~~Confidential~~

Helsinki, 20 June 2023

Addressees
Registrants of DAP_131-17-9 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
19/03/2020

Registered substance subject to this decision ("the Substance")
Substance name: Diallyl phthalate
EC/List number: 205-016-3

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 ***28 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 IX of REACH

4. Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: OECD TG 443) in rats, oral route, specified as follows:
   - Ten weeks premating exposure duration for the parental (P0) generation;
   - The highest dose level in P0 animals must be determined based on clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals as specified further in Appendix 1, Section 5.2., sub-section 5.2.3 or follow the limit dose concept. The reporting of the study must provide the justification for the setting of the dose levels;
   - Cohort 1A (Reproductive toxicity); and
   - Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

   You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

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

6. 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

The reasons for the request(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 request(s)
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 request(s)

Contents

Reasons related to the information under Annex VII of REACH

1. Short-term toxicity testing on aquatic invertebrates

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

1.1. Information provided

2 You have provided:

(i) a study on short term toxicity on aquatic invertebrates according to OECD TG 202 (2003) with the Substance;
(ii) a preliminary short-term toxicity study on aquatic invertebrates (1989) with a substance referred to as "phthalic acid diallyl ester". ECHA understands that you consider that the test material used in this study corresponds to the Substance.

1.2. Assessment of the information provided

1.2.1. The provided studies do not meet the specifications of the test guideline

3 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:

4 Technical specifications impacting the sensitivity/reliability of the test

a) the test duration is 48 hours or longer;

b) at least 20 animals are used at each test concentration and for the controls;

5 Characterization of exposure

c) analytical monitoring must be conducted. A reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range must be available;

6 Reporting of the methodology and results

d) adequate information on the test material is provided (i.e. identifiers, purity and presence of impurities)

e) the test design is reported (i.e., age of daphnids, concentrations tested, number of test animals and replicates)

f) the test conditions are reported (i.e., the test medium composition is reported and, in particular, the concentration in suspended solid and TOC)

g) the number of immobilised daphnids is determined at 24 and 48 hours. Data are summarised in tabular form, showing for each treatment group and control, the number of daphnids used, and immobilisation at each observation.

7 In studies (i) and (ii) described as short-term toxicity studies on aquatic invertebrates:

8 Technical specifications impacting the sensitivity/reliability of the test

a) for study ii., the test duration was only 24h;

b) for study i., you have reported that 10 animals were used per test concentration without replicates. In your comments, you submitted information for study i. (attachment [redacted].pdf) that shows that duplicate test vessels were used for each test concentration and control group. OECD TG 202 indicates that at least 20 animals, preferably divided into four groups of five animals should be used at each concentration and for the controls. ECHA takes note of the information provided which addresses the deficiency identified in the draft decision. However, as the information is currently not available in your registration dossier, the deficiency remains. You must submit this information in an updated registration dossier by the deadline set in the decision;

9 Characterization of exposure

c) for study ii., no analytical monitoring was performed;

10 Reporting of the methodology and results

d) for study ii., no identifiers are provided for the test material. For study ii., the purity of the test material and the presence of impurities are not reported;

e) for study i. and ii., key information on the test design is missing including the life-stage of test animals, the number of test animals, the number or replicates and the test concentrations. In your comments, you submitted information for study i. (attachment [redacted].pdf) that shows the life stage of the test species (young daphnids 24 hours old), number of test organisms (10 daphnids per vessel), replicates (duplicate) and validated test concentrations (measured test concentrations were 92-108% of nominal test concentration after 48 hours). The information you have provided in your comments addresses the deficiency identified in the draft decision for study i.. However, as the information is currently not available in your registration dossier, the deficiency remains. You must submit this information in an updated registration dossier by the deadline set in the decision;

f) for studies i. and ii, key information on the test conditions is missing and, in particular, the suspended solid and TOC content of the test medium. In your comments, you submitted information for study i. (attachment [redacted].pdf). The information provided does not include the suspended solid and TOC of the test medium. ECHA, however, takes note of the information provided for the test water addresses the deficiency identified in the draft decision for study i.. However, as the information is currently not available in your registration dossier, the deficiency remains. You must submit this information in an updated registration dossier by the deadline set in the decision;

g) for studies i. and ii, tabulated data on the number of immobilised daphnids after 24 and 48 hours for each treatment group and control are not reported. In your comments, you submitted information for study i. (attachment [redacted].pdf) in relation to the mentioned issues. The information you have provided in your comments addresses the deficiency identified in the draft decision for study i.. However, as the information is currently not available in your registration dossier, the data gap remains. You must submit this information in an updated registration dossier by the deadline set in the decision;

11 Based on the above and the information provided in your comments:

- there are critical methodological deficiencies resulting in the rejection of study ii. as currently reported in the dossier. More specifically,
    - for study ii., the test duration was shorter than the minimum requirement set out in the test guideline which may have significantly reduced the sensitivity of

this test. In addition, in the absence of analytical monitoring, adequate exposure to the test material is not demonstrated.

- the current reporting of the study i. and ii. in the dossier is not sufficient to conduct an independent assessment of their reliability. More specifically,
    - for study ii., you have not provided adequate information to support that the test material used in this study is representative of the Substance;
    - for studies i. and ii., key information on the test design and/or conditions are missing and therefore it is not possible to conduct an independent assessment as to whether these studies were conducted under conditions that are consistent with the specifications of the OECD TG 202;
    - for studies i. and ii, you have not provided adequate reporting of the study results and therefore it is not possible to verify that the validity criteria of the OECD TG 202 were met and to conduct an independent assessment of the interpretation of the results.

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

13 ECHA takes not of the additional information provided for study i. which addresses the deficiencies identified in the draft decision. However, as this additional information is not yet in your dossier, the data gap remains. . You must submit this information in an updated registration dossier by the deadline set in the decision.

2. Growth inhibition study aquatic plants

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

2.1. Information provided

15 You have provided:

(i) a growth inhibition study on algae according to DIN 38412, Part 9 (1980) with a test material referred to as "phthalic acid diallyl ester";
(ii) a non-guideline growth inhibition study on algae (1980) with a test material referred to as "phthalic acid diallyl ester".

16 ECHA understands that you consider that the test materials used in these studies correspond to the Substance.

2.1.1. The provided studies do not meet the specifications of the test guideline

17 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:

18 Validity criteria

a) exponential growth in the control cultures is observed over the entire duration of the test.

19 Technical specifications impacting the sensitivity/reliability of the test

b) for *Desmodesmus subspicatus* cells/mL the initial cell density is 2-5 x10^3 cells/mL;

20 Characterization of exposure

c) analytical monitoring must be conducted. Alternatively, a justification why the analytical monitoring of exposure concentrations is not technically feasible must be provided;

21 Reporting of the methodology and results

d) adequate information on the test material is provided (i.e. identifiers, purity and presence of impurities)

e) the test design is reported (e.g., number of replicates, number of test concentrations);

f) the test conditions are reported (e.g., composition of the test medium, test temperature, biomass density at the beginning of the test);

g) the method for determination of biomass and evidence of correlation between the measured parameter and dry weight are reported. Algal biomass is normally determined based on dry weight per volume, or alternatively as cell counts or biovolume using microscopy or an electric particle counter. If an alternative method is used (e.g. flow cytometry, *in vitro* or *in vivo* fluorescence, or optical density), a satisfactory correlation with biomass must be demonstrated over the range of biomass occurring in the test;

h) the results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form;

22 In studies (i) and (ii) described as growth inhibition study on algae:

23 Validity criteria

a) for study i., exponential growth in the control cultures was not observed over the entire duration of the test based on your statement that "*Algal growth in the controls showed a shift from exponential to stationary phase after 48h*". Technical specifications impacting the sensitivity/reliability of the test

24 Technical specifications impacting the sensitivity/reliability of the test

b) for study i., the test was conducted on *Desmodesmus subspicatus* and the initial cell density was 10000 cells/mL;

25 Characterisation of exposure

c) for studies i. and ii., no analytical monitoring of exposure was conducted, and you provided no justification for omitting this information;

26 Reporting of the methodology and results

d) for study i. and ii., no identifiers are provided for the test material. In addition, the purity of the test material and the presence of impurities are not reported;

e) on the test design, you have not specified for studies i., and ii.: the number of replicates, the test concentrations;

f) on the test conditions, you have not specified for studies i., and ii. the composition of the test medium and the test temperature. Initial cell density is not reported for study ii.

g) for study i., you report that algal biomass was determined using optical density (turbidity). However, you have not reported evidence of correlation between

the measured parameter and dry weight or cell numbers over the range of biomass occurring in the test. For study ii., you have not specified how cell density was determined;

h) for studies i. and ii., tabulated data on the algal biomass determined daily for each treatment group and control are not reported.

27 Based on the above,

- the validity criteria of OECD TG 201 are not met as exponential growth was not maintained over the entire exposure period;
- there are critical methodological deficiencies resulting in the rejection of the studies results. More specifically,
  - the initial biomass in study i. was higher than the maximum value specified in the OECD TG 201 which may have impacted the sensitivity of the test;
  - in the absence of analytical monitoring in studies i. and ii., adequate exposure to the test material is not demonstrated.
- the reporting of the study I. and ii. is not sufficient to conduct an independent assessment of its reliability. More specifically,
  - for studies i. and ii., you have not provided adequate information to support that the test material used in these studies is representative of the Substance;
  - for studies i. and ii., key information on the test design and conditions are missing and therefore it is not possible to conduct an independent assessment as to whether these studies were conducted under conditions that are consistent with the specifications of the OECD TG 201;
  - for studies i. and ii., you have not provided adequate information to support that the method used to determine algal biomass was adequate;
  - for studies i. and ii, you have not provided adequate reporting of the study results and therefore it is not possible to verify that the validity criteria of the OECD TG 201 were met and to conduct an independent assessment of the interpretation of the results.

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

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

3. Ready biodegradability

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

3.1. Information provided

31 You have provided:

(i) an inherent biodegradability study according to OECD TG 302C (2001) with the Substance.

32 In addition, you have adapted the standard information requirement by applying weight of evidence (WoE) adaptation in accordance with Annex XI, section 1.2:

(ii) A ready biodegradability study according to OECD TG 301C (1992) with the Substance.

3.2. Assessment of information provided

3.2.1. The study (i) does not qualify for a ready biodegradability test

33 Relevant information that can be used for the information requirement of Annex VII, Section 9.2.1.1. includes similar information that is produced by the OECD TG 301 or 310. OECD TG 301 and 310 require the study to investigate the following key element:

- the ultimate aerobic biodegradation (as measured by parameters such as DOC removal, CO2 production and oxygen uptake) of the test material under low inoculum concentration is measured at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation

34 The study (i) was conducted according to the OECD TG 302C (Inherent Biodegradability: Modified MITI test (II)).

35 As set out in the ECHA Guidance on IRs and CSA, Section R.7.9.5.1, "the optimum conditions in inherent biodegradability tests stimulate adaptation of the microorganisms thus increasing the biodegradation potential, compared to natural environments. Therefore, positive results in these tests should not be interpreted as evidence for rapid degradation in the environment".

36 Based on above, the study (i) cannot be used to conclude on the ready biodegradability of the Substance and does not fulfil the information requirement.

3.2.2. Weight of evidence adaptation rejected (study ii)

37 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.

38 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.

39 According to Guidance on IRs and CSA, Section 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.

40 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.

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

42 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.

43 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.

44 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex VII, Section 9.2.1.1 includes similar information that is produced by the OECD TG 301/310. OECD TG 301/310 requires the study to investigate the following key elements:

- the ultimate aerobic biodegradation (as measured by parameters such as DOC removal, CO2 production and oxygen uptake) of the test material under low inoculum concentration is measured at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

45 The source of information (ii) may provide relevant information on ultimate aerobic biodegradation.

46 However, the reliability of the source of information (ii) is affected by the following issue:

3.2.2.1. The reliability of the source information (ii), cannot be assessed

47 To fulfil the information requirement, normally a study according to OECD TG 301/310 must be provided. In the case of the source of information (ii), OECD TG 301C applies. The specifications of OECD TG 301C include:

48 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 (e.g., number of replicates, description of test conditions, such as temperature) is reported.
c) the test procedure (e.g., test medium composition, analytical method) is reported.
d) the results of measurements at each sampling point in each replicate is reported in a tabular form.
e) any observed inhibition phenomena and/or abiotic degradation are reported.
f) for a study conducted according to the OECD TG 301C, the determination of the biodegradation using a specific chemical analytical method is reported.

49 For study (ii), you have not provided any of the information listed under points a) to f) above.

50 In the absence of the above information, it is not possible to conduct an independent assessment as to whether the study was conducted under conditions that are consistent with the specifications of the OECD TG 301C, whether the validity criteria of the test guideline were met and whether the interpretation of the results is adequate.

51 Therefore, study (ii) cannot be considered a reliable source of information that could contribute to the conclusion on this key parameter investigated by the required study.

3.2.2.2. Conclusion on the weight of evidence

52 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) provides relevant information on ultimate aerobic degradation. However, this source of information is poorly documented, and its reliability cannot currently be assessed. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

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

Reasons related to the information under Annex IX of REACH

4. Extended one-generation reproductive toxicity study

54 An extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is an information requirement under Annex IX, Section 8.7.3., if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. Furthermore Column 2 defines the conditions under which the study design needs to be expanded.

4.1. Triggering of the information requirement

55 You claim that "this study does not need to be conducted as a one-generation screening study shows no adverse effects on fertility".

56 However, the screening study conducted in rats with the Substance (OECD TG 421, 2003) in your dossier indicates adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity, e.g. changes in gestation length.

57 In particular, the study reports 2 females killed in extremis and one female died, probably due to dystocia (an increase in gestation length) at 150 mg/kg bw/day (Highest dose, HD). Severe liver lesions are also observed in females (periportal hepatocyte necrosis (statically significant), enlargement and basophilia, bile duct proliferation and periportal fibrosis) at the same dose (HD) .

58 The same liver effects are reported in the repeated dose toxicity study conducted in rats with the Substance (13-weeks, NTP, 1985) at 200 and 400 mg/kg bw /day. However no female died.

59 Mortality in the screening study (OECD TG 421, 2003) at 150 mg/kg bw/day is not expected to be linked to the pregnancy because no mortality of dams was reported in a pre-natal developmental study with the Substance (OECD TG 414, 2008) up to 250 mg/kg bw/day.

60 In the repeated dose toxicity study, liver necrosis was observed at and above 200 mg/kg bw/day (after 13-weeks) while in the screening study liver necrosis was observed at 150 mg/kg bw/day. Even though the severities and incidences cannot be compared without the study report and individual data, the liver necrosis (and other liver toxicity) did not cause mortality in repeated dose toxicity study at 200 mg/kg bw/day or above (13-week exposure).

61 Therefore, it cannot be excluded that the mortality observed in females in the screening study is linked to the dystocia rather than to the pregnancy or the liver lesions.

62 In the comments to the draft decision, you indicate your intention to demonstrate that the information requirement is not triggered by conducting a pre-natal developmental toxicity study and an *ex vivo* rat uterine contractility assay as part of a weight of evidence approach with the source substance DAIP. We take note of your intended strategy. However, you do not provide any further justification. Therefore, no conclusion on the intended strategy can be made.

63 On this basis, the information requirement is triggered and a EOGRT study has to be provided.

4.2. Specification of the study design

4.2.1. Species and route selection

64 A study according to the test method OECD TG 443 must be performed in rats with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

4.2.2. Pre-mating exposure duration

65 The length of pre-mating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

66 Ten weeks pre-mating exposure duration is required to obtain results adequate for classification and labelling and/or risk assessment. There is no substance specific information in the dossier supporting shorter premating exposure duration (Guidance on IRs and CSA, Section R.7.6.).

67 Therefore, the requested pre-mating exposure duration is ten weeks.

4.2.3. Dose-level setting

68 The aim of the requested test must be to demonstrate whether the classification criteria of the most severe hazard category for sexual function and fertility (Repr. 1B; H360F) and developmental toxicity (Repr. 1B; H360D) under the CLP Regulation apply for the Substance (OECD TG 443, paragraph 22; OECD GD 151, paragraph 28; Annex I Section 1.0.1. of REACH and Recital 7, Regulation 2015/282), and whether the Substance meets the criteria for a Substance of very high concern regarding endocrine disruption according to Art.57(f) of REACH as well as supporting the identification of appropriate risk management measures in the chemical safety assessment.

69 To investigate the properties of the Substance for these purposes, the highest dose level must be set on the basis of clear evidence of an adverse effect on sexual function and fertility, but no deaths (i.e., no more than 10% mortality; Annex I, Section 3.7.2.4.4 of the CLP Regulation) or severe suffering such as persistent pain and distress (OECD GD 19, paragraph 18) in the P0 animals.

70 In case there are no clear evidence of an adverse effect on sexual function and fertility, the limit dose of at least 1000 mg/kg bw/day or the highest possible dose level not causing severe suffering or deaths in P0 must be used as the highest dose level. A descending sequence of dose levels should be selected to demonstrate any dose-related effect and aiming to establish the lowest dose level as a NOAEL.

71 In summary: unless limited by the physical/chemical nature of the Substance, the highest dose level in P0 animals must be as follows:

in case of clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals, the highest dose level in P0 animals must be determined based on such clear evidence, or

(1) in the absence of such clear evidence, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or

(2) if there is such clear evidence but the highest dose level set on that basis would cause severe suffering or death, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or

(3) the highest dose level in P0 animals must follow the limit dose concept.

72 You have to provide a justification with your study results demonstrating that the dose level selection meets the conditions described above.

73 Numerical results (i.e. incidences and magnitudes) and description of the severity of effects at all dose levels from the dose range-finding study/ies must be reported to facilitate the assessment of the dose level section and interpretation of the results of the main study.

4.2.4. Cohorts 1A and 1B

74 Cohorts 1A and 1B belong to the basic study design and must be included.

4.2.4.1. Histopathological investigations in Cohorts 1A and 1B

75 In addition to histopathological investigations of cohorts 1A, organs and tissues of Cohort 1B animals processed to block stage, including those of identified target organs, must be subjected to histopathological investigations (according to OECD TG 443, paragraph 67 and 72) if

- the results from Cohort 1A are equivocal,
- the test substance is a suspected reproductive toxicant or
- the test substance is a suspected endocrine toxicant.

4.2.4.2. Splenic lymphocyte subpopulation analysis

76 Splenic lymphocyte subpopulation analysis must be conducted in Cohort 1A (OECD TG 443, paragraph 66; OECD GD 151, Annex Table 1.3).

4.2.4.3. Investigations of sexual maturation

77 To improve the ability to detect rare or low-incidence effects, all F1 animals must be maintained until sexual maturation to ensure that sufficient animals (3/sex/litter/dose) are available for evaluation of balano-preputial separation or vaginal patency (OECD GD 151, paragraph 12 in conjunction with OECD TG 443, paragraph 47). For statistical analyses, data on sexual maturation from all evaluated animals/sex/dose must be combined to maximise the statistical power of the study.

4.2.5. Further expansion of the study design

78 The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Annex IX, Section 8.7.3., Column 2. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in Guidance on IRs & CSA, Section R.7.6.

79 You remain responsible for complying with this decision by the set deadline.

5. Long-term toxicity testing on aquatic invertebrates

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

5.1. Information provided

81 You have provided:

(i) a long-term toxicity study on aquatic invertebrates (1989) with a test material referred to as "phthalic acid diallyl ester". ECHA understands that you consider that the test material used in this study corresponds to the Substance.

5.2. Assessment of the information provided

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

82 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:

83 Technical specifications impacting the sensitivity/reliability of the test

a) for semi-static tests, at least 10 animals individually held at each test concentration and in the control series;

b) the test temperature is within 18°C and 22°C and not varying by over ±1°C;

84 Characterisation of exposure

c) Analytical monitoring must be conducted. A reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range must be available;

85 Reporting of the methodology and results

d) adequate information on the test material is provided (i.e. identifiers, purity and presence of impurities)

e) detailed information on feeding, including amount (in mgC/daphnia/day) and schedule is reported.

f) water quality monitoring within the test vessels (i.e., temperature and dissolved oxygen concentration, TOC and/or COD and hardness) is reported.

g) the full record of the daily production of living offspring during the test by each parent animal is provided.

h) the number of deaths among the parent animals (if any) and the day on which they occurred is reported.

86 In study (i) described as a long-term toxicity study on aquatic invertebrates:

87 Technical specifications impacting the sensitivity/reliability of the test

a) the test was conducted under semi-static conditions, but the organisms were not individually held (based on your record that number of the organism per vessel were 5);

b) the test temperature was 25°C hence above the maximum value set out in the test guideline;

88 Characterisation of exposure

c) analytical monitoring of exposure was conducted was not conducted;

89 Reporting of the methodology and results

d) no identifiers are provided for the test material. In addition, the purity of the test material and the presence of impurities are not reported;

e)-h) you have not provided any of this information.

90 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the studies results. More specifically,
  - the test temperature was too high which may have impacted the reliability of the test in an unpredictable way. Further, in the absence of information on mortality of parental animal, it cannot be assessed whether the fact that parental animals were not held individually may have biased the results of the study;
  - in the absence of analytical monitoring, adequate exposure to the test material is not demonstrated.
- the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically,
  - you have not provided adequate information to support that the test material used in these studies is representative of the Substance;
  - key information on the test conditions is missing and therefore it is not possible to conduct an independent assessment as to whether these studies were conducted under conditions that are consistent with the specifications of the OECD TG 211;
  - you have not provided adequate reporting of the study results and therefore it is not possible to verify that the validity criteria of the OECD TG 211 were met and to conduct an independent assessment of the interpretation of the results.

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

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

6. Further long-term toxicity testing

93 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 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).

6.1. Information provided

94 You have adapted this information requirement by using the following justification: "*Short term toxicity study shows the substance is toxic to fish (LC50 of 0.23mg/L) and it cannot be justified from the CSA*".

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

6.2.1. Your justification to omit the study has no legal basis

95 A registrant may only adapt this information requirement based on the general rules set out in Annex XI.

96 Your justification to omit this information does not refer to any legal ground for adaptation under Annex XI to REACH.

97 Therefore, you have not demonstrated that this information can be omitted and the information requirement of Annex IX, Section 9.1.6. is not met.

6.3. Justification for the further information required under Annex IX, Section 9.1., Column 2

98 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.

99 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; and
- 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; and
- 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).

100 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.

101 Your registration dossier provides the following:

- OECD CF Level 4 information:

  Data on mammalian species:

  - As already explained under Section 4.1., an increase in gestation length was observed in an OECD TG 421, parameter "sensitive to, but not diagnostic of, EATS" modalities for endocrine disruption according to the guidance provided in OECD GD 150 (OECD, 2018).

102 You have not provided any conclusion in your registration dossier on ED properties for the Substance.

[2] WHO/IPCS, 2002. Global assessment of the state-of-the-science of endocrine disruptors. https://apps.who.int/iris/handle/10665/67357.

In addition, the following information is publicly available for the Substance:

- OECD CF Level 2 information:
  - Nakai et al (1999) examined a series of ring and alkyl-chain isomers of dialkyl phthalates for their ability to displace [3H]17b-estradiol in the recombinant human estrogen receptor expressed on Sf9 baculovirus. All the ring isomers of C3-diallyl derivatives, the Substance, diallyl isophthalate, and diallyl terephthalate, exhibited a distinct receptor binding. Of these three phthalates the Substance was the most potent to bind to the estrogen receptor.
  - The CompTox Chemicals Dashboard v2.2[3] of the US EPA also reported a significant Estrogen and Androgen Receptor activity. The Substance was positive in nine ER assays (out of 19) and three AR assays (tested in 15).
  - In an *in vitro* E-screen assay, used for quantitative determination of the total estrogenicity in test compounds, the Substance showed stronger estrogenic effects than DBP (Kim and Ryu, 2006).

103 Furthermore, the above described estrogenic activity of the Substance in *in vitro* assays indicates similar estrogenic activity compared to the structurally similar substance dibutyl phthalate (DBP). DBP and the Substance shares the same orthophthalate structure but differs in the carbon side chains (side chain of DBP is saturated and longer by one carbon). DBP is currently identified as SVHC for its endocrine disrupting properties for human health[4], and there are several studies with DBP that have shown estrogenic, antiestrogenic and antiandrogenic effects in fish *in vivo* (Aoki et al., 2011; Bhatia et al., 2013 and 2014; Hu et al 2020).

104 In conclusion, there is *in vitro* evidence showing that the Substance has the potential to disrupt sex hormone balances through its binding affinity to estrogenic receptors and *in vivo* evidence showing changes in gestation length. This is further supported by *in vivo* data for structurally similar DBP with mammals and fish indicating to affect fish sexual development.

105 Therefore, this information indicates endocrine activity, but should be regarded as inconclusive with regard to endocrine disrupting properties due to the available studies only covering mechanistic parameters and not adversity.

106 On this basis, available information from OECD CF Level 2 and 4 indicate that the Substance may be an endocrine disruptor via EAS modalities in mammalian and (by analogy to DBP) non-mammalian species (including fish). However, this information does not allow to conclude whether or not the Substance may show adverse effects as a result of its endocrine activity.

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

6.4. Test selection and study specifications

108 As explained 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).

[3] https://comptox.epa.gov/dashboard/chemical/details/DTXSID7020392
[4] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017D1210

109 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.

110 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).

111 As explained 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

The following documents may have been cited in the decision.

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/.

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Kim Y, Ryu J. Evaluation of Estrogenic Effects of Phthalate Analogues Using *in vitro* and in vivo Screening Assays. 2006 Mol & Cell Tox, vol 2, 106 – 113

Nakai, M, Tabira Y, Asai D, Yakabe Y, Shimyozu T, Noguchi M, Takatsuki M, Shimohigashi Y. Binding Characteristics of Dialkyl Phthalates for the Estrogen Receptor, Biochemical and Biophysical Research Communications, Volume 254, Issue 2, 1999, Pages 311-314.

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).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).
All guidance documents are 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 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.

In your comments on the draft decision, you requested an extension of the deadline to provide information from 36 to 48 months from the date of adoption of the decision. In support of your request, you provided documentation from a CRO which includes a schedule for the requests including in the decision. This information indicates that the requested studies could be performed within a period of 37 months and ECHA exceptionally agrees to grant an extra 3 months to submit the information. On this basis, ECHA has extended the deadline to 39 months.

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

ECHA received proposals 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.

You have provided comments agreeing with the requests in the draft decision. These comments do not address the proposed amendment(s). Therefore, these comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee unanimously agreed on the draft decision during its MSC-82 meeting. 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[5].

(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[6].

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