EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 18 January 2021

Addressees
Registrants of JS_218-451-9 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
29/08/2018

Registered substance subject to this decision ("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 CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***24 October 2022***.

Requested information must be generated using the Substance unless otherwise specified.

A. Information required from all the Registrants subject to Annex VII of REACH

1. Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: OECD TG 301B/C/D/F or OECD TG 310)

B. Information required from all the Registrants subject to Annex VIII of REACH

1. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.; test method: OECD TG 473) or In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487)

2. If negative results are obtained in test performed for the information requirement of Annex VIII, Section 8.4.2. then: In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490)

3. Justification for an adaptation of a Short-term repeated dose toxicity (28 days) based on the results of the Sub-chronic toxicity study (90 days) requested under Testing Proposal Decision (TPE-D-XXXXXXXXXX-XX-XX/F) (Annex VIII, Section 8.6.1.)

C. Information required from all the Registrants subject to Annex IX of REACH

1. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit)

The reasons for the requests are explained in the following appendices:

- Appendices entitled "Reasons to request information required under Annexes VII to IX of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[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 to request information required under Annex VII of REACH

1. Ready biodegradability

Ready biodegradability is an information requirement in Annex VII to REACH (Section 9.2.1.1.).

You have provided:

i. OECD TG 301D, key study by [redacted] (2017);
ii. OECD TG 301B, supporting study by [redacted] (2017);
iii. an adaptation under Annex XI, Section 1.3 ((Q)SAR), provided as supporting information.

We have assessed this information and identified the following issues:

A. To fulfil the information requirement, a study must comply with the OECD TG 301 or 310 (Article 13(3) of REACH). Therefore, for a study according to OECD TG 301, the following requirements must be met:

Validity criteria
- the difference of extremes of replicate values of the removal of the test material at the plateau, at the end of the test or, if appropriate, at the end of the 10-d window is ≤ 20%;
- for OECD TG 301B, the total CO_2 evolution in the inoculum blank at the end of the test does not normally exceed 40 mg CO_2/L;

Technical specifications impacting the sensitivity/reliability of the test
- for an OECD TG 301D study, the concentration of the test material is in the range of 2-10 mg/L, corresponding to 5 to 10 mg ThOD/L;
- for an OECD TG 301D study, the concentration of the inoculum is set to reach a bacterial cell density of 10^4 to 10^6 cells/L in the test vessel. The concentration of added inoculum is ≤ 5 mL effluent/L;
- for an OECD TG 301B study, the concentration of the inoculum is set to reach a bacterial cell density of 10^7 to 10^8 cells/L in the test vessel. The suspended solid concentration is ≤ 30 mg/L;

Reporting of the methodology and results
- the results of measurements at each sampling point in each replicate is reported in a tabular form;

Your registration dossier provides an OECD TG 301D (study i. above) showing the following:
- the test was conducted at two test concentrations (1 and 3 mg test material/L corresponding to 2.18 and 6.54 mg ThOD/L);
- the inoculum density was 4 mL filtrate of secondary effluent per litre of final volume. No information on inoculum density expressed as cells/mL is provided;
- the results of measurements at each sampling point in each replicate is not provided;
- at both concentrations, you report that biodegradation reached ≥ 60% after 14 days but was below 60% after 28 days (52 and 55% at 1 and 3 mg test material/L, respectively). You have not provided a justification as to why oxygen consumption was reduced by the end of the test.

In addition, your registration dossier provides an OECD TG 301B (study ii. above) showing the following:

- on the inoculum density used to conduct the test, you report that the concentration of the sludge was 3.5 and 3.7 g/L in experiment A and B, respectively. For the suspended solids concentrations, your report the following information: "*10 mL supernatant liquid/L*", which indicates that the inoculum density as suspended solids was 35 and 37 mg/L in experiment A and B, respectively. No information on inoculum density expressed as cells/mL is provided;
- the relative biodegradation values calculated from the measurements performed during experiment A revealed 74% and 17% biodegradation (based on ThCO2), for the duplicate bottles tested. Results from experiment B revealed 67% and 36% biodegradation (based on ThCO2), for the duplicate bottles tested;
- the total CO_2 evolution in the inoculum blank at the end of the test is not provided.

Based on the above, study i. shows the following deficiencies:

- the test was conducted at two test substance concentrations (1 and 3 mg test material/L). However, only the test conducted at 3 mg test material/L meets the specifications of OECD TG 301D;
- as you have not provided information on the inoculum density expressed in cells/mL, it is not possible to verify that it falls within the acceptable range described by the guideline;
- as you have not reported the results of measurements at each sampling point in each replicate, it is not possible to verify that the validity criteria of OECD TG 301D were met and that the interpretation of the study results are adequate (for instance, fulfillment of the 10d-window criteria). Furthermore you have not provided any explanation as to why an overall reduction in oxygen consumption was observed at day 28 and why it does not impact your conclusion that the Substance should be regarded as readily biodegradable.

For study ii., the following deficiencies were identified:

- the inoculum density as suspended solids was above the maximum acceptable value of 30 mg/L. Furthermore, the information provided on inoculum density does not allow to verify that it falls within the acceptable range described by the guideline;
- this study does not meet all validity criteria of OECD 301B as the difference of extremes of replicate values of the removal of the test material was > 20%;
- as you have not provided information on the total CO_2 evolution in the inoculum blank, it is not possible to verify that it was below 40 mg CO_2/L at the end of the test.

Based on the above, none of these studies meet the information requirement of OECD TG 301.

B. ECHA Guidance R.7.9.5.1. specifies that (Q)SARs for predicting ready biodegradation are not yet sufficiently accurate to predict rapid degradation. However, when no useful information on degradability is available (either experimentally derived or estimated), (Q)SAR predictions can be used as supporting evidence that the substance is not rapidly degradable.

Your dossier provides (Q)SARs predictions. You have used this information to support that the Substance is readily biodegradable. As explained above, (Q)SARs predictions alone is not adequate to conclude on the persistence of the Substance. Therefore, this information does not fulfil the information requirement and your adaptation is rejected.

On this basis, the information requirement is not fulfilled.

Appendix B: Reasons to request information required under Annex VIII of REACH

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is a standard information requirement in Annex VIII to REACH.

Your dossier does not contain any study or adaptation in accordance with column 2 of Annex VIII, Section 8.4.2., for this information requirement. Instead you provide 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*".

Although you do not explicitly claim an adaptation, ECHA understands that the information you provided was submitted in order to meet the required information by way of adaptation under Annex XI, Section 2 of REACH (testing is technically not possible).

We have assessed this information and identified the following issue:

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.5[2] 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.

Based on the above, your adaptation is rejected and the information requirement is not fulfilled.

To fulfil the information requirement for the Substance, either *in vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) or *in vitro* micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487) are considered suitable.

2. In vitro gene mutation study in mammalian cells

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.

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

For Annex VIII, 8.4.3., you have not provided any study or adaptation according to the specific rules for adaptation of Annex VIII, 8.4.3. in your dossier. 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*".

Triggering of the study

Your dossier contains (i) a negative result for *in vitro* gene mutation study in bacteria, and (ii) no data for the other study (*in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study).

The adaptation for *the in vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study provided in the dossier is rejected for the reasons provided in section B.1.

The result of the request for information in section B.1.will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

Justification provided

Although you do not explicitly claim an adaptation, ECHA understands that the information you provided was submitted in order to meet the required information by way of adaptation under Annex XI, Section 2 of REACH (testing is technically not possible).

We have assessed this information and identified the following issue:

> 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. The reasons for which the same adaptation was rejected under Section 1 of Annex B above, apply equally to this information requirement.

Accordingly, your adaptation is rejected and the information requirement is not fulfilled.

Consequently, you are required to provide information for this endpoint, if the *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study provides a negative result.

To fulfil the information requirement for the Substance, either the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) or the thymidine kinase gene (OECD TG 490) are considered suitable.

3. Justification for an adaptation of the Short-term repeated dose toxicity study (28-day)

A Short-term repeated dose toxicity study (28 days) is a standard information requirement in Annex VIII to REACH. This information may take the form of a study record or a valid adaptation in accordance with either a specific adaptation rule under Column 2 of Annex VIII or a general adaptation rule under Annex XI.

You have provided an adaptation according to Column 2 of Annex VIII, Section 8.6.1.

We have assessed this information and identified the following issue:

As provided in Annex VIII, Section 8.6.1, Column 2, you may adapt the information requirement, provided you fulfil the following criterion:

- a reliable sub-chronic toxicity study (90-day) is available

However, you stated in your dossier that *"a short-term toxicity study does not need to be conducted because a sub-chronic (90 days) or chronic toxicity study is proposed to be conducted with an appropriate species, dosage, solvent and route of administration"*

Nevertheless, as the sub-chronic toxicity study (90-day) has not yet been submitted, your adaptation is rejected.

Therefore, the information you provided do not fulfil the information requirement.

While Column 2 of Annex VIII, Section 8.6.1. provides that an experimental study for this endpoint is not needed if a reliable sub-chronic (90 days) or chronic toxicity study is available, we take note of the fact that the testing proposal decision (communication no. TPE-D-2114538623-48-01/F) requests the registrants concerned to generate and submit a reliable sub-chronic toxicity study (90 days). According to Column 2 of Annex VIII, Section 8.6.1. and in order to prevent unnecessary animal testing, a short term toxicity study (28 days) does not therefore need to be conducted.

Instead, you are requested to submit a justification for the adaptation provided in Column 2 of that provision once the requested sub-chronic toxicity study (90 days) will be submitted.

Appendix C: Reasons to request information required under Annex IX of REACH

1. Pre-natal developmental toxicity study in one species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

You have provided a reference to a 28 days repeated dose study, including screening for effects on reproduction and development for Dibutyl Itaconate via oral route in rats, a key study ([redacted] 2018). You also concluded that *"No information on effects of repeated exposure to DBI are available. Data on analogues have been sought and evaluated, but were found not adequate to address this endpoint for the registered substance. Upon registration of Annex VIII, the registrant will update without delay to Annex IX. This dossier will be updated with a test proposal for a 90-day study combined with a screening study for developmental/ reproduction effects."*

Although you do not explicitly claim an adaptation, ECHA understands that the information you provided was submitted in order to meet the required information by way of adaptation.

We have assessed this information and identified the following issues:

A. In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, information provided has to meet the requirements of OECD TG 414 in one species, e.g external, skeletal and visceral malformations and variations has to be investigated as described in OECD TG 414.

   However, you have not provided a study based on OECD TG 414. Instead, you have provided a reference to 28-day study including screening for effects on reproduction and development. In addition, a study according to OECD 422 does not inform on skeletal and visceral malformations and variations as required by OECD TG 414.

B. Pursuant to Article 10(a)(vii) of the REACH Regulation, the information set out in Annex VII to XI must be provided in the form of a robust study summary. Article 3(28) defines a robust study summary as a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report.

   ECHA understands that you refer to a study similar to OECD TG 422. However, you have not provided a robust study summary; e.g. no information on the results is available in your dossier.

C. In paragraph 35 of its decision in case A-011-2018, ECHA Board of Appeal stated that a registrant who submits an adaptation must set out clearly, in the relevant part of its registration dossier, the provision of Annexes VII to XI on which the adaptation is based, the grounds for the adaptation, and the scientific information which substantiates those grounds.

   However, your dossier does not set out clearly the provision of Annexes VII to XI on which the adaptation for a PNDT study is based. In addition, your statement does not specify any grounds for an adaptation or any scientific information which would substantiate those grounds.

Therefore, the information you have provided in your dossier does not fulfil the information requirement.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[3] administration of the Substance.

[3] ECHA Guidance R.7a, Section R.7.6.2.3.2.

Appendix D: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

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

2. 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.

3. 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 on How to report robust study summaries[4].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - 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.

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[5].

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

Appendix E: Procedure

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

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

The compliance check was initiated on 13 February 2020.

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 F: List of references - ECHA Guidance[6] and other supporting documents

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 where relevant.

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 where relevant.

Read-across assessment framework (RAAF, March 2017)[7]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[8]

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.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

[6] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[7] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[8] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

OECD Guidance documents[9]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

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

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

[9] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix G: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.