~~Confidential~~

Helsinki, 20 June 2023

Addressees
Registrant(s) of JS_131-11-3 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
19/01/2022

Registered substance subject to this decision ("the Substance")
Substance name: Dimethyl phthalate
EC/List number: 205-011-6

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 listed below, by the deadline of ***25 September 2026***.

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

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

1. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202)

2. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201)

3. Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: EU C.4. A/B/C/D/E/F/OECD TG 301A/B/C/D/E/F or EU C.29./OECD TG 310)

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

4. 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)

5. In vivo genetic toxicity study also requested below (triggered by Annex VIII, Section 8.4., column 2)

6. 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 below (Annex VIII, Section 8.6.1.)

7. 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

8. In vivo genetic toxicity study (Annex IX, Section 8.4., column 2) to be selected according to the following specifications:

If the results of the *in vitro* cytogenicity study requested under 4. are **negative**:

Transgenic rodent somatic and germ cell gene mutation assay (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.
OR
In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum;

If the results of the *in vitro* cytogenicity study requested under 4. are **positive**:

In vivo mammalian alkaline comet assay (test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, or if justified, in mice, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

9. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats

10. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211)

11. Further long-term aquatic toxicity (Annex IX, Section 9.1., column 2; test method OECD TG 234) on Japanese medaka (Oryzias latipes) or zebrafish (Danio rerio). The test must be conducted with five test concentrations as specified in paragraph 30 of the OECD TG 234

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

12. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: OECD TG 414) by oral route, in a second species (rabbit)

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.

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

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

0. Reasons common to several requests

0.1. Read-across adaptation rejected

1 You have adapted the following standard information requirements by using grouping and read-across approach under Annex XI, Section 1.5:

- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study in a second species (Annex X, Section 8.7.2.)

2 ECHA has considered the scientific and regulatory validity of your read-across approach(es) in general before assessing the specific standard information requirements in the following sections.

3 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

4 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

0.1.1. Predictions for toxicological properties

5 You provide a read-across justification document in IUCLID Section 13.

6 You predict the properties of the Substance from information obtained from the following source substance(s):

DEP diethyl phthalate (CAS 84-66-2), EC 201-550-6.

7 You provide the following reasoning for the prediction of toxicological properties: "*This read across is based on the assumption that the potential human health hazard of the two phthalates would originate from the structural component of esterified phthalic acid present in both molecules. Of note, data available show only a very low or no toxicity up to limit doses and both substances are consequently not classified for human health. Both, the source substance and the target substance, belong to the chemical group of low molecular weight phthalates. Their common structural characteristic is that they are esters of phthalic acid of alcohols with short primary carbon length backbones. They only differ in the length of the carbon backbone of C1 or C2 for DMP and DEP, respectively*".

8 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source substance.

9 We have identified the following issues with the predictions of toxicological properties:

0.1.1.1. Missing supporting information to compare the properties of the substances

10 Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide supporting information to scientifically justify the read-across explanation for prediction of properties. The set of supporting information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6, Section R.6.2.2.1.f.).

11 Supporting information must include bridging studies to compare properties of the source substance.

12 As indicated above, your read-across hypothesis is based on the assumption that the structurally similar source substance(s) cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the source substance(s) is necessary to confirm that the substances cause the same type of effects. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

13 For the source substance, you provide the study used in the prediction in the registration dossier. Apart from that study, your read-across justification or the registration dossier does not include any robust study summaries or descriptions of data for the Substance that would confirm that both substances cause the same type of effects.

14 In the absence of such information, you have not established that the Substance and the source substance(s) are likely to have similar properties. Therefore you have not provided sufficient supporting information to scientifically justify the read-across.

0.1.1.2. Inadequate or unreliable source studies

15 According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results to be read across must:

(1) be adequate for the purpose of classification and labelling and/or risk assessment;

(2) have adequate and reliable coverage of the key parameters addressed in the corresponding study that shall normally be performed for a particular information requirement;

(3) cover an exposure duration comparable to or longer than the corresponding study that shall normally be performed for a particular information requirement if exposure duration is a relevant parameter.

16 Specific reasons why the studies on the source substance do not meet these criteria are explained further below under the applicable information requirement sections 6, 9 and 12. Therefore, no reliable predictions can be made for these information requirements.

0.1.2. Conclusion on the read-across approach

17 For the reasons above, you have not established that relevant properties of the Substance can be predicted from data on the source substance(s). Your read-across approach under Annex XI, Section 1.5. is rejected.

Reasons related to the information under Annex VII of REACH

1. Short-term toxicity testing on aquatic invertebrates

18 Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.).

1.1. Information provided

19 You have provided:

(i) a short-term toxicity study (EPA method for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians, 1975) on daphnia (1984) with the Substance.

1.2. Assessment of the information provided

1.2.1. The provided study does not meet the specifications of the test guideline

20 To fulfil the information requirement, a study must comply with OECD TG 202 (Article 13(3) of REACH). Therefore, the following specifications must be met:

21 Technical specifications impacting the sensitivity/reliability of the test

a) At least 20 animals are used at each test concentration and for the controls.

22 Reporting of the methodology and results

b) The test design is reported (*e.g.* age of the animal).
c) The test procedure is reported (*e.g.* composition of the test medium).
d) The method for deriving the effect concentrations is explained.

23 In study (i) described as acute toxicity of fourteen phthalate esters to Daphnia magna:

24 Technical specifications impacting the sensitivity/reliability of the test

a) Under the test condition section, you have stated that 5 organisms per vessel and 3 vessels per concentration were used in the study, indicating that 15 animals were used at each test concentration and for the controls.

25 Reporting of the methodology and results

b) On the test design, you have not specified the age of the animal used.
c) On the test procedure, you have not specified the composition of the test medium.
d) You have reported the effect concentrations based on measured concentrations. However, you did not specify how the effect concentration (LC50>52 mg/l) was derived. ECHA cannot verify the correctness of the reported effect concentrations, because there are discrepancies between the measured concentrations at the end of the test (48h) under the Material and method section of the IUCLID and those reported under the result section (i.e. the table titled "[redacted]") under the Results and discussion section.

26 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically, the number of animals used for each concentration is lower than required by the test guideline. Consequently, the study is compromised due to low statistical power.

- the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically, it is not possible to verify that the test was conducted on neonates and that the test medium comply with the requirement of the test guideline. You have not demonstrated that the effect concentration was correctly reported.

27 In the comments to the draft decision, you agree that the study provided does not meet the current standards of an OECD TG 202 study. Instead of performing a new OECD TG 202 study as requested, you propose to adapt the information requirement by using Annex VII section 9.1.1, column 2.

28 This provision specifies that the short-term toxicity study does not need to be conducted if a long-term aquatic toxicity study on invertebrates is available. At present no long-term toxicity study on aquatic invertebrates is provided in the IUCLID dossier, therefore no conclusion on the compliance can currently be made. You remain responsible for complying with this decision by the set deadline.

29 Therefore, the requirements of OECD TG 202 are not met, and the information requirement is not fulfilled.

2. Growth inhibition study aquatic plants

30 Growth inhibition study on aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

2.1. Information provided

31 You have provided:

(i) a growth inhibition study to algae (DIN 38412 L9, 1992) with the Substance

(ii) a statement that a new OECD TG 201 study with the Substance is currently underway.

32 As the study you refer to under (ii) has not yet been provided, ECHA is not in a position to assess its validity.

2.2. Assessment of the information provided

2.2.1. The provided study (study (i)) does not meet the specifications of the test guideline

33 To fulfil the information requirement, a study must comply with OECD TG 201 (Article 13(3) of REACH). Therefore, the following specifications must be met:

34 Technical specifications impacting the sensitivity/reliability of the test

a) For *Desmodesmus subspicatus* the initial cell density is 2-5 x10^3 cells/mL.

35 Characterisation of exposure:

b) Analytical monitoring must be conducted.
c) The results can be based on nominal or measured initial concentration only if the concentration of the test material has been maintained within ±20 % of the nominal or measured initial concentration throughout the test.

36 Reporting of the methodology and results:

d) Method for determination of biomass and evidence of correlation between the measured parameter and dry weight are reported.
e) Results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form.
f) Microscopic observation performed to verify a normal and healthy appearance of the inoculum culture are reported. Any abnormal appearance of the algae at the end of the test is reported.

37 In study (i) described as acute static growth inhibition test:

38 Technical specifications impacting the sensitivity/reliability of the test

a) The test was conducted on *Desmodesmus subspicatus* and the initial cell density was 10000 cells/mL.

39 Characterisation of exposure

b) No analytical monitoring of exposure was conducted.
c) You have reported effect concentrations based on the nominal concentrations although you have not demonstrated that the concentration of the test material has been maintained throughout the test.

40 Reporting of the methodology and results:

d)-f) You did not provide any information listed above.

41 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically,
  - High biomass of the test organism applied at the start of the study may impact the sensitivity of the test.
  - In the absence of analytical monitoring, you have not demonstrated that the test material has been satisfactorily maintained throughout the test.
- the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically, it is not possible to confirm the requirements of the test guideline and the validity of the study based on the information provided in the dossier.

42 In your comments to the draft decision, you have provided the information on the new OECD TG 201 study (i.e. study (ii)), in the format of an attached copy of the modified Robust Study Summary (RSS). You have updated your dossier with the modified RSS for the OECD TG 201 study.

43 As explained above, to fulfil the information requirement, a study must comply with OECD TG 201 (Article 13(3) of REACH).

44 Reporting of the methodology and results:

45 The point e) above is missing in the provided copy of the modified RSS for study (ii).Therefore, the information you have provided on the study (ii) is not sufficient to conduct an independent assessment of its reliability. You should therefore submit the information in an updated registration dossier by the deadline set out in the decision.

46 Therefore, the requirements of OECD TG 201 are not met, and the information requirement is not fulfilled.

3. Ready biodegradability

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

3.1. Information provided

48 You have provided:

(i) a ready biodegradation study (OECD TG 301E, 1990) with the Substance.

3.2. Assessment of information provided

3.2.1. The provided study does not meet the specifications of the test guideline

49 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 301E, the following requirements must be met:

50 Reporting of the methodology and results

a) The source of the inoculum, its concentration in the test and any pre-conditioning treatment are reported.
b) The test design is reported (e.g. the test temperature).
c) The results of measurements at each sampling point in each replicate is reported in a tabular form.
d) Any observed inhibition phenomena and/or abiotic degradation are reported;

51 In study (i) described as study report - modified OECD Screening Test (1990):

52 Reporting of the methodology and results

a) The origin of the inoculum is described as effluent from a domestic STP. However, you did not specify whether:
   - a dilute inoculum without sludge flocs was used;
   - the inoculum was derived from the secondary effluent of a treatment plant;
   - the concentration of the inoculum was approx. 10^5 cells/L in the test vessel;
   - the concentration of added inoculum was ≤ 0.5 mL/L;
   - the inoculum was pre-adapted to the test material.
b) On the test design, you did not report the test temperature, pH and whether the measurement of DOC in the test suspension and inoculum blanks are done in parallel.
c) and d): You did not provide any information listed above.

53 Based on the above, the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically, it is not possible to confirm whether the study was conducted under conditions that are consistent with the test guideline requirements, whether the validity criteria were met and whether the interpretation of the study results is adequate.

54 Therefore, the requirements of OECD TG 301E are not met and the information requirement is not fulfilled.

55 In your comments on the draft decision, you agree to perform the requested study.

Reasons related to the information under Annex VIII of REACH

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

56 An in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is an information requirement under Annex VIII, Section 8.4.2.

4.1. Information provided

57 You have provided the following information on the Substance:

(i) an in vitro chromosomal aberration study according to OECD 473 (1990)

(ii) an in vitro SCE study (1986)

(iii) a micronucleus study in rat via ip route (1986)

(iv) a micronucleus study in mice via ip route (1986)

4.2. Assessment of the information provided

4.2.1. The study (i) does not meet the specifications of the test guidelines

58 To fulfil the information requirement, the study has to be an in vitro chromosomal aberration test, or an in vitro micronucleus test conducted in mammalian cells. The study must comply with the OECD TG 473 or the OECD TG 487, respectively (Article 13(3) of REACH). Therefore, the following specifications must be met:

a) the maximum concentration tested induces 55+5% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration corresponds to 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest;
b) at least 300 well-spread metaphases are scored per concentration;
c) the positive controls induce responses compatible with those generated in the historical positive control database;
d) the positive controls produce statistically significant increase compared with the negative control;
e) the negative control data is ideally within the 95% control limits of the distribution of the laboratory's historical negative control database;
f) data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures is reported;

59 In study (i) described as an in vitro chromosomal aberration study:

a) the maximum tested concentration did not induce 55+5% of cytotoxicity compared to the negative control, and it did not induce the precipitation of the tested substance, and it was less than 10 mM, 2 mg/mL or 2 µL/mL;
b) 200 metaphases (i.e., less than 300 metaphases) were scored per concentration;
c) no positive control data compatible with those generated in the historical positive control database is reported;
d) you did not report if the positive control did produce a statistically significant increase in the induced response when compared with the concurrent negative control;
e) you did not report if the negative control did show a response within the historical

control range of the laboratory;
f) data on the cytotoxicity and/or the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures were not reported;

60 The information provided does not cover the specifications required by the OECD TG 473.

61 Therefore, the information requirement is not fulfilled.

4.2.2. The study (ii) is not adequate for the information requirement

62 (Eco)toxicological studies must comply with a recognised test method (Art. 13(3) of REACH), in this case an in vitro chromosomal aberration test or an in vitro micronucleus test, conducted in mammalian cells and comply with the OECD TG 473 or the OECD TG 487. Such study must cover the key parameters of the corresponding OECD test guideline (Art. 13(3) of REACH).

63 The study (ii) is described as a SCE (sister chromatid exchange) study. This study is neither an in vitro cytogenicity study in mammalian cells nor an in vitro micronucleus study. Therefore, the information provided does not cover the key parameters required by the OECD TG 473 or 487.

64 The study is not adequate for the information requirement and is therefore rejected.

4.2.3. The provided in vivo studies (studies (iii) and (iv)) do not meet the criteria of Annex VIII, Section 8.4.2., column 2

65 Under Annex VIII, Section 8.4.2., Column 2, the study usually does not need to be conducted "*if adequate data from an in vivo cytogenicity test are available*". The Guidance on IRs and CSA, Section R.7.7.6.3 and Table R.7.7–3 clarifies that the in vivo somatic cell cytogenicity test must be either a micronucleus test or a chromosomal aberration test, performed according to the OECD TG 474 or 475, respectively.

66 For the data from an in vivo somatic cell cytogenicity test to be considered adequate, the in vivo studies you submitted has to meet the requirements of the OECD TG 474. Therefore, the following specifications must be met:

a) the highest dose studied is the maximum tolerated dose (MTD), i.e. the highest dose that is tolerated without evidence of toxicity (e.g. body weight depression or hematopoietic system cytotoxicity, but not death or evidence of pain, suffering or distress necessitating humane euthanasia). The highest dose can also be a dose that produces toxicity in the bone marrow (e.g. a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood);
b) the proportion of immature erythrocytes among total (immature + mature) erythrocytes is determined for each animal by counting a total of at least 500 erythrocytes for bone marrow and 2000 erythrocytes for peripheral blood;
c) at least 4000 immature erythrocytes per animal are scored for the incidence of micronucleated immature erythrocytes;
d) the proportion of immature erythrocytes among total (immature + mature) erythrocytes and the mean number of micronucleated immature erythrocytes are reported for each group of animals;
e) a clear negative outcome is concluded when the data available shows that bone marrow exposure to the Substance occurred;
f) the negative control data is ideally within the 95% control limits of the distribution of the laboratory's historical negative control database;
g) the positive controls or scoring controls induce responses compatible with those generated in the historical positive control database;
h) the positive controls or scoring controls produce statistically significant increase

compared with the negative control.

67 In studies (iii) and (iv) described as micronucleus studies:

a) you did not demonstrate that the highest dose studied was the maximum tolerated dose/the highest dose studied was not the maximum tolerated dose and it did not produce toxicity in the bone marrow;
b) you did not report the total of number of erythrocytes for bone marrow and the number of erythrocytes for peripheral blood to determine the proportion of immature erythrocytes among total (immature + mature) erythrocytes for each animal;
c) you did not report the number of immature erythrocytes per animal (i.e. less than 4000 immature erythrocytes) scored to determine the incidence of micronucleated immature erythrocytes;
d) the proportion of immature erythrocytes among total (immature + mature) erythrocytes and the mean number of micronucleated immature erythrocytes were not reported for each group of animals;
e) you did not demonstrate that bone marrow exposure to the Substance, or its metabolites, occurred;
f) the negative control did not show a response within the historical control range of the laboratory;
g) the positive controls or scoring controls did not induce responses compatible with those generated in the historical positive control database;
h) the positive control (or scoring control) did not produce a statistically significant increase in the induced response when compared with the concurrent negative control.

68 The information provided does not cover the specifications required by the OECD TG 474. As a result, the column 2 criteria are not met, and your adaptation is rejected.

69 In your comments on the draft decision, you agree to perform the requested study.

4.3. Specification of the study design

70 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.

5. In vivo mammalian genetic toxicity study

71 Appropriate in vivo mutagenicity studies must be considered under Annex VIII, Section 8.4., column 2 in case of a positive result in any of the in vitro genotoxicity studies under Annex VII or VIII to REACH.

5.1. Triggering of the information requirement

72 Your dossier contains positive results for the in vitro gene mutation study in mammalian cells (1986) which raise the concerns for gene mutations.

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

74 The assessment of the information provided, and the specifications of the study design are addressed below under Request 8.

6. 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)

75 A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII, Section 8.6.1. 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.

6.1. Information provided

76 ECHA understands that you have adapted this information requirement by using Annex VIII, Section 8.6.1, Column 2. To support the adaptation, you have provided the following information:

(i) a one-year repeated dose toxicity study, via dermal route, in mice (1993) with the Substance;

(ii) a 2-year chronic toxicity study, via oral route, in rats (1955), with the Substance which you report in IUCLID as under "adequacy of study" weight of evidence;

(iii) a sub-chronic toxicity study, via oral route, in rats (1978) with the source substance DEP EC 201-550-6.

6.2. Assessment of the information provided

77 The studies (i), (ii), and (iii) are rejected for the reasons explained under request 9.

78 Therefore, the information requirement is not fulfilled.

6.2.1. Specification of the study design

79 Annex VIII, Section 8.6.1., Column 2 provides that an experimental study for this information requirement is not needed if a reliable sub-chronic (90 days) or chronic toxicity study is available.

80 The present decision requests the registrants concerned to generate and submit a reliable sub-chronic toxicity study (90 days) (see request 9). According to Annex VIII, Section 8.6.1., Column 2 and to prevent unnecessary animal testing, a short-term toxicity study (28 days) does not therefore need to be conducted.

81 Because you still must comply with the information requirement in Annex VIII, Section 8.6.1., you are requested to submit a justification for the adaptation provided in Column 2 of that provision.

82 In your comments on the draft decision, you agree to perform the requested study.

7. Short-term toxicity testing on fish

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

7.1. Information provided

84 You have provided:

(i) a short-term toxicity to fish (Comparable to EPA-660/3-7500, 1981) with the Substance.

7.2. Assessment of the information provided

7.2.1. The provided study does not meet the specifications of the test guideline

85 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:

86 Reporting of the methodology and results

a) The test procedure is reported (*e.g.* composition of the test medium, fish loading).
b) Adequate information on the analytical method (including performance parameters of the method) are provided.
c) Tabulated data on mortalities and sub-lethal effects (*e.g.* with regard to equilibrium, appearance, ventilator and swimming behaviour) are reported. The frequency of observations includes at least 2 observations within the first 24 hours and at least two observations per day from day 2 to 4.

87 In study (i) described as a short-term toxicity study to fish:

88 Reporting of the methodology and results

a)-c) You did not provide information listed above.

89 Based on the above, the reporting of the study is not sufficient to conduct an independent assessment of its reliability.

90 In your comments you agree that the study provided does not meet the current standards of an OECD TG 203 study. Instead of performing a new OECD TG 203 study as requested, you propose to perform a long-term toxicity testing on fish.

91 REACH Annex VII section 9.1.3. column 2 specifies that the short-term toxicity study does not need to be conducted if a long-term aquatic toxicity study on fish is available. At present no long-term toxicity study on aquatic fish is provided in the IUCLID dossier, therefore no conclusion on the compliance can currently be made. You remain responsible for complying with this decision by the set deadline

92 Therefore, the requirements of OECD TG 203 are not met, and the information requirement is not fulfilled.

Reasons related to the information under Annex IX of REACH

8. In vivo mammalian genetic toxicity study

93 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.

8.1. Triggering of in vivo mutagenicity studies

94 In relation to the first condition, your dossier contains positive results for the in vitro gene mutation study in mammalian cells (1986) which raise the concern for gene mutation.

8.2. Information provided and its assessment

95 In relation to the second condition, your dossier contains the following in vivo studies:

(i) a micronucleus study in rat (1986) with the Substance;

(ii) a micronucleus study in mice (1986) with the Substance

96 For the assessment of studies (i) and (ii), see Request 4 (the corresponding studies are studies (iii) and (iv) under Request 4). For the reasons already explained under request 4., the information provided does not cover the specification(s) required by the OECD TG 474.

97 Therefore, the conditions set out in Annex IX, Section 8.4., column 2 are met and the information requirement for an appropriate in vivo somatic cell genotoxicity study is triggered and an appropriate in vivo follow up mutagenicity study is necessary to address the concern identified in vitro.

98 In the comments to the draft decision, you agree to perform the requested study.

8.3. *Test selection*

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

100 As explained above, under Request 4, in the dossier there is no adequate information from an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study, according to the requirements of Section 8.4.2., Annex VIII to REACH. Therefore, by this decision, ECHA also requests an in vitro cytogenicity study or an in vitro micronucleus study, which may raise a concern for chromosomal aberration in the case of positive results.

101 If there is also a concern for chromosomal aberration, the comet assay can be combined with an in vivo mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) in a single study (see OECD TG 489 para. 33; OECD TG 474 para. 37c; Guidance on IRs & CSA, Section R.7.7.6.3). While the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations, the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy). A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

102 The combined study, together with the results of the in vitro mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing in vivo mutagenicity and lack thereof. Furthermore, the combined study can help reduce the number of tests performed and the number of animals used while addressing (structural and numerical) chromosomal aberrations as well as gene mutations.

103 Therefore, you must wait for the results of the in vitro test requested under Request 4 and, depending on these results, to conduct either a) the TGR assay or Comet assay if the test results of Request 4 are negative; or b) Comet assay combined with MN test if the test results of Request 4 are positive. The deadline set in this decision allows for sequential testing.

8.4. Study design

*8.4.1. Comet assay (if the test results of request 4 are **negative**)*

104 If 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, para. 23).

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

106 In line with the test method OECD TG 489, the test must be performed by analysing tissues from the liver as the primary site of xenobiotic metabolism, and from the 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.

*8.4.2. TGR assay (if the test results of request 4 are **negative**)*

107 If 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.

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

109 You are requested to follow the new 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.

110 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, 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.

*8.4.3. In vivo mammalian alkaline comet assay combined with In vivo mammalian erythrocyte micronucleus test (if the test results of request 4 are **positive**)*

111 According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.

112 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.

113 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.

114 The combination of OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen et al. 2011 [1]).

[1] Bowen D.E. et al. 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. Mutation Research 722 7–19.

8.4.4. Germ cells

115 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483, depending on the concern raised by the substance) 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.

8.4.4.1. Comet assay or Comet assay combined with MN test

116 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other 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.

8.4.4.2. TGR assay

117 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 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.

9. Sub-chronic toxicity study (90-day)

118 A sub-chronic toxicity study (90 days) is an information requirement under Annex IX, Section 8.6.2.

9.1. Information provided

119 You have provided:

(i) a one-year repeated dose toxicity study, via dermal route, in mice (1993) with the Substance.

120 In addition, you have adapted this information requirement by using Annex XI, Section 1.2. (weight of evidence) based on the following experimental data:

(ii) a 2-year chronic toxicity study, via oral route, in rats (1955), with the Substance.

121 In addition, you have adapted this information requirement by using Annex XI, Section 1.5. (Grouping of substances and read-across approach) based on experimental data from the following substance:

(iii) a sub-chronic toxicity study, via oral route, in rats (1978) with the source substance DEP EC 201-550-6.

9.2. Assessment of the information provided

9.2.1. Study (i) is not adequate for the information requirement

122 Under Annex VIII, Section 8.6.1, Column 1, the study must be performed using the most appropriate route of administration, having regard to the likely route of human exposure.

123 According to the 'Guidance on IRs and CSA, Section R.7.6.2.3.2.', the default route is oral. However, the dermal or the inhalation route may be more appropriate, depending on the physico-chemical properties of the Substance, the most relevant route of human exposure, and other toxicological considerations.

124 The study (i) was performed with exposure via the dermal route. You did not provide a justification for the choice of the route of exposure.

125 The oral route is the most appropriate route, because it is the default route, and the Substance is a liquid of moderately low vapour pressure (0.13 Pa at 20°C) and no uses with spray application are reported that could potentially lead to aerosols of inhalable size.

126 Therefore, the information provided is not performed using the most appropriate route of exposure and this study does not meet the information requirement.

9.2.2. Weight of evidence adaptation rejected (study ii)

127 Annex XI, Section 1.2. states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

128 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

129 According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the 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 and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency, and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude on the corresponding information requirement.

130 As specified under Annex XI, Section 1.2, a weight of evidence must rely on several independent sources of information to conclude on the information requirement.

131 However, your weight of evidence adaptation relies on a single source of information (study ii).

132 Given that you have submitted only one source of information, your adaptation does not meet the requirements of Annex XI, Section 1.2 and is therefore rejected for that reason alone.

133 However, in addition to this critical deficiency of your weight of evidence adaptation, ECHA has identified issues with the information you provided as such. These issues are further detailed below.

134 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex IX, Section 8.6.2. includes similar information that is produced by the OECD TG 408 with a design as specified in this decision. OECD TG 408 requires the study to investigate the following key elements:

1. In-life observations
2. Blood chemistry
3. Organ and tissue toxicity

135 The source of information (ii) may provide relevant information on some of these key parameters.

136 However, the reliability of the source of information (ii) is significantly affected by the following deficiency:

137 To fulfil the information requirement, the sub-chronic toxicity study (90 day) has to meet the requirements of the OECD TG 408. Therefore, the following specifications must be met:

a) at least 10 male and 10 female animals are used for each concentration and control group;
b) body weight and food consumption are measured at least weekly;
c) clinical signs are observed daily, and functional observations (i.e. sensory activity, grip strength and motor activity assessments) are made during week 11 or later;
d) haematological and clinical biochemistry tests are performed as specified in paragraphs 30-38 of the test guideline;
e) the oestrus cycle in females is examined at necropsy;
f) terminal organ and body weights are measured;
g) gross pathological examinations as specified in paragraphs 43-46 of the test guideline are performed;
h) full histopathology is performed as specified in paragraphs 47-49 of the test guideline.

138 In study (ii) described as a chronic repeated dose toxicity study:

a) only 10 females and no males were included in each test and control group;
b) food consumption, body weights and body weight changes were not assessed;
c) clinical signs and functional aspects were not assessed;
d) haematology and clinical biochemistry were not performed;
e) oestrus cyclicity was not assessed;
f) terminal organ weights and organ/body weight ratios were not recorded;
g) gross pathology was not assessed;
h) histopathology was not performed.

139 The information provided does not cover the specifications required by the OECD TG 408, in particular with regards the investigations to be conducted.

140 Therefore the provided study cannot be considered a reliable source of information that could contribute to the conclusion on this key parameter investigated by the required study.

141 In summary, as explained above, your adaptation relies on a single source of information and therefore does not qualify for a weight of evidence as set out under Annex XI, Section 1.2 of REACH. Furthermore, the source of information (ii) lacks essential elements on in-life observations, blood chemistry, organ and tissue toxicity. Therefore, this source of information cannot contribute to the conclusion on the information requirement for sub-chronic toxicity.

142 On this basis, your adaptation is rejected.

9.2.3. Read-across adaptation rejected (study iii)

143 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected. In addition, ECHA identified endpoint-specific issue addressed below.

9.2.4. Source study (iii) not adequate for the information requirement

144 Under Annex XI, Section 1.5., the study to be read across must have an adequate and reliable coverage of the key parameters addressed/ cover an exposure duration comparable to or longer than the one specified in the corresponding test method referred to in Article 13(3), in this case OECD TG 408. Therefore, the following specifications must be met:

a) dosing of the Substance is performed daily for a minimum of 90 days;
b) body weight and food consumption are measured at least weekly;
c) clinical signs are observed daily and functional observations (i.e. result of ophthalmological examination, sensory activity, grip strength and motor activity assessments) are made during week 11 or later;
d) clinical biochemistry tests are performed;
e) the oestrus cycle in females is examined at necropsy.

145 In study (iii) described as a sub-chronic toxicity study:

a) the Substance was administered 5 times a week for 16 weeks, i.e. 80 days;
b) food consumption, body weights and body weight changes were not assessed weekly;
c) the following clinical signs and functional aspects were not assessed: nature, severity and duration; In particular, the following investigations are missing: ophthalmological examination, sensory activity, grip strength and motor activity assessments;
d) clinical biochemistry was not performed;
e) oestrus cyclicity was not assessed.

146 The information provided does not cover the specifications required by the OECD TG 408.

147 Based on the above, the study does not provide an adequate and reliable coverage of/cover an exposure duration comparable to or longer than the one specified in the key parameter(s) addressed by the OECD TG 408 and this study is not an adequate basis for your read-across predictions.

148 Therefore, the information requirement is not fulfilled.

149 In your comments on the draft decision, you agree to perform the requested study.

9.3. Specification of the study design

150 Following the criteria provided in Annex IX, Section 8.6.2, Column 2, and considering the guidance on IRs and CSA, Section R.7.5.6.3.2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity of the Substance, because the Substance is a liquid of moderately low vapour pressure (0.13 Pa at 20°C) and no uses with spray application are reported that could potentially lead to aerosols of inhalable size.

151 According to the OECD TG 408, the rat is the preferred species.

152 Therefore, the study must be performed in rats according to the OECD TG 408 with oral administration of the Substance.

10. Long-term toxicity testing on aquatic invertebrates

153 Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

10.1. Information provided

154 You have provided:

(i) a long-term toxicity to daphnia (comparable to EPA guideline OTS 797.1330, 1995) with the Substance; and

(ii) other information: statement that a new OECD TG 211 study with the Substance is currently underway.

155 As the study you refer to under (ii) has not yet been provided, ECHA is not in a position to assess its validity.

10.2. Assessment of the information provided

10.2.1. The provided study (i) does not meet the specifications of the test guideline

156 To fulfil the information requirement, a study must comply with the OECD TG 211 (Article 13(3) of REACH). Therefore, the following specifications must be met:

157 Reporting of the methodology and results

a) The test procedure is reported (test medium composition including TOC)
b) Detailed information on feeding, including amount (in mgC/daphnia/day) and schedule is reported.
c) The nominal test concentrations are reported.
d) The full record of the daily production of living offspring during the test /in each

replicate is provided.
e) The coefficient of variation for control reproductive output is reported.

In study (i) described as a Chronic toxicity of 14 phthalate esters to Daphnia magna (1995)*:*

158 Reporting of the methodology and results:

a)-e) You did not provide information listed above.

159 Based on the above, the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically, it is not possible to confirm the requirements of the test guideline and the validity of the study based on the information provided in the dossier.

160 In your comments to the draft decision, you have provided information on a new OECD TG 211 study (i.e. study (ii)), in the format of an attached copy of the modified Robust Study Summary (RSS). You have updated your dossier with the modified RSS for the OECD TG 211 study.

161 As explained above, to fulfil the information requirement, a study must comply with OECD TG 211 (Article 13(3) of REACH).

162 Reporting of the methodology and results

163 The points b) and d) above are missing in the provided copy of the modified RSS for study (ii).Therefore, the information you have provided on the study (ii) is not sufficient to conduct an independent assessment of its reliability. You should therefore submit the information in an updated registration dossier by the deadline set out in the decision.

164 Therefore, the requirements of OECD TG 211 are not met, and the information requirement is not fulfilled.

11. Further long-term aquatic toxicity

165 Long-term toxicity testing on fish is an information requirement under Annex IX Section 9.1.6. Further studies than those listed in Column 1 of Section 9.1.6. of Annex IX may be required must be proposed if the chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the effects on aquatic organisms (Annex IX, Section 9.1., Column 2).

11.1. Information provided

166 You have provided:

(i) a long-term toxicity to fish (EPA-TSCA 40 CFR, Part 797.1600, 1995) with the Substance.

11.2. Assessment of the information provided

11.2.1. Assessment of the information provided against the requirements of Annex IX, Section 9.1.6., Column 1

11.2.1.1. The provided study does not meet the specifications of the test guideline

167 To fulfil the information requirement, a study must comply with the OECD TG 210 (Article 13(3) of REACH). Therefore, the following specifications must be met:

168 Technical specifications impacting the sensitivity/reliability of the test

a) At least 80 eggs, divided equally between at least four replicate test chambers, are used per concentration.

169 Reporting of the methodology and results

b) The test procedure is reported (*e.g.* composition of the test medium, the stage of embryonic development at the start of the test, chamber volume, fish loading).
c) Evidence that controls met the overall survival acceptability standard of the test species is reported.
d) Data on mortality at each stage (embryo, larval and juvenile) measured daily and cumulative mortality are reported.
e) Days to hatch, numbers of larvae hatched each day, and end of hatching are reported.
f) The number of healthy fish at end of test is reported.
g) Data for length (specify either standard or total) and weight of surviving animals at the end of the test are reported.
h) The incidence, description and number of morphological abnormalities, if any, are reported.
i) Adequate information on the analytical method (including performance parameters of the method) and on the results of the analytical determination of exposure concentrations is provided.

170 In study (i) described as "a long-term toxicity to fish" (1995):

171 Technical specifications impacting the sensitivity/reliability of the test

a) You reported that 40 to 60 embryos at study initiation, on day 25 embryos were reduced to 20 eyed embryos per vessel, and only two replicates per vessel were used. ECHA therefore understands that only 40 test animals were used at the end of the study.

172 Reporting of the methodology and results

b) On the test procedure, you have not specified:
  - on the composition of the test medium, DOC and/or TOC content,
  - Use of vehicle: You stated that acetone or Dimethylformamide was used to prepare test solution. However, you did not specify their concentrations nor whether the solvent control was run in parallel.
  - fish loading (i.e. biomass per volume of test solution, for flow-through test the loading rate should be ≤ 0.5 g/L wet weight per 24 hours and 5 g/L of solution at any time).
  - frequency of analytical monitoring

c) -h) You did not provide information listed above.
i) On the analytical method adequate information, i.e. performance parameters of the method, as well as, the results of the analytical determination of exposure concentrations, are not reported.

173 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically, the number of eggs and replicates were fewer than required by the OECD TG 210. Therefore, the statistical power of the provided study (i) is lower than that from standard OECD TG 210 studies.

- the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically, it is not possible to confirm the requirements of the test guideline and the validity of the study based on the information provided in the dossier.

174 Therefore, the requirements of OECD TG 210 are not met and the information requirement of Annex IX, Section 9.1.6. is not met.

11.3. Justification for the further information required under Annex IX, Section 9.1, column 2

175 The chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the effects on aquatic organisms (Annex IX, Section 9.1., Column 2). This can be the case, for instance, if there are indications that the Substance may be an endocrine disruptor. None of the three studies listed under Column 1 of Section 9.1.6. of Annex IX allows to conclude whether the Substance may have endocrine disrupting properties.

176 According to IPCS/WHO[2], "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations". Based on this definition, the Substance may be an endocrine disruptor (ED) if the following conditions are met:

- It shows endocrine activity, *i.e.* it has the potential to alter the function(s) of the endocrine system.
- It shows adverse effects(s) in (an intact) organism, or its progeny, or (sub)populations which include, among others, change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.
- There is a biologically plausible link between the adverse effects and the endocrine activity, *i.e.* the Substance has an endocrine disrupting mode of action (ED MoA).

177 Based on the above definition, further information to investigate the endocrine disrupting properties of the Substance is needed if there are indications that the above criteria may be met but without conclusive information on all elements of that definition. Such indications can be grouped according to the Conceptual Framework (CF) described in OECD GD 150.

The following information is publicly available for the Substance:

- Information equivalent to OECD CF Level 2:
  - In an androgen receptor (AR) inhibition study with dihydrotestosterone (DHT), the Substance (dimethyl phthalate, DMP) was shown to inhibit DHT-stimulated AR activity *in vitro* (Engel et al., 2017). The study demonstrated a clear dose-response relationship for AR inhibition with the Substance. Furthermore, at the maximum non-cytotoxic concentration (100 µM), the Substance caused a complete AR inhibition, which is comparable to the level of inhibition observed using the AR antagonist flutamide. In the same study there was no activation of ERα or ERβ at 100 µM, and marginal (non significant) inhibition at 100 uM of ERα or Erβ.
  - In an *in vitro* study (Lee et al., 2019), the Substance was shown to significantly increase E2/T ratio in H295 cells, while in the MVLN cell line there was no direct ER agonistic effects.

[2] WHO/IPCS, 2002. Global assessment of the state-of-the-science of endocrine disruptors. https://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/.

- Information equivalent to OECD CF Level 3:
    - In an *in vivo* embryonic zebra fish assay (Lee et al., 2019), the Substance induced an up-regulation of all tested genes related to steroidogenesis, and led to significant transcriptional changes even at lower concentrations of 0.01 or 0.1 mg/L. Furthermore, the exposure of the Substance modulated the gene regulating steroid hormone balance at lower concentrations than that of di(2-ethylhexyl) phthalate (DEHP), a known endocrine disruptor, suggesting greater endocrine disruption potency of the Substance in zebrafish.

178 In conclusion, there is *in vivo* evidence showing that DMP has the potential to disrupt sex hormone balances through modulating key steroidogenic genes in zebrafish embryos. In addition, there is *in vitro* evidence suggesting that the DMP has endocrine activity via the oestrogenic, androgenic and steroidogenic (EAS) modalities. Although this information indicates potential endocrine activity, ECHA consider it inconclusive with regard to endocrine disrupting properties because the available studies only cover mechanistic parameters, but not apical endpoints.

179 In your comments on the draft decision, you disagree with the request and propose to perform an OECD TG 210, instead of TG 234. As summarised below, you have provided your reasoning as to why you consider the available information from the publications mentioned by ECHA is invalid/ unassignable and, hence it is not suitable to justify the ED concern for the Substance.

180 For the OECD CF Level 2 study (Engel et al., 2017), you state that the study may be suitable for screening purposes for the ED. However, you doubt that these studies are suitable basis for the suspected ED activity. In particular, you express the following concerns:

(i) *Test material information*: You state that the purity of the test material is unclear and the test material used in the study is unlikely to be representative for the Substance from industrial highly controlled production process (purity > 99.5%).

(ii) *Relevance of the human cell line receptors:* You have argue that the study may not be relevant for the environmental assessment, as the relevance of the human cell line receptors in ecological assessment has not been verified.

(iii) *Contradicting evidence*: you argue that there are contradicting evidence from *in vivo* data from the studies of [redacted] (1998), [redacted] (2000), [redacted] (2014) and the uterothropic assay ([redacted] 1999) that show a lack of estrogenic or antiandrogenic activity for the Substance.

181 For the OECD CF Level 2/3 study from Lee et al. (2019), you raise the same concern as mentionned above under point (i) with regard the available information on the test material used in this study. You also express the following concerns:

(iv) *Inappropriate use of solvent*: you consider that the use of solvent was not appropriate for the following reasons:

- "*DEP has a water solubility of 4000 mg/L, and is definitively not poorly soluble, is not hydrolytically unstable and is not highly viscous*";
- the concentration of DMSO used (i.e. 0.1% (v/v)) exceeds the concentration recommended for endocrine screens and fish reproduction studies;
- solvent control is missing in the study and therefore, it is not clear whether the observed effects are due to the tested substance or caused by the solvent.
- You consider there are publications available giving the concern that DMSO may be endocrine disruptive (e.g., [redacted] (2020), [redacted] (2004), [redacted] (2006)).

(v) The test concentrations are not appropriate you state that "[t]*he authors establish LC25 at 100mg/L and so testing concentrations are too high, with lower exposure doses needed to evaluate chronic effects; therefore, observed effects could be systemic toxicity and not mediated by an ED MOA at such high concentrations*".

182 With regards your concern under point (i) above, ECHA points out that there is no evidence that the observed effects in the studies (Engel *et al.*, 2017 andd Lee *et al.*, 2019) are caused by the (potential) presence of impurities and/or constituent(s) which are not present in the industrially produced Substance.

183 With regards your concern under point (ii) above, ECHA notes that the revised guidance OECD 150 states that *It should be remembered that due to the molecular similarities of endocrine systems and receptor homologies across the vertebrates, there may be some potential for using information from non-mammalian vertebrate test assays for assessing endocrine activity in mammals (and vice versa), and especially for extrapolation between various in vitro screens (see Section B.3). ….[…]… The in vitro screens in question (although at present based largely on mammalian receptors and/or enzymes) are generally capable of providing information applicable to both humans and vertebrate wildlife (OECD, 2010d)"*. Therefore, ECHA maintains that these studies support the need to investigate further potential effects in non-mammalian species. The requested OECD TG 234 study would provide further information to the OECD TG 210 to clarify whether the Substance may have endocrine disrupting poperties in the environnement.

184 Regarding the point (iii) above, ECHA notes the following:

- [redacted] (1998): the Substance was not tested in this study.
- [redacted] (1999): the uterotrophic assay is designed specifically to detect a single endocrine mechanism, i.e. oestrogenicity, therefore other modalities cannot be excluded, in particular anti-androgenic pathway reported for other phthalates.
- [redacted] (2000) particularly focused on androgenic or antiandrogenic effects and showed that the Substance did not alter the sexual differentiation of the male rat. [redacted] (2014) focussed on the development and validation of a protocol to screen the ability to disrupt testis endocrine function in utero and showed that the Substance did not reduce fetal testosterone production. However, the lack of effects in these toxicological studies does not exclude the possibility that the Substance may be is an endocrine disruptor to the environment.

185 With regards your concern under point (iv) above, ECHA cannot speculate why the authors of the paper have used a solvent control. ECHA agrees that a solvent controlled is indeed needed and that would be a noted deficiency if the results of such study were to be used as equivalent or replacement of OECD TG 234 as such. This is not the case as the study from Lee *et al.* (2019) is not used to draw a firm conclusion on the ED properties, but rather indicates along other sources of information the need to investigate further the EAS modalities in fish.

186 On your claim that DMSO may have endocrine disruptive properties, ECHA notes that:

- None of the studies mentioned by you claim that DMSO is an endocrine disruptor (which could only be identified if the three conditions mentioned above would be fulfilled). In addition, DMSO is not formally identified as an endocrine disruptor, its use is not explicitly excluded from the OECD TGs investigating endocrine disrupting properties and it is even used as solven in some OECD TGs for investigating endocrine disrupting properties recently approved by OECD (e.g. OECD TG 251).
- The DMSO concentration used in Christou et al., study (≥0.55% DMSO) is even higher than the one used in Lee et al., 2019 study (0.1 %).

187 With regards your concern under point (v) above, ECHA notes that, while the top dose induced lethal effects, the effects observed on ED related endpoints were observed also at

lower doses where no acute effects were detected. The requested OECD TG 234 study with five different concentrations, as specified under Section 3.4. ('Test selection and study specifications) below, would provide the information required to evaluate the chronic ED effects.

188 ECHA reiterates that the studies discussed above are not used to conclude that the Substance is an ED as there is not possible to draw a firm conclusion yet. Nevertheless, these studies show consistent effects that support the need to investigate further the EAS modalities. These deficiencies do not invalidate the conclusions taken from the analysis of the overall available data on the substance nor the request for an OECD TG 234 as explained underneath.

189 On this basis, available information from studies which are equivalent to OECD CF Level 2-3 indicate that the Substance may be an endocrine disruptor via EAS modalities. However, as explained above, this information does not allow to conclude whether or not the Substance may show adverse effects as a result of its endocrine activity.

190 Therefore, the chemical safety assessment (CSA) indicates the need for further long-term toxicity testing on aquatic organisms.

11.4. Test selection and study specifications

191 As explained under Section 11.3 above, there are indications that the Substance may have endocrine disrupting properties through EAS modalities. In addition, there is currently no indication that the Substance may be more toxic to reproduction than to sexual development. Therefore, the Fish Sexual Development test (test method: OECD TG 234) is considered adequate to investigate further the ED properties of the Substance (OECD GD 150).

192 A Fish Sexual Development test (test method: OECD TG 234) is an in vivo assay (OECD Conceptual Framework Level 4) providing apical information on phenotypic sex ratio which is fixed during fry or juvenile stages of the species used in this test.

193 As explained in the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, the assessment of gonad histopathology (e.g. staging of gonads, severity of intersex) is needed for investigating EAS modalities as it may inform on adversity. The test should be conducted on the Japanese medaka (Oryzias latipes) or the zebrafish (Danio rerio). As the test is to be used for hazard and risk assessment, it must not be conducted on stickleback because the validation data available so far showed that in this species the alterations of phenotypic sex ratio were uncommon (OECD GD 234).

194 As explained under Section 1.1 above, the information requirement on long-term toxicity to fish under Annex IX, Section 9.1.6. is not met. Therefore, adequate information on long-term toxicity to fish is also needed for the purpose of the risk assessment. In such case, the concentration range needs to be adjusted in order to investigate both potential endocrine disrupting effects of the Substance (in the absence of significant non-endocrine mediated effects) and apical endpoints normally measured in an OECD TG 210 study (including hatching rate, survival, length and body weight). Therefore, to minimize vertebrate testing and to avoid the need to conduct additionally a Fish, Early-Life Stage (FELS) Toxicity Test (test method: OECD TG 210), you must conduct the test with five test concentrations as specified in paragraph 30 of the OECD TG 234.

References

Engel et al. (2017). Agonistic and antagonistic effects of phthalates and their urinary metabolites on the steroid hormone receptors ERα, ERβ, and AR. Toxicology Letters, 277 (2017) 54–63.

Lee et al. (2019). Comparative analysis of endocrine disrupting effects of major phthalates in employed two cell lines (MVLN and H295R) and embryonic zebrafish assay. Environmental research, 172: 319-325.

Reasons related to the information under Annex X of REACH

12. Pre-natal developmental toxicity study in a second species

195 Pre-natal developmental toxicity (PNDT) studies (OECD TG 414) in two species is an information requirement under Annex X, Section 8.7.2.

12.1. Information provided

196 You have adapted this information requirement by using Annex XI, Section 1.5. (Grouping of substances and read-across approach) based on experimental data from the following substance:

(i) a prenatal developmental toxicity study (1984) via dermal route with the source substance DEP, EC 201-550-6.

12.2. Assessment of the information provided

12.2.1. Read-across adaptation rejected

197 As explained in Section 0.1., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected. In addition, ECHA identified endpoint-specific issue addressed below.

12.2.2. Source study not adequate for the information requirement

198 Under Annex XI, Section 1.5., the results to be read across must have an adequate and reliable coverage of the key parameters addressed in the test guideline for the corresponding study that shall normally be performed for a particular information requirement, in this case OECD TG 414. Therefore, the following specifications must be met:

a) at least 20 female animals with implantation sites for each test and control group are included;

199 In study (i) described as a pre-natal developmental toxicity study:

a) only 12 females were included in each test and control group;

200 The information provided does not provide an adequate and reliable coverage of the specifications required by the OECD TG 414. Therefore, this study is not an adequate basis for your read-across predictions.

12.2.3. Source study not adequate for the purpose of classification and labelling and/or risk assessment

201 Under Annex XI, Section 1.5., the results to be read across must be adequate for the purpose of classification and labelling and/or risk assessment.

202 As ECHA Guidance on IRs and CSA, Section (R.7.6.2.3.2.) specifies, according to the test methods for reproductive toxicity which focus on the detection of reproductive hazards, the oral route (gavage, in diet, or in drinking water) is the "default" route, except for gases. If another route of administration other than oral is used, the registrant should provide justification and reasoning for its selection. Testing via dermal route might be necessary

under specific circumstances, for example for substances with high dermal penetration and indications for a specific toxicity following dermal absorption.

203 In addition, OECD 414 specifies that if another administration route than oral is used, a justification and reasoning for its selection should be provided (OECD TG 414, para. 18).

204 The study (i) is described as prenatal developmental toxicity study (1984) via dermal route with the source substance DEP, EC 201-550-6. You justified the use of dermal route with the following statement: *"The test substance is known to be dermally absorbed. Thus, dermal application guarantees systemic exposure".*

205 As pointed out above, according to ECHA Guidance and OECD TG 414, concerning reproductive hazards including those investigated under the pre-natal developmental toxicity testing the oral route is the default route while if another route than oral is used adequate justification and reasoning for the selection must be provided. In particular for the use of dermal route, it must be demonstrated that the substance has high dermal penetration and there are indications for a specific toxicity following dermal absorption.

206 You did not provide proof (e.g. study data) that the source substance has high dermal penetration and that it causes specific toxicity following dermal absorption. Therefore, you have not provided adequate justification and reasoning to support the use of the dermal route of exposure.

207 Therefore study (i) does not constitute a reliable basis to predict the properties of the Substance. Consequently the information from study (i) is not adequate for the purpose of classification and labelling and risk assessment for the Substance.

208 Therefore, the information requirement is not fulfilled.

209 In the comments to the draft decision, you agree to perform an OECD 414 study in a second species (rabbit) with the Substance.

12.3. Specification of the study design

210 A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species. The study in the first species was carried out by using a rodent species (rat).

211 Therefore, a PNDT study in a second species must be performed in the rabbit as preferred non-rodent species.

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

213 Based on the above, the study must be conducted in rabbits with oral administration of the Substance.

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

The information requirement for an Extended one-generation reproductive toxicity study (EOGRTS; Annexes IX or X, Section 8.7.3.) is not addressed in this decision. This may be addressed in a separate decision once the information from the Sub-chronic toxicity study (90-day) requested in the present decision is provided; due to the fact that the results from the 90-day study is needed for the design of the EOGRTS. Similarly the information requirement for a Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.) is not addressed in this decision; as the EOGRTS will cover the same parameters.

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 07 December 2021.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It 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 notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the requests.

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 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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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[3].

(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

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[4].

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