~~Confidential~~

Helsinki, 27 October 2021

Addressees
Registrant(s) of JS_405-520-5 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
13/11/2020

Registered substance subject to this decision ("the Substance")
Substance name: 4-(4-isopropoxyphenylsulfonyl)phenol
EC number: 405-520-5
CAS number: 95235-30-6

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***2 August 2024***.

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

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

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: EU B.13/14. /OECD TG 471) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102

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

1. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) by oral route, in rats

2. Activated sludge respiration inhibition testing (Annex VIII, Section 9.1.4.; test method: EU C.11/ OECD TG 209)

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

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

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

Reasons for the request(s) are explained in the following appendices entitled "Reasons to request information required under Annexes VII to IX of REACH", respectively.

Information required depends on your tonnage band

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

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

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

How to comply with your information requirements

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

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

Appeal

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

Failure to comply

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

Authorised[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix A: Reasons to request information required under Annex VII of REACH

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is an information requirement under Annex VII to REACH (Section 8.4.1.).

You have provided a key study in your dossier:

i. [redacted] (1987) with the following strains, TA 98, TA 100, TA 1535, TA 1537, and TA 1538 which all gave negative results.

We have assessed this information and identified the following issue:

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471[2] (1997). One of the key parameters of this test guideline includes that the test must be performed with 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

The reported data for the study you have provided did not include results for the appropriate 5 strains, as it does not include the required fifth strain, S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101).

The information provided does not cover one of the key parameters required by OECD TG 471. Therefore, the information requirement is not fulfilled.

In your comments on the draft decision, you agreed to conduct the requested study.

Study design

To fulfil the information requirement for the Substance, the *in vitro* gene mutation study in bacteria (OECD TG 471) should be performed using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

[2] ECHA Guidance R.7a, Table R.7.7–2, p.557

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

1. Screening for reproductive/developmental toxicity

A Screening for reproductive/developmental toxicity study (test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) is an information requirement under Annex VIII to REACH (Section 8.7.1.), if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant.

There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

You have provided a key experimental study according to OECD TG 415 (One-Generation Reproduction Toxicity Test) with the Substance ([redacted], 2009). You consider that this study includes OECD TG 421 parameters and therefore an additional screening reproductive/developmental toxicity study was not performed.

To be considered compliant and to generate information concerning the effects of the Substance on male and female reproductive performance, the study has to meet the requirements of EU B.63/OECD TG 421 or EU B.64/OECD TG 422. The study you provided covers the parameters of OECD TG 421.

However, the criteria of this test guideline include that the highest dose level should aim to induce toxic effects.

The highest dose level in the study did not induce any toxicity and you have not shown that the aim was to induce toxicity. Therefore, the dose level selection was too low.

In your comments on the draft decision, you state that *"data on relevant parameters for reproductive toxicity are already available and together with the data from the newly to perform OECD 414 study the Registrants claims that this endpoint (REACH Annex VIII Section 8.7.1) is sufficiently covered. In conclusion, adequate information on the endpoint of reproductive toxicity as required under REACH Annex VIII will be provided by performing the OECD TG 414, for which ECHA already has accepted the Testing Proposal."*

Regarding your comment on the relevant parameters, ECHA already acknowledged that these are in principle covered by a study according to OECD TG 415. However, as already explained above, the selected dose of the provided OECD TG 415 is considered too low in order to accept the results with confidence, as a no observed adverse effect level (NOAEL) was not established but rather a NOEL of 125 mg/kg bw/day. You state in the dossier *"In conclusion, D-8 administered daily by oral gavage to Wistar rats at least during premating and mating periods in male animals and premating, mating, gestation and 21-day lactation periods in female animals did not lead to any toxicologically adverse effects at dose levels of 0, 5, 25 and 125 mg/kg bw/day in either the parent or offspring generation. Under the conditions of this study, the no observed effect level (NOEL) for D-8 for parental and F1 offspring effects is 125 mg/kg bw/day."*

Regarding your further reference to the performance of a study according to OECD TG 414, ECHA acknowledges that under Annex VIII, Section 8.7., Column 2, first paragraph, fourth indent, the standard information (OECD TG 421) does among others not need to be conducted if a pre-natal developmental toxicity study (OECD TG 414) is already available. However, the study you refer to is not yet available and it is thus not possible for ECHA to check whether such information may comply with the REACH requirements.

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

Information on study design

A study according to the test method EU B.63/OECD TG 421 or EU B.64/OECD TG 422 must be performed in rats with oral administration of the Substance (ECHA Guidance R.7.6.2.3.2.).

2. Activated sludge respiration inhibition testing

Activated sludge respiration inhibition testing is an information requirement under Annex VIII to REACH (Section 9.1.4.).

You have adapted this information requirement based on the following justification: "*According to REACH Annex XI Section 1 ("The study is scientifically not necessary; other information available") no study on the toxicity to microorganisms has to be conducted. Following REACH Annex XI Section 1.2 "Weight of Evidence" other information is available that is reliable and sufficient to cover the endpoint of toxicity to microorganisms according to REACH Annex VIII Section 9.1.1 Column 1. The performance of the toxicity testing of D-8 to aquatic microorganisms is scientifically not justified. D-8 was tested for ready biodegradability in a biodegradation screening test according to EU Method C.4-C and OECD 301B, and in a simulation test of biodegradation in water and sediment according to OECD Guideline 303A. In both tests, D-8 showed no signs of toxic activity to the activated sludge. Thus, it can be concluded that D-8 is non-toxic to aquatic microorganisms. Therefore, no test on toxicity to microorganisms was performed. A NOEC of 15 mg/L was chosen as a key values (from the OECD 303A study) for further risk assessment*".

From the above ECHA understands that you have adapted this information requirement under Annex XI, Section 1.2. of REACH ('weight of evidence'). In support of your adaptation, you have provided the following sources of information:

i. an adaptation under Annex XI, Section 1.1. ('use of existing data') with no further justification;
ii. a statement that no toxicity to the inoculum was observed in studies conducted according to OECD TO 301B and OECD TG 303A.

We have assessed this information and identified the following issues:

A. Your weight of evidence adaptation does not rely on any relevant source of information

Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of 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 of the information for the given regulatory information requirement. Subsequently, relevance, reliability, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach.

However, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has or has not a particular dangerous property.

In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation and identified the following issue:

To fulfil the information requirement, normally a study performed according to OECD TG 209 must be provided (ECHA Guidance R.7.8.17.). OECD TG 209 requires the study to investigate the following key parameter:

- the respiration rates of samples of activated sludge fed with synthetic sewage after a contact time of at least 3 hours.

None of the sources of information provided inform on the above key parameter. Accordingly, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an OECD TG 209 study. Therefore, your adaptation is rejected.

B. *Your adaptation under Annex XI, Section 1.1. does not meet the requirements of that provision*

Under Annex XI, Section 1.1., the information requirement may be omitted based on existing data if the available data meet, among other the following condition:

- it provides adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred in Article 13(3), in this case the OECD TG 209.

As already explained above under issue A., you have provided no relevant information on the respiration rates of samples of activated sludge fed with synthetic sewage after a contact time of at least 3 hours. Therefore, your adaptation is rejected.

C. *The justification provided does not meet the requirements of Annex VIII, Section 9.1.4., third indent.*

While you have not explicitly claimed such adaptation, ECHA has also assessed your adaptation in line with the specific rule for adaptation of Annex VIII, Section 9.1.4., third indent. Under this provision, a study may be omitted if a substance is found to be readily biodegradable and the applied test concentrations are in the range that can be expected in the influent of a sewage treatment plant. ECHA Guidance R.7.8.18.2. clarifies that the assumption that the substance under investigation is not inhibitory to the micro-organisms when dosed in the test system is implicit in ready biodegradability testing (i.e., EC C.4A-F, OECD 301A-F (OECD, 1992) and OECD 310 (2006)). If a compound degrades well in a ready biodegradability test, or does not inhibit the degradation of a positive control at a certain concentration, this concentration can be used as a NOEC value. In some case, the results of an OECD TG 303A may also be considered acceptable to derive a PNECstp. However, to be considered acceptable, parameters such as BOD/COD removal, N-removal, sludge settling, etc., as compared to a parallel non-dosed control must be monitored.

Your registration dossier provides a ready biodegradability study according to OECD TG 301B with the Substance ([redacted], 1990). Biodegradation reached 31% and 45% after 28 days at 10 and 20 mg/L, respectively. No information on inhibition of a positive control is provided. On the study, you state that "*typical exponential phase of degradation of the test item was not obtained. Until day 20 a slow degradation at both*

concentrations was observed. Thereafter, a diauxic behaviour was obtained for the concentration 10 mg/L, whereas at the concentration of 20 mg/L the degradation stopped".

To support your adaptation you also refer to a study according to OECD TG 303A with the Substance ([redacted], 2004). On this study you state that "*The running-in period was from day 0 (addition of D-8) to day 54. It was divided into three stages (I to III) because the D-8 concentration was changed three times. At test start the D-8 test concentration was set at 25 mg/L with the intention to use a concentration level high enough to see effects on DOC removal, but still below the maximum solubility in waste water (30 mg/L). As at this concentration level no effects on DOC removal could be observed after 22 days, the test item concentration was increased to 30 mg/L in a second stage to get the maximum solubility value. At this stage clear indications for degradation/elimination were noted*".

The ready biodegradability study from your dossier shows that the Substance cannot be regarded as readily biodegrable and cannot therefore be concluded to degrade well in a ready biodegradability test. Lower biodegradation was observed at 20 mg/L compared to 10 mg/L which may be indicative of an inhibition phenomenon. Further in the absence of information on inhibition of a positive control you have not demonstrated that the Substance in not inhibitory to STP micro-oragnisms. Finally, the OECD TG 301A study indicates that inhibition may have occurred at least during the first phase of the study. You have not provided the results of the monitoring of BOD/COD removal, N-removal and sludge settling as compared to a parallel non-dosed control and therefore you have not demonstrated that no inhibition occurred during this test. As a result the information provided does not allow concluding that the Substance is not inhibitory to STP micro-organisms. Therefore, your justification does not meet the requirements of Annex VIII, Section 9.1.4., third indent.

On this basis, the information requirement is not fulfilled.

In your comments on the draft decision, you agreed to conduct the requested study.

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

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

A Sub-chronic toxicity study (90 day) is an information requirement under Annex IX to REACH (Section 8.6.2.).

You have provided a key experimental study according to OECD TG 408 with the Substance ([redacted], 2009).

We have assessed this information and identified the following issue:

To be considered compliant and enable concluding whether the Substance has dangerous properties and supports the determination of the No-Observed Adverse Effect Level (NOAEL), a study has to meet the requirements of OECD TG 408. The key parameter(s) of this test guideline include, among others, that the highest dose level should aim to induce some systemic toxicity, but not death or severe suffering.

The highest dose level in the study did not induce any systemic toxicity. You have reported a no observed effect level (NOEL) of 50 mg/kg bw/day (top dose) stating that D8 administration did not lead to any toxicologically adverse effects. Therefore, the dose level selection was too low, and the study does not fulfil the criterion set in OECD TG 408.

In your comments on the draft decision you state that "*The dose setting for this study was based on available data for a 28-day study according to OECD TG 407 from 1988 ([redacted])*". You also quote the study report which states that "[…] *D-8 was administrated in the diet to male and female F344 rats. Applied dose levels were 0, 120 ppm (low), 1200 ppm (mid) and 12000 ppm (high) (corresponding to approximately 10, 100 and 1000 mg/kg bw/d) for 4 weeks (plus 2 weeks recovery).* […] *Liver weight and its ratio to body weight were increased in the mid and/or high dose males.* […] *The incidence of tubular regeneration in kidneys was higher in males of the mid and high dose group than that of the control group. Renal calcium deposition was found in males of mid and high dose group and also in females of the high dose group."*

Among all the toxicity findings you listed, only few were significant after exposure to the mid-dose (1200 ppm): increased liver weight ratio to body weight in males, and higher tubular regeneration in kidneys in males. According to the report "[redacted].pdf" provided in Section 7.5.1 in IUCLID, the calcification and tubular regeneration were only significant for males exposed to the high-dose (12000 ppm), and were not significant for males exposed to mid-dose (1200 ppm). As you report in the dossier "*There were no macroscopic or microscopic adverse findings, no statistically or toxicologically significant changes in organ weight values or any pathology changes that could be ascribed to test item administration. In conclusion, D-8 administered daily by oral gavage for 90 days in Wistar rats did not lead to any toxicologically adverse effects at dose levels of 5, 10 or 50 mg/kg bw/day"* therefore a no observed adverse effect level (NOAEL) was not established but rather a NOEL of 50 mg/kg bw/day.

In your comments on the draft decision you further state that "*In the performed OECD TG 408 study the administration route was chosen to be via oral gavage. It is known that the kinetic profiles of the same substance differ in animals treated via gavage versus administration via other oral routes [2] and that oral gavage results in higher tissue concentrations in rats when compared with animals receiving comparable dietary exposure [3,4].*". You conclude that "*the selection of 50 mg/kg bw/day as highest dose for the 90-day study is justified because the induction of some systemic toxicity could be expected considering the adverse kidney effects at 100 mg/kg bw/day in the 28-day study, the influence of gavage versus diet treatment and the longer exposure time.*".

As you explain, the route of administration of the Substance and the duration of the study are different between the 28-day study and the 90-day study. ECHA considers your statement "*It is known that the kinetic profiles of the same substance differ in animals treated via gavage versus administration via other oral routes [2] and that oral gavage results in higher tissue concentrations in rats when compared with animals receiving comparable dietary exposure [3,4]*" to be too speculative to allow for adequate dose selection, especially given the large interval between doses (factor of 10) that was used in the 28-day study. In addition, you did not take into account the differences in the strain of the rats between the 28-day study and the 90-day study in your considerations for dose selection.

For all these reasons ECHA maintains that the dose level selection was too low, and that the study does not fulfil the criterion set in OECD TG 408.

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

Information on the design of the study to be performed (route/ species/ strain)

Referring to the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the Substance is reported to occur as a dust without a significant proportion (>1% on weight basis) of particles of inhalable size (MMAD < 50 µm).

Therefore the sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and with oral administration of the Substance

2. Further long-term aquatic toxicity

Long-term aquatic toxicity testing 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). This is the case if, for instance, there are indications that the Substance may be an endocrine disruptor.

1.1. On the need for further investigation: Indications of endocrine disrupting activity

According to IPCS/WHO[3], *"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 cumulative conditions are met:

- 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;
- it shows endocrine activity, *i.e.* it has the potential to alter the function(s) of the endocrine system;
- 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).

Available information to be considered in the assessment of ED properties can be grouped according to the Conceptual Framework (CF) described in OECD GD 150. Evidence from information falling under CF levels 1 to 3 may be considered as indications of endocrine activity.

[3] 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/.

Your registration dossier provides the following:

- OECD CF Level 1 information:

  a. an intermediate receptor binding probability was predicted for Androgen Receptor (AR) antagonistic, Glucocorticoid Receptor (GR) agonistic as well as for Thyroid hormone Receptor (TR) α and TRβ using the Endocrine Disruptome Software. A low probability of binding was obtained for all other receptors, including Estrogen Receptor α (ERα) and Erβ.
  b. Using the DANISH QSAR database, the Substance is predicted positive for ERα Binding when a balanced training set is used but negative based on the full training set. CASE Ultra reveals a positive alert for ERα activation within the model's applicability domain. The Substance is concluded either positive or inconclusive by the other models but is outside of the applicability domains. All models predict a negative result for AR antagonism. Finally, the Substance is predicted positive in two Leadscope QSAR models for TPO inhibition but falls into the applicability domain of only one of these models.
  c. According to the OECD QSAR toolbox profiler v.4.2., the Substance is predicted as a strong binder for the ER and its simulated metabolites are predicted to be very strong binders for the ER. No alerts were found by the rainbow trout ER binding (rtER) expert system from the US EPA.
  d. Using VirtualToxLab™, the toxic potential (TP), which reflects binding affinity to proteins, was calculated to be 0.386 (i.e. moderate affinity). The main target of the Substance was predicted to be ERβ with a lower predicted binding affinity (1.33 µM) in comparison to BPA (0.084 µM) ([redacted], 2015).
  e. Based on a molecular docking model, the Substance was predicted as an ER binder.

You state that "*a binding affinity to the estrogene receptor cannot be fully excluded*" and that "*there are some indications for thyroid receptor binding and TPO inhibition*".

Based on the above, ECHA concludes that there are structural alerts indicating that the Substance may show endocrine activity.

- OECD CF Level 2 information:

  f. the Substance was tested for estrogenic activity in a screening test with yeast cells (*Saccharomyces cerevisiae*) transfected with the human ER (hER) gene ([redacted], 1998). No evidence of estrogenic activity was obtained at the top dose of 100 mg/L.
  g. ER agonist activity was measured in a yeast two-hybrid estrogenicity assay using yeast cells (*Saccharomyces cerevisiae* Y190) transfected with hERa or medaka (med) ERa, and with TIF2 coactivator and β-galactosidase reporter gene ([redacted], 2007). There was no agonist activity neither in the presence nor in the absence of the S9 mix.
  h. the estrogenic activity and/or endocrine modulating activity of the Substance was assessed in MCF-7 cells (E-screen assay) ([redacted], 1998). Cellular growth as a possible consequence of estrogenic activity was assessed using the sulforhodamine B cellular protein staining method. A slight but statistically significant induction of cell proliferation was observed at a concentration of 10 µg/mL in the absence of cytotoxicity.
  i. in an H295R steroidogenesis assay according to OECD TG 456 ([redacted], 2015), the concentration of 17β-estradiol or free testosterone was not modified by the Substance.
  j. in a competitive enzyme-linked immunosorbent assay (ER-ELISA) both with and without metabolic activation by a rat liver S9 mix ([redacted], 2007), no

binding affinity to hERα was observed at the top dose of 1 mg/L.

k. in a GFP-expression system ([redacted], 2005), Human breast cancer MCF-7 cells were stably transfected with an estrogen responsive GFP reporter gene construct. No estrogen activity was observed but antiestrogenic activity was seen at the higher concentration of 50 µM in co-exposure with E2 at the EC50 concentration (10 pM) (about 60% activity relative to E2 alone), in the absence of cytotoxicity.
l. extracts from cash receipts and other thermal paper products were analyzed using a LC-nanofractionation platform in combination with a cell-based ER-luciferase reporter gene bioassay ([redacted], 2017). The results show low or absence of estrogenic activity. Furthermore, the study was not conducted on pure substances.

You state that despite the fact that the Substance "*slightly induce cell proliferation in an MCF-7 cell proliferation assay*" ([redacted], 1998), the Substance "*neither exhibits ER binding affinity nor transactivation activity in vitro*". You further note that the MCF-7 cell proliferation assay has never been adopted by the OECD and that "*no impairment of steroidogenesis as indicated by the intracellular estradiol or testosterone levels was determined*". You consider the antiestrogenic effects observed in the study by [redacted] (2005) as unreliable as there is no information on the purity of the test material in this study and there are no indications for an antiestrogenic effect in other *in vitro* tests or *in vivo*.

ECHA notes that there are some *in vitro* evidence suggesting that the Substance has endocrine activity for the EAS modalities, but negative results were also observed in a number of *in vitro* assays. Therefore, this information provides some indication of endocrine activity but it is regarded as inconclusive with regard to endocrine disrupting properties.

- OECD CF Level 3 information:

Data on mammalian species

m. In an uterotrophic assay according to OECD TG 440 and GLP ([redacted], 1998), the Substance when administered orally to 5 rats per dose at 100 and 1000 mg/kg bw for 4 days produced no marked or statistically significant increases in uterine weight when compared with control animals.

You consider that this result is "*in line with the findings of the in vitro mechanistic studies in which no ER-transactivation potential was detected*" and that experimental evidence contradicts "*the results of the in silico models providing indications of ER binding*".

Data on non-mammalian species

n. in a modified Fish, juvenile growth test according to OECD 215 and GLP ([redacted], 1999), no estrogenic effect, as evidenced by the absence of oviduct formation in male fish, was observed up to the top dose of 2 mg/L[4].
o. a non-GLP and non-OECD guideline study is available for the Substance to determine adverse effects on egg production, relative organ weights, plasma levels of sex hormones, and transcription of genes related to the hypothalamus-pituitary-gonad (HPG) axis in zebrafish (*Danio rerio*) ([redacted], 2018). In male fish, the gonadosomatic index was significantly decreased at concentrations of 5 and 50 µg/L. Estrogenic (increase in E2/T ratio) and antiandrogenic (decrease in T) effects were observed. In general, males were more sensitive to the effects than females.

[4] While such study is not referred to in the OECD GD 150, it provides *in vivo* data on selected endocrine mechanism(s) and is therefore included OECD CF Level 3 information. However, in the absence of a validation of the test protocol, the reliability of this information is considered low.

The changes in sex hormones were supported by the regulation of genes along the HPG axis, such as cyp19, 17βhsd, and cyp17 transcripts.

p. a non-GLP and non-OECD guideline study is available for the Substance to determine the developmental and neurotoxicity effects of the Substance on zebrafish embryos (*Danio rerio*) ([redacted], 2018). Two test concentrations were examined (100 nM and 100 µM, corresponding to 29 µg/L and 29 mg/L, respectively). Phenotypical malformations, abnormal development, and an increase of coagulated (dead) eggs were observed at the top dose of 29 mg/L. At 29 µg/L no effects on development were observed.

With regard non-mammalian species, you state that the findings of the study by [redacted] (2018) are inconclusive since the most appropriate and sensitive endpoint for estrogenicity according to OECD TG 229, Vitellogenin, was not examined. You also consider that, as the fish strain was not specified, the use of an inappropriate strain for estrogenicity can therefore not be excluded.

ECHA agrees that the available OECD CF Level 3 information indicate no *in vivo* evidence for ED activity in mammalian species. However, ECHA considers that there is evidence that the Substance may be an endocrine disruptor in non-mammalian species. In particular, despite some limitations, the study by [redacted] (2018) raises concern for endocrine disruption in fish (i.e. reduced gonadosomatic index under low exposure concentrations). This study includes *in vivo* mechanistic parameters but does not cover apical endpoints.

- OECD CF Level 4 information:

Data on mammalian species

q. in a study according to OECD TG 407 and GLP ([redacted], 1988), a statistically significant decrease of ovary weight (absolute and relative weights) was observed at 1000 mg/kg bw/d. The absolute testis weights were also decreased but the testis weight relative to organ weight was increased in animals of the high dose group. After two weeks of recovery the decreased testis weight (absolute weight) was still present whereas the testis weight ratios recovered due to the regeneration of body weights in animals at 1000 mg/kg bw/day. Additionally, no differences in ovary weights between control and high dose females were observed at the end of the recovery phase. You consider that "*since all findings in organs putatively affected by the hormonal system occurred at excessive systemic toxicity in the high dose groups only and are mostly reversible after two weeks of recovery they are considered of no toxicological relevance*".

r. in a study according to OECD TG 407 and GLP ([redacted], 2009), uterus and/or ovaries showed slightly higher absolute and relative to body and/or brain weights mean values at 50 mg/kg bw/day. However, you consider these observations as incidental as no dose-response was observed and they were not correlated with pathological findings.

s. in a study according to OECD TG 415 and GLP ([redacted], 2009). No effects were observed up to the top dose of 125 mg/kg bw/day.

You consider that the above data on mammalian species indicate no *in vivo* evidence for ED activity in mammalian species. However, you acknowledge that "[in available repeated-dose toxicity studies], *determination of hormone levels (T3/T4, E, A) and sperm analysis were not performed*" and "*a study on prenatal development is not available*".

ECHA agrees that the available short-term repeated dose toxicity did not investigate all relevant ED related parameters (e.g., hormone levels (T3/T4, E, A) and sperm analysis). Also, your registration dossier does not meet the information requirements for Screening for reproductive/developmental toxicity and Sub-chronic toxicity study (90-day), as

explained under Appendices B.1. and C.1., respectively. Finally, your registration dossier currently include a testing proposal for pre-natal developmental toxicity. Therefore, as your dossier currently stands, ED activity in mammalian species is not indicated but cannot be excluded either.

In conclusion, there are structural alerts indicating that the Substance may show endocrine activity. There are some *in vitro* evidence suggesting that the Substance has endocrine activity for the EAS modalities, but negative results were also observed in a number of *in vitro* assays. Therefore, this information indicates endocrine activity but should be regarded as inconclusive with regard to endocrine disrupting properties. There is currently no *in vivo* evidence for ED activity in mammalian species in your dossier. However, as acknowledged by you, the available repeated dose toxicity did not investigate all relevant ED related parameters (e.g., hormone levels (T3/T4, E, A) and sperm analysis). Furthermore, your dossier does not comply with the information requirements for an number of endpoints relevant to assess endocrine disrupting properties (See appendices B1. and C1). Therefore, ECHA concludes that no conclusion can be reached on whether or not the Substance may be an endocrine disruptor in mammalian species. Finally, there is evidence that the Substance may be an endocrine disruptor in non-mammalian species. In particular, the study by [redacted] (2018) raises concern for endocrine disruption in fish (i.e. reduced gonadosomatic index under low exposure concentrations). This study includes in *vivo* mechanistic parameters but does not cover apical endpoints.

On this basis, there are indications from information on OECD CF Level 1 to 3 that the Substance may be an endocrine disruptor via EAS modalities in non-mammalian species (including fish). Therefore, the chemical safety assessment (CSA) indicates the need for further long-term toxicity test on aquatic organisms.

There is currently no indication that reproduction may be a more sensitive endpoint to assess the potential endocrine disruptive properties of the Substance compared 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).

1.2. Available information on long-term toxicity to fish

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

i. a Fish, Juvenile Growth Test according to OECD TG 215 (modified to investigate oviduct formation as an additional parameter) with the Substance.

We have assessed this information and identified the following issues:

A. Under ECHA Guidance R.7.8.2., the Fish, Juvenile Growth Test (test method: OECD TG 215) is considered to provide adequate information on long-term fish toxicity only if there are well founded justifications indicating that growth inhibition is the most relevant effect in fish.

You have not provided any justification as to why growth inhibition may be considered as the most relevant effect in fish.

Therefore, you have not demonstrated the Fish, Juvenile Growth Test is an acceptable test method to meet the information requirement set out under section 9.1.6. of Annex IX for the Substance. Furthermore, as explained above, there are indications that the Substance may have endocrine disrupting effects and it cannot be excluded that other life-stage or other more sensitive endpoints may be more relevant.

B. To fulfil the information requirement, a study must comply with the OECD TG 234 [and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test] (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.

   The study i. above investigates the impact of the exposure to the test material on juvenile fish growth and on oviduct formation (as an additional parameter to OECD TG 215) after 28 days exposure.

   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 specified in OECD TG 234.

On this basis, the information provided is rejected.

In your comments on the draft decision, you specify that you have "*performed an ED Assessment following the recommendations of the Guidance Document (GD) for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (ED GD; ECHA and EFSA, 2018) and further relevant regulatory documents regarding the assessment of ED chemicals (EDC)*". You consider that:

- "*based on the modified OECD TG 215 study it was concluded that exposure towards D-8 does not lead to a feminisation of male carp*";
- "*the available publication by* [redacted]*, 2018* [has] *various shortcomings in this non-"OECD and non- GLP study. Mainly there is no information on strain, age and health status of the fish used in this study. It is known from literature that parasites can affect the endocrine system in fish, like tape worm Ligula intestinalis and a few parasites from the micropsora phylum [5]. Furthermore, Vitellogenin was not examined and no historical control data for the endpoints measured were presented. Therefore, the Registrant is of the opinion that the findings presented by* [redacted] *are inconclusive for the overall ED Assessment in non-mammalian species*".

However, you specify that it cannot be excluded the other life-stages or other sensitive endpoints may be relevant for the overall ED Assessment in fish. Therefore, you agree to conduct the requested study.

1.3. Study design

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.

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).

Adequate information on long-term toxicity to fish is also needed for the purpose of the risk assessment. As specified in OECD GD 150, the OECD TG 234 may also support an evaluation whether specific endocrine-mediated effects may be influenced by general toxicity. In such case, the concentration range needs to be adjusted in order to investigate potential endocrine disrupting effects of the Substance (in the absence of significant non-endocrine mediated effects) and in the same study to investigate other apical endpoints that should be measured 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.

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

A. Test methods, GLP requirements and reporting

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

2. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[5].

B. Test material

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

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

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

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

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[6].

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

Appendix E: Procedure

The Substance is listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2023.

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 10 June 2020.

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

ECHA took into account your comments and amended the deadline.

In your comments on the draft decision, you requested an extension of the deadline to provide information from 18 to at least 36 months from the date of adoption of the decision. In support of your request you provided documentary evidence from a Contract Research Organisation (CRO) indicating the the study corresponding to request C.2. above cannot be started before Q1 2023. Accordingly, you consider that it is not feasible to submit the results before the end of Q1 2024.

ECHA acknowledge limitations regarding lab capacities for conducting studies according to OECD TG 234. However, ECHA notes that the deadline set out in the draft decision will apply from the date the final decision is adopted. On this basis, ECHA has extended the deadline to 30 months.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix F: List of references - ECHA Guidance[7] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[8]

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

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

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

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

[7] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[8] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[9] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

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

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

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

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

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

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

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