not possible under ECHA Guidance R.5. Therefore you have not demonstrated that it is technically not possible to conduct the study as a consequence of the properties of the Substance.

Consequently, your testing proposal for an *in vivo* mammalian alkaline comet assay with the Substance is not required as currently in your dossier there are no positive results that might indicate that the Substance induces chromosomal aberrations and/or gene mutations.

Hence, considering that there are no positive results in any of the *in vitro* genotoxicity studies in Annex VII or VIII, the *in vivo* somatic cell genotoxicity study (Annex IX, Section 8.4., column 2) is not triggered.

Outcome

Therefore, under Article 40(3)(d) of REACH, your proposed test is rejected.

[2] ECHA Guidance Chapter R.5: Adaptation of information requirements (December, 2011)

Appendix B: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 29 August 2019.

ECHA held a third party consultation for the testing proposal(s) from 24 February 2020 until 9 April 2020. ECHA did not receive information from third parties.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA did not receive any comments within the notification period.

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 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 and other guidance/ reference documents[5]

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.

ECHA Read-across assessment framework (RAAF, March 2017)[6]

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.

OECD Guidance documents
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

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]

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.