~~Confidential~~

Helsinki, 20 June 2023

Addressees
Registrant(s) of [redacted] as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
03/04/2014

Registered substance subject to this decision ("the Substance")
Substance name: Diethyl phthalate
EC/List number: 201-550-6

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 ***27 March 2026***.

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

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

2. 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

3. 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 decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

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

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

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes

to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

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

Failure to comply

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

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the 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. Growth inhibition study aquatic plants

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

1.1. Information provided

2 You have provided the following information on the Substance:

(i) a study on Growth inhibition study on aquatic plants equivalent to OECD TG 201 (1982)
(ii) a study on Growth inhibition study on aquatic plants according to OECD TG 201 (1990)

1.2. Assessment of the information provided

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

3 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:

4 Characterisation of exposure

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

5 Reporting of the methodology and results

b) the test design is reported (*e.g.*, number of replicates, number of test concentrations and geometric progression used);
c) the test conditions are reported (*e.g.*, composition of the test medium, test temperature, test species, biomass density at the beginning of the test);
d) 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;
e) the results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form;
f) 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.

6 In studies (i) and (ii) described as growth inhibition studies on aquatic plants/algae:

7 Characterisation of exposure

a) no analytical monitoring of exposure was conducted for study (i) and no justification was provided;

8 Reporting of the methodology and results

b) on the test design, you have not specified the number of replicates, number of test concentrations for study (i) and (ii);
c) on the test conditions, you have not specified the composition of the test medium, test temperature, biomass density at the beginning of the test for study (i) and (ii);
d) for study (i), you report that algal biomass was determined using optical density. 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;
e) tabulated data on the algal biomass determined daily for each treatment group and control are not reported for study (i) and (ii);
f) information on the analytical method (including performance parameters of the method) and on the results of the analytical determination of exposure concentrations is not provided for study (ii).

9 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically, no analytical monitoring was conducted in study (i) and therefore you have not demonstrated that exposure was satisfactorily maintained throughout the test. For study (ii), you claim that analytical monitoring of exposure was conducted. However, you reported not information and therefore an independent assessment is not possible.
- the reporting of the studies is not sufficient to conduct an independent assessment of their reliability. More specifically, you have not provided biomass data for study (i) and (ii). Therefore, it is not possible to independently assess whether validity criteria of the test guideline were met and that the interpretation of the results is adequate. Then, key information is missing to verify that the test design and procedure were consistent with the requirements of the test guideline. Finally, for study (i), you have not provided adequate information to support that the method used for biomass determination was adequate.

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

2. Ready biodegradability

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

2.1. Information provided

12 You have provided:

(i) a study on assessment of ultimate biodegradability following the EPA560/6-82-003 test method (1984) with the Substance.

2.2. Assessment of information provided

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

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

14 Technical specifications impacting the sensitivity/reliability of the test

a) the inoculum is not be pre-adapted to the test material;

15 Reporting of the methodology and results

b) the source of the inoculum, its concentration in the test are reported;
c) the test temperature is reported;
d) the results of measurements at each sampling point in each replicate is reported;
e) the calculation of the $ThCO_2$ is described;
f) the inorganic carbon content (IC) and total carbon content (TC) of the test material suspension in the mineral medium at the beginning of the test is reported.

16 In study (i) described as a study on ultimate ready biodegradability:

17 Technical specifications impacting the sensitivity/reliability of the test

a) the inoculum was pre-adapted to the test material;

18 Reporting of the methodology and results

b) the source of the inoculum, its concentration in the test are not reported;
c) the test temperature is not reported;
d) the results of measurements at each sampling point in each replicate is not reported;
e) the calculation of the $ThCO_2$ is not described;
f) the inorganic carbon content (IC) and total carbon content (TC) of the test material suspension in the mineral medium at the beginning of the test is not reported.

19 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically the inoculum of was acclimated for 2 weeks and exposed to the Substance, therefore the test does not qualify for ready biodegradability test;
- as you have not reported the information listed under points b) to f) the reporting of the study is not sufficient to conduct an independent assessment of its reliability.

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

Reasons related to the information under Annex IX of REACH

3. Further long-term aquatic toxicity

21 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).

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

3.1.1. Information provided

22 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided following justification: "*Annex IX, Column 2, long-term tests on fish need only be conducted if the outcome of the Chemical Safety Assessment indicates such a need*".

23 You have also provided the following information on long-term toxicity to fish:

(i) a non-guideline long-term toxicity study on fish (2007) on the substance

3.1.2. Assessment of the information provided

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

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

25 Your adaptation is therefore rejected.

3.1.2.2. The provided study does not meet the specifications of the OECD TG 210

26 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:

27 Key parameter to be measured

a) parameters related to the survival and development of fish in early life stages from the stage of fertilized egg until the juvenile life-stage following exposure to the test substance are measured, including:
   (i) the stage of embryonic development at the start of the test, and
   (ii) hatching of fertilized eggs and survival of embryos, larvae and juvenile fish, and
   (iii) the appearance and behaviour of larvae and juvenile fish, and
   (iv) the weight and length of fish at the end of the test.

28 You have provided a non-guideline long-term toxicity study on fish showing the following:

29 Key parameter to be measured

a) the test was conducted on *Cyprinus carpio* and the test animals were 4-months old. Therefore, the test did not cover the adequate life-stages for the test species (*i.e.*, from the stage of fertilized egg until the juvenile life-stage).

30 Based on the above, the information provided does not cover any of the key parameters required by the OECD TG 210.

31 Therefore, the study does not meet the specifications of the OECD TG 210 and the information requirement set out under Annex IX, Section 9.1.6., Column 1 is not met.

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

32 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.

33 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).

34 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.

35 Your registration dossier provides the following information indicating a potential ED hazard for the environment :

- Information equivalent to OECD CF Level 4:

  In study (i), a significant (P<0.01) and dose dependant vitellogenin induction effect was observed following an exposure of adult male fish to 0.1, 1 and 5 ppm of the Substance.

36 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://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/.

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

- Information equivalent to OECD CF Level 2:
  - In an *in vitro* study using human adrenocortical carcinoma (H295R) cells (Sohn *et al*., 2016), reduced T concentrations and increased E2/T ratio were observed after 48 hours exposure to the Substance. Gene expression changes were observed in H295R cells with significant down-regulation of StAR gene and up-regulation of CYP19A gene, that supported depressed synthesis of sex hormones in the adrenal cell.
  - In an *in vitro* study (Lee *et al*., 2019) showed that the Substance induced some affinity (up to 16% of E2 affinity at high dose of 100 mg/L) in MVLN cell line. A dose response was observed from 0.16 mg/L to 100 mg/L for the cell line. In the same study, an increase in E2 production and in E2/T at 20 mg/L and above was also observed in H295R cells, while inhibition of T production at 4 mg/L and above was observed but without dose response.
  - In an androgen receptor (AR) inhibition study with dihydrotestosterone (DHT), the Substance (DEP) 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 a marginal (non-significant) activation of Erα, but not of Erβ, and a marginal (no-significant) inhibition at 100 µM of ERα or Erβ in presence of E2.
- Information equivalent to OECD CF Level 3:
  - In an *in vivo* study using male zebrafish, Sohn *et al*. (2016) showed that after 14 days exposure to The Substance, there was significant reduction of plasma E2 and T concentration at 10 mg/L, but no significant change in E2/T ratio; expression of several steroidogenic genes were significantly affected in gonads of male zebrafish: *star* gene was significantly down-regulated at 10 mg/L of the Substance (leading to decreased steroid hormone synthesis) and marginally significant negative trends in *cyp11a* gene expression and significant up-regulation of *cyp19a* gene (responsible for T to E2 conversion) were observed. In liver, a significant down-regulation of *Vtg* gene expression was observed at 10 mg/L.
  - In an *in vivo* embryonic zebra fish assay, Lee *et al*. (2019) showed that the exposure to the Substance (DEP) resulted in a significant up-regulation of *vtg1* and *esr1* at 0.1 mg/L and *esr2* at 1 mg/L which could explain the increased E2 level in the H259R assay. Up-regulation *cyp19a1a* and *cyp19a1b* genes (involved in conversion to T to E2) provides a further support for the increased E2/T ratio observed *in vitro*. No modulation of the expression of genes involved in the hypothalamic-pituitary-gonadal (HPG) axis (i.e. *fshr* and *lhb* which promote steroid-hormone production in gonads) was observed.
  - In a study from Bisseger *et al*. (2018), embryos of *S. tropicalis* (from stage NF11 to stage NF46) were exposed to three phthalates tested (DEP, DBP, DEHP). All three phthalates increased the expression of androgen related genes, such as steroid-5α-reductase 1, 2, 3, steroid-5β-reductase, and androgen receptor at concentrations ranging from 0.1 to 10 µM depending on the phthalate tested and gene. In addition, phthalate exposure increased malformations rates of incomplete gut coiling, eye malformation, tail and oedema occurrence.

38 In your comments on the draft decision, you disagree with the request and propose to perform an OECD TG 210, instead of the requested OECD TG 234. As summarised below, you have provided your reasoning as to why you consider the available information from

the publications mentioned by ECHA as invalid/unassignable. You consider that this information is not suitable to justify an ED concern for the Substance.

39 For the OECD CF Level 2 studies from Sohn *et al.* (2016) and Engel *et al.* (2017), 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 studies may to be representative for the Substance from industrial standardardized processes. Furthermore, you note the absence of "*data on purity and* [on] *substance characterization*".

(ii) *Relevance of the human cell line receptors*: you argue that the studies may not be relevant for the environmental assessment, as the relevance of the human cell line receptors in ecological assessment has not been verified. You also argue that EDEG in the 18th meeting concluded that DEP is not an endocrine disruptor for humans.

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

40 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 932 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.

(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*".

41 For the other study used as OECD CF Level 3 from Bisseger *et al.* (2018), 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 specific concern:

(vi) *Relevance of the study*: you question the validity of amphibian larvae of of S. *tropicalis* to detect E and A effects when other species, such as *Xenopus* have shown alterations of hormonal activities when maintained in captivity and are primarily used to detect thyroid effects for this reason. You also argue that it is not possible to link the noted malformations to an ED MoA

42 Finally, concerning the non-guideline long-term toxicity study on fish (study (i) in Section 3.1.1. above) from Barse *et al.* (2007) that you used to fulfil the information requirement for long-term toxicity on fish, you now consider that the study is invalid as no indication on data purity or substance characterisation was given as already mentioned under point (i) above. You also consider that similar deficiencies in relation to the use of solvent (point (iv) above) also apply to this study. Finally, you also express the following specific concerns:

(vii) *Control animals*: you consider that the number of control animals was too low which challenges the statistical interpretations of the results;

(viii) *Lack of analytical monitoring of exposure*: you state that "*While analytical measurements of DEP are discussed in the methods, there is no reporting of these data*";

(ix) *Metric selection*: you consider that measurement of VTG from dorsal muscle needs to be explained and defended as it is generally measured in the liver or in plasma. You also consider that "*standard errors for VTG induction look too good to be true*" and you consider the observed increase in VTG concentration as "*not actually a major absolute increase*". You also consider some of the conclusions from the authors as speculative.

43 With regards your concern under point (i) above, ECHA points out that there is no evidence that the observed effects in the studies (Barse *et al.*, 2007; Sohn *et al.*, 2016; Engel *et al.*, 2017; Bisseger *et al.*, 2018; and 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.

44 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.

45 With regards your concern under point (iii) above, ECHA notes the following:

- [redacted] (1998): the Substance was not tested in this study.
- [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.

46 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.

47 The requested OECD TG 234 study without the use of a solvent would clarify whether relevant effects are caused by the Substance.

48 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.

49 With regards to points (vi) to (ix), ECHA acknowledges the deficiencies raised by you. 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.

50 In conclusion, there is *in vitro* and *in vivo* evidence showing that the Substance has the potential to disrupt sex hormone balances by modulating key steroidogenic genes in the human adrenal cells and in zebrafish embryos. In addition, there is evidence that the Substance elicits androgenic activity in frog embryos. 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, but not apical endpoints.

51 On this basis, available information from OECD CF Level 2 to 4 indicate that the Substance may be an endocrine disruptor via estrogenic, androgenic and steroidogenic (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.

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

3.3. Assessment of the information provided on further aquatic toxicity testing

3.3.1. Information provided

53 ECHA has also assessed the study (i) listed under Section 3.1.1. against the requirements of the OECD TG 234 that is required for the Substance to conclude whether or not it may show adverse effects as a result of its endocrine activity.

3.3.2. Assessment of the information provided

54 To fulfil the information requirement, a study must comply with the OECD TG 234 (Article 13(3) of REACH). Therefore, the following specifications must be met:

- fish is exposed, from newly fertilized egg until the completion of sexual differentiation (i.e. 60 dph);
- observations and measurements include the stage of embryonic development, hatching of fertilised eggs and survival or larvae and juvenile fish, recording of abnormal appearance and behaviour, fish weight and length, VTG analysis and sex determination via histological evaluation.

55 The study (i) above investigates the impact of the exposure to the test material on growth and on vitellogenin production in young adult fish after 28 days.

56 This study does not provide information on effects of the test material to all relevant sensitive life-stages (i.e. juveniles, eggs and larvae). Furthermore, it does not provide equivalent exposure length, observations and measurements as those specified in the OECD TG 234.

57 As this study does not provide equivalent information to a study conducted according to the OECD TG 234, the information requirement is not met.

3.4. Test selection and study specifications

58 As explained under Section 3.2 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).

59 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.

60 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).

61 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.

3.5. References

62 Barse *et al.* (2007). Endocrine disruption and metabolic changes following exposure of *Cyprinus carpio* to diethyl phthalate. Pesticide Biochemistry and Physiology 88 (2007) 36–42.

63 Bisseger *et al.* (2018). Phthalates modulate steroid 5-reductase transcripts in the Western clawed frog embryo. Comparative Biochemistry and Physiology Part C 213:39-46.

64 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, 77:54-63

65 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.

66 Sohn *et al.* (2016). Alteration of sex hormone levels and steroidogenic pathway by several low molecular weight phthalates and their metabolites in male zebrafish (Danio rerio) and/or human adrenal cell (H295R) line. Journal of Hazardous Materials 320 (2016) 45–54.

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).
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.

In your comments on the draft decision, you requested an extension of the deadline to provide information from 30 to 36 months from the date of adoption of the decision based on laboratory capacities. You provided no documentation from a testing laboratory to support your request.

ECHA has already exceptionally extended by 12 months the standard deadline which cover the time needed to perform the tests requested in the decision, including the performance of an OECD TG 234, in parallel to the other tests requested. In the absence of any documentation to support your request, no further extension is granted.

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

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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

~~Confidential~~

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