~~Confidential~~

Helsinki, 09 February 2023

Addressee
Registrant of JS_EC_210-519-6 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
17/02/2016

Registered substance subject to this decision ("the Substance")
Substance name: Dimethyl itaconate
EC number: 210-519-6

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 below, by the deadine of ***16 February 2026***.

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

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

1. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: EU C.1./OECD TG 203)

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

2. *In vivo* mammalian alkaline comet assay (Annex IX, Section 8.4., Column 2; test method: EU B.62./OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

OR

Transgenic rodent somatic and germ cell gene mutation assay (Annex IX, Section 8.4., Column 2; test method: OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

3. 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)

4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210)

The reasons for the decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

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 requirements for testing and reporting new tests under REACH, see Appendix 4.

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 Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[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 1: Reasons for the decision

Contents

Reasons related to the information under Annex VIII of REACH

1. Short-term toxicity testing on fish

1 Short-term toxicity testing on fish is an information requirement under Annex VIII to REACH (Section 9.1.3.).

1.1. Information provided

2 You have provided:

i. an OECD TG 236 study (2015) with the Substance;

3 Further, you have also adapted this information requirement by using Column 2 of Annex VIII, Section 9.1.3. To support the adaptation, you have provided the following information:

ii. a claim that a fish early-life stage (FELS) toxicity test according to OECD TG 210 is available for the Substance;

1.2. Assessment of the information provided

4 We have assessed this information and identified the following issues:

1.2.1. The provided study (i) does not meet the information requirement

5 To fulfil the information requirement, a study must comply with OECD TG 203 (Article 13(3) of REACH). Therefore, the following specifications must be met:

6 Key parameter to be measured:

7 In the OECD TG 203 studies, the key parameter to be measured is the concentration of the test material leading to the mortality of 50% of the juvenile fish at the end of the test.

8 Your registration dossier includes an OECD TG 236 study with the Substance.

9 Key parameter to be measured:

10 In the OECD TG 236 studies, the newly fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hours. Every 24 hours, up to four apical observations are recorded as indicators of lethality: 1. coagulation of fertilised eggs, 2. lack of somite formation, 3. lack of detachment of the tail-bud from the yolk sac, and 4. lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC50 is calculated.

11 Hence, in the OECD TG 236 studies, the key parameter to be measured is the concentration of the test material leading to the mortality of 50% of the fish embryos, not the juvenile, at the end of the test.

12 Based on the above,

- the information provided does not cover the key parameter(s) required by the OECD TG 203

13 Therefore, the requirements of OECD TG 203 are not met.

1.2.2. The Column 2 adaptation is not supported by data

14 Your registration dossier does not include an OECD TG 210 study with the Substance.

15 On this basis, the information requirement is not fulfilled.

16 In the comments to the draft decision, you agree that study (i) does not meet the requirements of OECD TG 203. Instead of conducting a new OECD TG 203 study as requested, you state your intention to conduct the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) study requested in request 4.

17 For your information, REACH Annex VIII, section 9.1.3, column 2 specifies that the registrant does not have to conduct a short-term toxicity test on fish if a long-term toxicity test on fish is available. At present, no long-term toxicity study on fish is provided in the IUCLID dossier, therefore currently no conclusion on the compliance can be made. You remain responsible for complying with this decision by the set deadline.

1.3. Study design and test specifications

18 To fulfil the information requirement for the Substance, the Fish, Acute Toxicity Test (test method OECD TG 203) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

Reasons related to the information under Annex IX of REACH

2. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

19 Under Annex IX, Section 8.4, Column 2, 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.

2.1. Trigger

20 In relation to the first condition, your dossier contains positive results for the in vitro gene mutation study in bacteria which raises the concern for gene mutation.

21 In relation to the second condition, your dossier contains the following in vivo study:

a) In Vivo Liver Unscheduled DNA Synthesis (UDS) Assay (2013).

22 You conclude on the basis of study (a) that its negative results 'overule' the positive results in the in vitro study.

23 The study (a) is described as a UDS test. This is an indicator test that detects some DNA repair mechanisms (measured as unscheduled DNA synthesis in liver cells). However, as reminded in the Guidance on IRs & CSA, R.7a, Section R.7.7.6.3 (page 571-572), the UDS test is sensitive to some (but not all) DNA repair mechanisms and not all gene mutagens are positive in the UDS test. The sensitivity of the UDS test has been questioned (Kirkland and Speit, 2008 [1]) and its lower predictive value towards rodent carcinogens and/or in vivo genotoxicants has been confirmed in comparison with the TGR assay and comet assay (EFSA, 2017 [2]). Therefore, a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation. Moreover, though a positive result in the UDS assay can indicate exposure of the liver DNA and induction of DNA damage by the substance under investigation, it is not sufficient information to conclude on the induction of gene mutation by the substance.

[1] Kirkland D and Speit G (2008) Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing *in vivo*. Mutat Res 654:114-32.

[2] EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, Gurtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, van Benthem J, Carfì M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi. org/10.2903/j.efsa.2017.5113.

24 Therefore, the study (a) does not provide appropriate results.

25 ECHA considers that an appropriate in vivo follow up genetic toxicity study is necessary to address the concern identified in vitro.

26 We have assessed this information and identified the following issue(s):

2.2. Information provided and its assessment

27 Only information addressed in the trigger section was provided and it only shows that the trigger is met.

28 On this basis, the information requirement is not fulfilled.

29 In the comments to the draft decision, you indicate your intention to adapt this information requirement by means of weight of evidence according to Annex XI, Section 1.2, and of grouping and read-across according to Annex XI, Section 1.5, of the REACH Regulation.

30 However, the information in your comments is not sufficient for ECHA to make an assessment, because while you have described your intentions, you have not provided any new scientific information addressing the information requirement or supporting your intended adaptation. You remain responsible for complying with this decision by the set deadline.

2.3. Test selection

31 According to the Guidance on IRs & CSA, Section R.7.7.6.3 either the in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) or the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) are suitable to follow up a positive in vitro result on gene mutation.

2.4. Specification of the study design

2.4.1. Comet assay

32 In case you decide to perform the comet assay, according to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified (OECD TG 489, paragraph 23).

33 Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

34 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.

2.4.1.1. Germ cells

35 A subsequent germ cell genotoxicity study (TGR/OECD TG 488) may still be required under Annex IX, in case 1) an in vivo genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

36 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, 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 the comet assay, 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.

2.4.1.2. Cross-linking properties

37 You are reminded that you may decide to take into account the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay in order to detect cross links. Therefore, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in the OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA crosslinks. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23 *[1]* in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS); or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39 *[2-5]* in the OECD TG 489 or Pant et al. 2015 *[6]*). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

[1] Nesslany *et al.* (2007) *In vivo* comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds *Muta Res*;630(1-2):28-41.
[2] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.
[3] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.
[4] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.
[5] Spanswick *et al.* (2010) Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay. *Methods Mol Biol*;613:267-282.
[6] Pant K et al. (2015) Modified in vivo comet assay detects the genotoxic potential of 14-hydroxycodeinone, an α,β -unsaturated ketone in oxycodone. Environ Mol Mutagen;56(9):777-87.

2.4.2. TGR assay

38 In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats.

39 Also, according to the test method OECD TG 488, the test substance is usually administered orally.

40 Based on OECD TG 488, you are requested to follow the 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

41 According to the test method OECD TG 488, the test must be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, from glandular stomach and duodenum as rapidly proliferating tissue and site of direct 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 mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

2.4.2.1. Germ cells

42 A subsequent germ cell genotoxicity study (TGR/OECD TG 488) may still be required under Annex IX , in case 1) an in vivo genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

43 In case you choose to perform a TGR assay, you must collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, in accordance to Annex IX, Section 8.4., Column 2, to decide on the need for assessment of mutation frequency in the collected germ 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.

3. Pre-natal developmental toxicity study in one species

44 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX, Section 8.7.2.

3.1. Information provided

45 You have adapted this information requirement by using Annex XI, 1.1.2 using

i. an OECD TG 408/415 study (2014).

3.2. Assessment of the information provided

3.2.1. Assessment of the information provided

46 Annex XI Section 1.1.2 applies only to studies conducted prior to 1 June 2008 (Annex XI, Section 1.1.).

47 Your study was conducted after that date. Therefore, the adaptation is rejected.

48 For the sake of completeness, the study is assessed below.

3.2.2. The provided study does not meet the information requirement

49 To fulfil the information requirement, a study must comply with OECD TG 414 (Article 13(3) of REACH). Therefore, the following specifications must be met:

a) the exposure duration is at least from implantation until one day prior to scheduled caesarean section;
b) the foetuses are examined for body weight, number and percent of live and dead foetuses and resorptions, sex ratio, external, skeletal and soft tissue alterations (variations and malformations), measurement of anogenital distance in all live rodent foetuses.

50 However, the following specifications are not according to the requirements of the OECD TG 414:

a) the exposure duration was approximately 17 weeks and there were no planned caesarean sections;
b) data on the examination of the foetuses (i.e. number and percent of resorptions, skeletal and soft tissue alterations (variations and malformations), measurement of anogenital distance in all live rodent foetuses), including incidence and severity, are missing.

51 Based on the above, the information you provided do not fulfil the information requirement.

52 In the comments to the draft decision, you agree with the request.

3.3. Specification of the study design

53 A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species.

54 The study must be performed with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

55 Therefore, the study must be conducted in rats or rabbits with oral administration of the Substance.

4. Long-term toxicity testing on fish

56 Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

4.1. Information provided

57 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1.

4.2. Assessment of the information provided

58 We have assessed this information and identified the following issue:

4.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

59 Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on long-term toxicity to fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

60 Your adaptation is therefore rejected.

61 On this basis, the information requirement is not fulfilled.

62 In the comments to the draft decision, you agree with the request.

4.3. Study design and test specifications

63 To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; (ECHA 2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).

All Guidance on REACH is available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF), ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs), ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: 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 17 November 2021.

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

In your comments you indicated that a deadline extension of 12 months would be needed. You did not provide any justification from a testing laboratory to support your request. However, the deadline of the decision has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

ECHA took into account your comments and amended the deadline.

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

Your comments agreeing on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee unanimously agreed on the draft decision in its MSC-81 written procedure. ECHA adopted the decision under Article 51(6) of REACH.

Appendix 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- 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;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

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.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. 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[2].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. Test material

(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 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.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[3].

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