Helsinki, 12 December 2022

Addressee
Registrant of Isopentyl p-methoxycinnamate listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: Isopentyl p-methoxycinnamate
EC number: 275-702-5
CAS number: 71617-10-2

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

DECISION ON SUBSTANCE EVALUATION

Under Article 46 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below:

A. Information required to clarify the potential risk related to Endocrine disruption

1. Combination of Fish short term reproduction assay (FSTRA - according to OECD TG 229) and Fish Sexual Development Test (FSDT – according to OECD TG 234) must be conducted on the Substance, using the Zebrafish (*Danio rerio*) or Japanese medaka (*Oryzias latipes*) or Fathead minnow (*Pimephales promelas*), with the following specifications:
   - A range finding study must be performed before the main (combined) study;
   - At least four test concentrations must be used;
   - The highest test concentration must be selected according to the OECD TG 234 (The maximum test concentration should be 10% of the LC_{50} on the larval/juvenile life stage as determined in the range finding study);
   - The exposure must take place via testing water and the use of a solvent must be avoided;
   - If the test is conducted using Japanese medaka, the genetic sex and secondary sex characteristics must be examined.
   - The test must be started according to the protocol laid down in OECD TG 229 using adult fish and must cover all standard endpoints of OECD TG 229 and OECD TG 234;
   - The OECD TG 234 protocol must be started with the eggs collected from the breeding pairs of the OECD TG 229 study**,** for each concentration and control**,** in the 4**-** or 8**-**cell stage;
   - The same exposure concentrations must be used in the OECD TG 234 part as in the OECD TG 229;
   - You must document the fertility (the number of fertilised and viable eggs) after 21 days (in the OECD TG 229);
   - The number of vessels or replicates per treatment must follow the specifications in the OECD TG 229:
     - Zebrafish: two vessels or replicates per control or treatment must be used (each vessel containing 5 males and 5 females); these 2 vessels are divided to set up 4 replicates per control or treatment in the OECD TG 234 protocol.

- Fathead minnow: four vessels or replicates per control or treatment must be used (each vessel containing 2 males and 4 females).
- Japanese medaka: four vessels or replicates per control or treatment must be used (each vessel containing 3 males and 3 females).

- Histopathology of gonads (evaluation and staging of oocytes and spermatogenic cells) must be conducted at the end of the OECD TG 234 in all concentrations and the control(s) and at the end of OECD TG 229 only when there is an impact seen on fecundity and fertility in F0 fish, unless plasma vitellogenin (VTG) or secondary sex characteristics are clearly impacted;
- The histopathology of the liver must be included in both tests (at the end of OECD TG 229 and OECD TG 234) in all concentrations and the control(s).

Deadline
The information must be submitted by **19 December 2025**.

Conditions to comply with the information requested
To comply with this decision, you must submit the information in an updated registration dossier, by the deadline indicated above. The information must comply with the IUCLID robust study summary format. You must also attach the full study report for the corresponding study in the corresponding endpoint of IUCLID.

You must update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You will find the justifications for the requests in this decision in the Appendix entitled ''Reasons to request information to clarify the potential risk'.
You will find the procedural steps followed to reach the adopted decision and some technical guidance detailed in further Appendices.

Appeal
This decision 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] by Mike Rasenberg, 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.

Basis for substance evaluation

The objective of substance evaluation under REACH is to allow for the generation of further information on substances suspected of posing a risk to human health or the environment ('potential risk').

ECHA has concluded that further information on the Substance is necessary to enable the evaluating Member State Competent Authority (eMSCA) to clarify a potential risk and whether regulatory risk management is required to ensure the safe use of the Substance.

The ECHA decision requesting further information is based on the following:

(1) There is a potential risk to human health or the environment, based on a combination of hazard and exposure information;
(2) Information is necessary to clarify the potential risk identified; and
(3) There is a realistic possibility that the information requested would allow improved risk management measures to be taken.

The Appendices entitled 'Reasons to request information' describe why the requested information is necessary and appropriate.

Appendix A – Reasons to request information to clarify the potential risk related to Endocrine disruption

Introduction/background

The Substance was previously evaluated in parallel to the structurally similar substance OMC (2-Ethylhexyl trans-4-methoxycinnamate, List No 629-661-9, CAS RN 83834-59-7).

At the end of this initial parallel evaluation, ECHA sent two similar substance evaluation decisions (one for OMC (ECHA 2018a), and one for the Substance, IPMC (ECHA 2018b)) requesting, among other information, a Fish Sexual Development (FSDT) study and an Amphibian Metamorphosis Assay (AMA) or Larval Amphibian Growth and Development Assay (LAGDA). The decisions mentioned that it may be possible to test only one of the two substances, provided a scientifically reasoned case justifying read-across of the results from one to the other was made.

Consequently, you chose to perform both assays requested with the substance OMC and to use a read-across approach from these study results to conclude on the properties of the Substance and to fulfil the information request.

In your comments to the draft decision, you stated that the present decision challenges the read-across which has previously been accepted, and that it is not clear why and for which endpoints the rejection of the read across applies. You stated that it is not clear if this conclusion is based on the results and the conclusion of the OMC study, as those are questioned by ECHA, or if it is due to the difference in water solubility or if both are the reasons for this change of the original assessment. You further stated that only few *in vitro* data and no *in vivo* studies in fish are available for ED-specific endpoints for the Substance. Thus, the concern and request for higher-tier vertebrate studies with the Substance in this decision are mainly triggered after read-across to OMC for which data are available.

ECHA notes that read-across from OMC to IPMC has not been accepted previously, but the alternative of applying read-across was available to you if a reasonable scientific case could be made to support it. You have undertaken new physicochemical tests that showed considerable differences in the water solubilities of the two substances and did not confirm your initial assumption that the read-across could be justified.

Therefore, ECHA considers the results obtained in the FSDT and AMA with OMC as unsuitable to conclude on the ED properties for IPMC as well.

ECHA considers that it is highly likely that adverse effects of the Substance could occur at higher test concentrations above the water solubility of OMC. These would be overlooked when the assessment of IPMC is solely based on the results of the study obtained for OMC tested up to its considerably lower water solubility. Therefore, a new test with IPMC conducted at higher concentrations than OMC is necessary to conclude on the concern identified.

Furthermore, you noted that the two esters, OMC and the Substance, differ in the alkyl-side chain only and degrade to the same structure trans-4-methoxycinnamic acid and the respective alcohol, none of which being considered as ED with the current knowledge. This is important for the hazard assessment *in vivo* and is the basis for the read-across.

ECHA agrees that metabolism must be considered when setting up a scientifically reasoned hypothesis justifying the application of read-across. However, it is unclear which chemicals

(i.e., parent compound or metabolites) exert endocrine activity. The available *in vitro* data suggest that endocrine activity is mediated via the parent compounds since both, OMC, and the Substance, were tested positive in assays lacking metabolic capacity (Kunz and Fent, 2006). Furthermore, other substance-specific metabolites may be formed beside trans-4-methoxycinnamic acid, and the respective alcohol as shown by Zhou et al. (2019a).

Prior to performing the tests requested (a FSDT study and an AMA or LAGDA) in the previous decisions, you stated that the water solubility of OMC was 220 to 750 µg/L. However, when the respective dossiers were updated, you also submitted new information on water solubility for OMC (solubility limit of 50 µg/L).

The requested tests were performed up to that limit: (i.) the AMA was clearly negative whereas (ii.) not statistically significant effects were seen in the FSDT ([redacted], 2020) conducted with OMC on skewed sex ratio and change of gonadal stage in females.

Therefore, the results of this FSDT do not allow to definitively conclude on the Substance with respect to its endocrine disrupting properties, since OMC has a much lower water solubility compared to IPMC (2.47 mg/L).

The current decision is therefore requesting testing with the Substance because (i.) the Substance has a markedly higher solubility in water (2.47 mg/L) than the highest concentration tested in the FSDT with OMC, it is very likely that the effects in the FSDT study observed when testing OMC will be much more pronounced; and (ii.) to be considered as significant adverse effects relevant at the population level in fish, when testing the Substance at higher test concentrations up to its water solubility.

1. Potential risk

1.1 Potential hazard of the Substance

a) Potential endocrine disrupting properties in the environment

Following its assessment of the available relevant information on the Substance, the evaluating MSCA (eMSCA) and ECHA have identified a potential hazard which must be clarified.

The information contained in the registration dossier and from scientific literature show that the Substance may have endocrine disrupting (ED) properties in the environment, more specifically *in vitro* studies with the Substance suggest an interaction with the HPG axis.

In your chemical safety assessment, you have claimed that it is possible to use the data on the structurally similar substance, 2-ethylhexyl trans-4-methoxycinnamate (OMC) to also predict and determine the ED potential of the Substance (or IPMC). The main difference between OMC and the Substance is the nature of the alkyl chain.

Additionally, OMC and the Substance can both be biodegraded to the same structure: trans-4-methoxycinnamic acid (CAS No 830-09-1) after hydrolytic cleavage of the alkyl chains in a first biodegradation step (cf. the EAWAG pathway prediction results[2]. Similarly to the Substance, effects seen in *in vitro* and *in vivo* fish studies with OMC point towards an interaction with the HPG axis. Therefore, in general, the MoA of both substances is

[2] http://eawag-bbd.ethz.ch/predict/

considered to be similar. Therefore, due to the high structural similarity of the Substance and OMC, the effects caused by OMC give indications for the endocrine properties of the Substance.

In vitro data
In vitro data from transactivation assays provide some indication that the Substance shows endocrine activity with regards to the HPG axis (anti-oestrogenic and (anti-)androgenic activity), whereby the anti-androgenic effects were most pronounced. Furthermore, OMC shows also endocrine activity with regards to the HPG axis in transactivation assays, which were inconclusive as they appeared in some assays, but not in others. OMC was negative in an oestrogen receptor binding assay but showed oestrogenic effects in a cell proliferation assay. More details are provided below.

i. Oestrogenic activity
The Substance showed no significant oestrogenic effects in two transactivation assays (Kunz and Fent, 2006; Kunz et al. , 2006), but anti-oestrogenic effects with an IC_{50} of 0.3 mM (Seidlova-Wuttke *et al.,* 2006a; Kunz et al., 2006). The Substance showed no binding to the ER in a binding assay using hERa ([redacted], 2002b).

The structurally similar substance OMC was evaluated in nine *in vitro* studies (Seidlova-Wuttke *et al.,* 2006a; Kunz and Fent, 2006; Kunz et al., 2006; Gomez. et al., 2005; Heneweer et al., 2005; Schreurs et al., 2002; Schreurs et al., 2005; Ma et al., 2003; Schlumpf et al., 2001) with inconclusive results. OMC showed no oestrogenic activity in one binding study (Seidlova-Wuttke *et al.,* 2006a) and two transactivation assays (Kunz et al., 2006; Kunz and Fent, 2006), but showed oestrogenic effects in three other transactivation studies (Gomez et al., 2006; Heneweer et al., 2005; Schreurs et al., 2002). In one proliferation assay, OMC showed an oestrogenic effect with EC_{50} 2.4 µM (Schlumpf et al., 2001). In one transactivation assay with OMC anti-oestrogenic effects were seen (Kunz and Fent , 2006).

ii. Androgenic activity
The Substance was tested for binding of hAR and is considered a non-binder ([redacted], 2002a). The affinity for the AR was very weak, with a displacement of ligand up to max 30%.

Results from yeast transactivation assays (Kunz and Fent, 2006) point to an anti-androgenic activity:

- The Substance showed androgenic effects (EC_{50} 0.4 mM), but more pronounced anti-androgenic effects were seen in the same study with an IC_{50} of 8.1 µM.
- OMC showed androgenic effects (EC_{50} 10 mM) and antiandrogenic effects (IC_{50} 0.3 mM).

In two other transactivation assays with OMC (Schreurs et al., 2005), (Ma et al., 2003), no effects were seen neither on androgenicity nor on anti-androgenicity.

iii. Comparison of anti-oestrogenic and anti-androgenic activity of IPMC and OMC:
In the ER and AR transactivation assay conducted by Kunz and Fent (2006) both OMC and the Substance were tested: the Substance showed stronger anti-oestrogenic effects than OMC on the hERα (IC_{50} 297 µM versus 4.3 mM), and stronger anti-androgenic effects than OMC on the hAR (IC_{50} 8.1 µM versus 312 µM).

In your comments to the draft decision, you stated that "*the in vitro data with regard to ED show some differences in the potency and the mode of action between OMC and IPMC*

with OMC being often by orders of magnitude more potent." This is explained by the bulkier alkyl side-chain of OMC compared to the Substance which can modify the interaction with the E/A receptors in such tests. Furthermore, you mentioned that for (anti-) androgen effects the data are different and not conclusive for both substances with sometimes positive results at high test concentrations and sometimes no effects up to the highest test concentrations. You stated that "*in one assay IPMC seemed to be more potent than OMC. However, this was not confirmed in other assays and did not trigger in a former assessment the need to test IPMC instead of OMC as a worst-case substance."*

ECHA notes that in the available *in vitro* tests where the test design allows for a direct comparison between OMC and IPMC (Kunz and Fent, 2006), IPMC shows higher activity:

- In an ER transactivation assay, where both OMC and IPMC were examined, no oestrogen agonistic effects for either substance were seen (Kunz and Fent, 2006). However, in the same publication (Kunz and Fent, 2006), IPMC has a stronger anti-oestrogenic activity (IC_{50}: 297 µM) than OMC (IC_{50}: 4300 µM).
- The androgenic and the anti-androgenic activity of IPMC was stronger than for OMC (androgenic activity IPMC EC_{50}: 429 µM, OMC EC_{50}: 10 mM; anti-androgenic activity IPMC IC_{50}: 8.12 µM, OMC IC_{50}: 312 µM) in Kunz and Fent (2006).

In summary, IPMC shows a 14 to 38-fold higher activity than OMC in *in vitro* assays which allows for a direct comparison of both substances. A proposal for amendment (PfA) was submitted with the proposal to include the IC_{50} values for OMC and IPMC from the study conducted by Kunz and Fent (2006). The draft decision was changed accordingly. You stated that this data indicates that there is a difference between OMC and IPMC. We agree to your statement. This underlines the relevance of the request for new data on the Substance, since these *in vitro* data demonstrate that the Substance might be more active than OMC. Hence, a conclusion on IPMC solely based on data for OMC cannot be justified. Irrespective of this, the available data for OMC, even if not conclusive for adversity, support the concern for the Substance.

You further stated: "*In addition, IPMC represents a racemic mixture in comparison to OMC. E.g., for OMC hints for estrogenic activity were found at µM, but not for IPMC. Anti-estrogen effects were noted for IPMC at very high test concentrations (mM) whereas the data for OMC are not conclusive with regard to this."*

ECHA notes that a racemic mixture is defined as a 1:1 mixture of enantiomers. Hence, your above statement is unclear since the Substance does not split-up into enantiomers (the potential E/Z isomers depending on double-bond geometry would constitute diastereomers) as opposed to OMC which does split up into enantiomers owing to its molecular chirality.

In vivo data
There are no ED-specific *in vivo* data for fish available where the Substance was tested. However, OMC was tested in seven *in vivo* studies using various fish species:

i. Fathead minnow

- Christen et al., 2011:
  - No guideline study (equivalent to OECD CF level 3)
  - Oestrogenic effects at high concentrations (plasma vitellogenin (VTG) level increased at 244 µg/L after a 14-day exposure of adult males),
  - Effects on the histology of male gonads were observed, as spermatogenesis seemed to be inhibited (proportion of spermatogonia increased, and significantly less spermatocytes were seen).

- The estrogen receptor alpha (ERα) and 3β-hydroxysteroid dehydrogenase (3β-HSD) genes were significantly down-regulated at high concentrations whereas the androgen receptor (AR) gene was consistently down-regulated also on low concentrations (beginning at 37.5 µg/L OMC) in the liver of female fish.

ii. Zebrafish

- Zhou et al., 2019a :
  - No guideline study (equivalent to OECD CF level 3)
  - Adults were exposed for 21 days.
  - Amount of VTG and E2 were decreased after a 21-day exposure at 1, 10, 100 µg OMC/ L. Testosterone was significantly increased at all 3 doses.
  - Gene expression was examined: VTG1 and CYP19a1 were significantly down-regulated, whereas AR was up-regulated at 1 to 100 µg/L.
  - Several signs for oxidative stress were seen, e.g., increased catalase and superoxide dismutase.

- Zhou et al., 2019b :
  - No guideline study (equivalent to OECD CF level 4)
  - Zebrafish embryos were exposed for 4 months until sexual maturation; fish were paired at 120 dpf (days post fertilization). F1; eggs were divided in two groups with and without continued exposure for 5 dpf.
  - Amounts of VTG and E2 were decreased at 1, 10, 100 µg OMC/L at 40 dpf (F0).
  - Effects were seen on gene expression: VTG1, CYP19a, CYP19b, ERα, PR were significantly down-regulated at 1, 10, 100 µg/L, whereas AR was up-regulated at 10 and 100 µg/L.
  - Malformation in F0 at 5dpf were significantly increased at 100 µg/L, as well as in F1 at 10 and 100 µg/L with continued exposure. Without continued exposure in F1, no effect on malformation appeared.
  - Body weight decreased dose dependently, with a significant decrease at 100 µg/L (F0).
  - In F0 and F1 (without continued exposure) hatching rates were significantly decreased at 10 and 100 µg/L, and in F1 with continued exposure at 1, 10 and 100 µg/L.
  - Survival at 5 dpf was significantly decreased at 100 µg/L in the F0 generation, whereas no effects were seen in the F1 generation.
  - OMC was transferred from parents to the eggs.

- Zucchi, 2011:
  - No guideline study (equivalent to OECD CF level 3)
  - Adult male fish were exposed for 14 days at 0, 3, 3000 µg/L (nominal concentrations)
  - VTG gene expression was up-regulated in one study in liver of adult males, whereas VTG gene expression in brain and testes was down-regulated.

- [redacted], 2020:
  - FSDT according to OECD TG 234 (OECD CF level 4)
  - The study was conducted with a single concentration of OMC: 50 µg/L (nominal), 46.9 µg/L (measured). The exposure began within 4 hours after fertilization and lasted until 60 dph (days post hatching) using a flow-through test design. Four replicates of 30 embryos were used.
  - OMC caused statistically significantly decreased body weights and length in female and male fish at 46.9 µg/L.
  - Decreased mean ovarian stage score (stage 0.0 ovaries in 66 % of treated females, whereas 61% of control females were in ovarian stage 1.0, no statistics).

- The study authors connected this with treatment-induced decrease in somatic growth. There was a statistically not significant increase in the ratio of females to males, which was -according to the study authors- related to the delayed transition from the female to male phenotype and treatment-induced decrease in somatic growth.

iii. Japanese medaka

- Lee et al. (2019), equivalent to OECD CF level 5 (based on OPPTS 850.1500)
  - Two-generation reproduction study using the following test concentrations 0.05, 0.158, 0.5, 1.58, and 5 mg/L.: exposure began with fertilized eggs until 154 days (F0). Mating at 106 dpf, at 120 dpf eggs were collected and exposed further as F1 generation until 38 dpf. No analysis of testing concentrations was performed.
  - Significant decrease of the number of eggs at 50 µg/L and higher. No effects were seen on mRNA expression of ERα, ARα and VTG1 in the liver, as well as on hatching and survival.

- Inui et al. (2003), equivalent to OECD CF level 3
  - VTG gene expression was up regulated in one study in male adults after 7 days exposure at 0.034 mM and higher concentrations.

Observed effects on gene expression of the ER and AR (partly up-regulated or down-regulated) were inconsistent in several studies.

In your comments to the draft decision, you noted that the OECD TG 234 study on OMC is available now and revealed no effects up to clear toxic concentrations. You concluded that OMC is not an ED for the environment.

ECHA agrees that the new FSDT ([redacted], 2020) does not show significant effects allowing for an identification of OMC as SVHC. However, ECHA disagrees that the test did not show any effects up to toxic concentrations. The provided FSDT study shows some ED related activity, albeit not statistically significant: the percent of males at 46.9 µg/L was decreased compared to control (at 46.9 µg/L 34.7 %, in control 44.4 %) and the number of undifferentiated fish was increased at 46.9 µg/L compared to control (at 46.9 µg/L 10.2 %, in control 4 %). The ovarian stage score at 46.9 µg/L was decreased compared to control. Most control females (32 of 52) were in the ovarian stage score of 1.0 (stage immature ovaries: characterized by the presence of both cortical alveolar and peri nucleolar phase oocytes), the females at 46.9 µg/L (36 of 54 animals) were in stage 0.0 (the most developed oocytes were in peri nucleolar phase). In addition, in the control, no female displayed germ cell degeneration whereas at 46.9 µg/L 24 % of females (13 of 54) showed germ cell degeneration.

Furthermore, there is the peculiar finding that in control males there were 3 fish with testicular oocytes and one fish with gonadal duct feminization (of 44 males), whereas no single male had such properties at 46.9 µg/L.

Additionally, there are other new fish *in vivo* tests with OMC that show endocrine effects as decreased VTG and E2 content (Zhou et al. 2019 a and b), as well as effects on reproduction (Lee et al. 2019).

Thus, the ECHA considers that the available data for OMC point to an endocrine activity as well as to related adverse effects. It cannot be excluded that this activity is also observed with the Substance at higher test concentrations as requested in this decision and will lead to significant adverse effects.

OMC was tested in different *in vivo* studies with mammals.

iv. Mammals

- OMC was tested negative in an uterotrophic assay (similar to OECD TG 440) conducted in immature Wistar rats ([redacted], 2001).
- OMC caused increased uterine weights in an uterotrophic assay (similar to OECD TG 440) conducted with immature Long-Evans rats (Schlumpf et al., 2001), and in a mechanistic non-guideline study with ovariectomised Sprague Dawley rats (Klammer et al., 2005). OMC is much less potent than 17β oestradiol.

Overall, ECHA concludes that OMC has a weak oestrogenic effect in *in vivo* mammalian studies equivalent to tier 3 of the OECD conceptual framework.

- Thyroid
  - Several non-guideline studies in rat show changes in thyroid hormone and/or TSH levels (Axelstad et al., 2011; Ferraris et al., 2019; Klammer et al., 2007; Schmutzler et al., 2004; Seidlova-Wuttke et al., 2006a), raising concern regarding thyroidal activity in the environment.
  - Effects were seen on thyroid hormones or on the DIO gene expression in the studies by Chu et al. (2021), and Lee et al. (2019).

These effects might also be relevant for the Substance and hence might require further follow-up testing in amphibians if a conclusion on the ED properties of the Substance for the environment (based on the requested fish assay) is not possible (see section 2.1).

Summary
From the data available for the Substance (*in vitro)* and for the structurally closely related substance OMC (*in vitro* and *in vivo* from fish to mammalian species), a potential hazard for the Substance with respect to its endocrine disruption properties in the environment is indicated. The *in vitro* studies with the Substance and OMC and the *in vivo* studies in fish with OMC indicate that the Substance might act on the HPG axis in an (anti-)oestrogenic or (anti-)androgenic way.

In vivo data for OMC with fish species show that zebrafish appears to be more sensitive than fathead minnow or Japanese medaka.

The results obtained in the FSDT study on OMC ([redacted], 2020) are not considered conclusive for the endocrine properties of the Substance, as OMC has a much lower solubility (51 µg/L) in water than the Substance (2.47 mg/L). As the Substance can be tested at higher concentrations than OMC, more pronounced effects are expected at exposure levels above the solubility level of OMC.

In addition, available studies with OMC in mammals show endocrine activity with regards to the EAS (oestrogenic, androgenic, steroidogenesis) and the T (thyroid) modality.

Therefore, the available and current information is not sufficient to draw a conclusion on the hazard. Further information is needed on potentially adverse and population-relevant effects mediated via endocrine modes of action of the Substance.

The current request aims at clarifying the EA concern (the most substantiated one) and not the T MoA, which may potentially need to be further investigated at a later stage.

1.2 Potential exposure

According to the information you submitted in the registration dossier for the Substance, its aggregated tonnage manufactured or imported in the EU is in the range of 10-100 tonnes per year.

Furthermore, you reported that the Substance is used as an ingredient in cosmetics and personal care products. As a cosmetics ingredient, the Substance fulfils the technical function of [redacted].

Beside these open and widespread uses, other releases to the environment of the Substance are likely to occur from outdoor and indoor use as processing aid.

Available monitoring data show the presence of the Substance in the environment:
Chisvert et al. (2017), Benede et al. (2014) and Roman et al. (2011) detected the Substance in river water and sea water in Spain. The Substance was additionally detected in swimming pool water in one study (Chisvert et al., 2017) and in another investigation in tap water (Roman et al., 2011).

Cunha et al. (2018) showed that the Substance occurs in marine animals as fish and mussels. Moreover, it was detected in canned fish and in aquaculture fish in Europe (not specified). A study by Tsui et al. (2014) showed the occurrence of the Substance in sea water near Hong Kong.

Therefore, exposure of the Substance to the environment has been shown.

In your comments to the draft decision, you stated that the Substance is only used in cosmetics and therefore vertebrate testing should be considered as last resort.
ECHA notes that all registered substances are regulated under REACH, even if exclusively used in cosmetics and vertebrate testing may have to be performed to conclude on environmental hazards. The Substance presents potential risk to the environment that needs to be clarified, as laid out in Section 1.2., so further testing, as required in this decision, is necessary.

1.3 Identification of the potential risk to be clarified

Based on all information available in the registration dossier and information from the published literature, the Substance may be an endocrine disruptor (ED) in the environment.

The information you provided on manufacture and uses demonstrates a potential for exposure of the environment.

Based on this hazard and exposure information the Substance poses a potential risk to the environment.

As explained in section 1.1, the available information is not sufficient to conclude on the hazard. Consequently, further data on the Substance are needed to clarify the potential risk related to ED properties for the environment.

Hence, to conclude on the environmental ED properties of the Substance, a long-term fish assay combining the Fish short term reproduction assay (according to OECD TG 229) and

the Fish Sexual Development Test (according to OECD TG 234) is requested by this decision.

1.4 Further risk management measures

If the properties(s) of the Substance are confirmed, the eMSCA will analyse the options to manage the risk(s). New regulatory risk management measures could be the identification as substance of very high concern (SVHC) and authorisation or further restrictions of the use of the Substance due to its ED properties in the environment.

This would result in stricter risk management measures, such as improved measures at manufacturing sites, better waste management and revised instructions on safe use, if appropriate.

In addition, SVHC identification would trigger additional information duties of producers and importers to ECHA according to Article 7(2) of REACH as well as information duties in the supply chain and for consumers according to Article 33 of REACH.

2. How to clarify the potential risk

2.1 Development of the testing strategy

Two kinds of effects trigger the concern for ED for the Substance (i.e., via the EAS and T modalities). There is no data available to conclude on them – there are tests available with OMC showing effects for both modalities, but a conclusive read-across regarding ED properties from OMC to the Substance is not possible due to the differences in water solubility, with OMC being much less soluble than the Substance.

As explained in section 1.1., the AMA test conducted using OMC was negative, after it was tested up to a concentration of 44.2 µg/L OMC (measured). However, ECHA considers that testing the Substance (having a higher water solubility) may cause significant effects at higher concentrations. As a first step the current decision addresses with the requested study one kind of effects (elicited via EAS modalities). If the conclusion on the ED properties via EAS modalities cannot be made from the combined OECD TG 229/OECD TG 234 test or if this combined test is negative, then further testing in a subsequent decision-making process may be considered to address the thyroid modality.

Thyroidal effects were seen in at least one rat study and in two fish studies with OMC. We acknowledge your comments concerning the T concern. As this concern is not followed with testing in the current decision, there is no need to discuss them here in detail. Any discussion can take place in a potential future decision-making process.

2.2 Combination of Fish short term reproduction assay (according to OECD TG 229) and Fish Sexual Development Test (FSDT – according to OECD TG 234) using the Zebrafish (*Danio rerio*) or Japanese medaka (*Oryzias latipes*) or fathead minnow (*Pimephales promelas*)

a) Aim of the study

As detailed in section 1.1., information on endocrine activity and subsequent adverse effects in environmental species are required to conclude on the potential hazard. More specifically, the available data point to potential adverse effects of the Substance on the sexual development and reproduction capacity of fish. Therefore, a study that investigates

potential ED properties in fish is required.

As described in more detail under section 2.2(c), the requested study design is the most appropriate and least burdensome approach to clarify whether the Substance meets the criteria to be identified as SVHC due to its ED properties in the environment, following REACH Article 57(f).

b) Specification of the requested study

Test material
The test material must be representative for the Substance as manufactured and put on the market as a substance or in mixtures.

Range finding study
Two MSCAs submitted PfAs noting the need for a dose range finding study.

Since only an LC_{50} is available for the structurally related substance OMC and no chronic data for the Substance are available, a range finding study is required to be performed before setting the concentrations for the requested main (combined) study.

In your comment to the PfAs of the two MSCAs, you raised the question according to which protocol and for which part of the different OECD studies the range finding study should be done. Further you noted it will be difficult for the combined assay because the amended draft decision requires that the same test concentrations to be used for the two study parts to be combined.

The aim of the range finding study, which is to be conducted before the main (combined) study can be run, is to find suitable concentrations. As the OECD TG 234 part of the combined test is the part of the study where the life stages with higher sensitivities. i.e., juvenile stages, are tested, then the range finding study needs to be run to find the suitable concentrations considering the OECD TG 234 part. These concentrations obtained from the range finding study will be then used for both parts of the combined study.

The Fish Toxicity Testing Framework (OECD No. 171, 2012) provides general guidance on how to perform a range-finding study. Paragraph 102 of this document refers to chronic tests and describes that the test duration of a range finder must not be as long as the full duration of the definitive test, but only sufficient time to assess the relevant parameters (e.g., 14 days instead of 28 days for a FELS test) is needed. Durations longer than acute tests are recommended as well as assessment of indicators of systemic toxicity (mortality and symptoms of toxicity).

Test concentrations
At least four test concentrations must be used to:
- properly distinguish the potential endocrine-related adversity from the systemic toxicity in the requested FSDT.
- obtain a robust concentration-response setting that significantly reduces the risk of inconclusive results with respect to regulatory decision-making.
- reduce the possibility for further data requests to come to a regulatory conclusion.

The highest test concentration should cause clear systemic (i.e., non-endocrine-specific) toxicity.

As for the second part of the combined study life stages with higher sensitivities, i.e., juvenile stages, are tested, the maximum test concentration should be chosen according to OECD TG 234. In para 31 of this test guideline, it is stated that *"[…] Concentrations of the chemical higher than 10% of the acute adult LC_{50} or 10 mg/l, whichever is lower, need not to be tested. The maximum test concentration should be set at 10% of the LC_{50} on the larval/juvenile life-stage."*

In your comments to the draft decision, you disagreed with the request of at least four test concentrations. You pointed out that neither the OECD TG 229 nor the OECD TG 234 require four or more concentrations, but at least three concentrations are proposed in the respective guidelines. Secondly, you noted that *"the requirement for four test concentrations is not justified in the draft decision. If the concentration range is considered problematic (we assume mainly for an OECD 234 as this has been discussed already for quite a while), the sequential approach as proposed by us would certainly provide a better basis than the proposed combined assay especially as according to ECHA requirements the same concentrations have to be used for both parts of the combined assay."* Thirdly, you consider the request of four test concentrations as not scientifically substantiated, nor in line with the animal welfare provision of REACH, as this would require 25 % more animals for any of the tests discussed above meaning e.g., 140 to 144 more animals for the test proposed by ECHA. Consequently, you asked ECHA to change the wording in the final decision to at least three concentrations.

ECHA disagrees with your proposal to perform the requested study with only three test concentrations. In addition to the reasons mentioned above, the need for at least four test concentrations is specified as follows:

- to minimise the risk that this study yields inconclusive results, and hence would be followed-up by another animal study, four test concentrations ensure that the highest concentration tested evokes systemic toxic effects. This will allow for an adequate concentration spacing and will minimise the risk of not including the highest possible concentration at which ED specific effects are most prominent.
- such a concentration setting will allow for a regulatory sound assessment of all effects evoked by the substance.
- to derive a full dose-response curve, which makes it easier to interpret possible effects for the ED identification.

Furthermore, the revised OECD Guidance Document 150 states that: "*some of these assays (e.g., the Fish Sexual Development Test and the Peripubertal Assays) may test relatively few concentrations or dose levels, thus limiting the precision of the results, and hence their usefulness for identifying a no-observed-effect-concentration/lowest-observed effect-concentration/x% effect concentration (NOEC/LOEC/ECx) for all relevant types of adverse effects in environmental species*" (OECD, 2018). Thus, to avoid limiting the precision of the assay results and to ensure that the results can fully be used for regulatory purposes, at least four test concentrations are requested.

This reference to OECD GD 150 was proposed by a MSCA. In your comment to this PfA you disagree that the additional rational given is of support for the requested four concentrations. OECD TG 229 and 234 request a minimum of three test concentrations to identify the endocrine hazard of a substance. However, for regulatory purposes we must distinguish systemic toxic from endocrine specific effects. Hence at least four test concentrations are needed. This would ensure a full dose-response curve (including the derivation of a NOEC/LOEC for systemic toxicity) and a minimum of three test concentrations to properly identify ED specific effects. Choosing the three lower

concentrations as near as possible to the LOEC for systemic toxicity would reduce the risk of inconclusive results with respect to ED specific effects.

Route of exposure
The Substance is soluble in water (2.47 mg/L). Therefore, the exposure must take place as described in OECD TG 229 and OECD TG 234 via testing water. The use of a solvent must be avoided.

Fish species
Since effects of the structural analogue OMC on the HPG axis have already been evaluated using Japanese medaka (*Oryzias latipes*), Zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*), the requested study can be performed with all three fish species.

In your comments to the draft decision, you disagreed with *Danio rerio* as the fish species to be used independently from the test finally requested and considered one of the three standard species suitable for the test to be performed claiming: "a) *The statement by ECHA […] that the Danio rerio is more sensitive than Japanese medaka or Fathead minnow is not substantiated and even then, being only relevant for OMC. However, even for OMC the two-generation study in Japanese medaka reported toxicity at 50µg/L similar to the OECD 234 with Danio rerio, thus indicating no difference in the sensitivity. b) OECD 229 states on page 1 in the introduction that this test is fully validated for Fathead minnow but not for the two other fish species. Especially with regard to Danio rerio it is stated on page 2: "There are limitations to the use of zebrafish in this assay, due to the absence of quantifiable secondary sex characteristics responsive to androgenic acting substances". Considering that potential (anti)androgen effects are considered as the main concern, this is clearly an argument that Danio rerio is not the preferred species for this test and supports the use of one of the other species mentioned above."*

ECHA agrees that all three fish species, i.e., *Oryzias latipes, Danio rerio* and *Pimephales promelas* can be used to perform the requested study.

Regarding the choice of species, according to OECD TG 229, para 1 (introduction) describes that: *"All endpoints of the Test Guideline have been validated on the fathead minnow, and a subset of endpoints have been validated in the Japanese medaka (i.e., vitellogenin and secondary sex characteristics) and the zebrafish (i.e., vitellogenin)."* On page 2 (para 7) the OECD TG 229 describes that secondary sex characteristics in male fish are externally visible in fathead minnow and medaka *"but not for zebrafish which does not possess quantifiable secondary sex characteristics."* The limitation you cited from the TG in this assay is *"due to the absence of quantifiable secondary sex characteristics responsive to androgenic acting substances"*. However, the OECD TG 229 also describes that "a decrease in secondary sex characteristics in males [in fathead minnow or medaka] should be interpreted with caution because of low statistical power […]".

The OECD TG 234 para 1 (introduction) states that: *"All endpoints of the Test Guideline have been validated on the fathead minnow, and a subset of endpoints have been validated in the Japanese medaka (i.e., vitellogenin and secondary sex characteristics) and the zebrafish (i.e., vitellogenin)."* In para 5 of the OECD TG 234 it is stated that: *"Several measurement methods have been successfully developed and standardised for routine use to quantify VTG in blood, liver, whole body or head/tail homogenate samples collected from individual fish."*

ECHA further notes that zebrafish showed a high sensitivity in the test with OMC (already at the F0 generation from 10 µg/L; F1 generation: from 1 µg/L, Zhou et al., 2019b). The

study performed with OMC with Japanese medaka showed effects at a slightly higher concentration in the F1 generation (Lee et al., 2019). However, ECHA agrees that secondary sex characteristics can be better monitored using medaka. Hence, the request was amended to include all three fish species mentioned in OECD TGs 229 and 234.

If Japanese medaka is chosen as test species, the test must include genetic sex determination, as well as reporting of any change of the secondary sex characteristics. The presence of a genetic sex marker is a considerable advantage as it increases the power of the sex ratio statistics and enables the detection of individual phenotypic sex reversal.

In your comments to the PfAs you agree to investigate the genetic sex. However, you disagree to investigate secondary sex characteristics as this "*is not a mandatory parameter according to our interpretation of the OECD 234 study*". ECHA disagrees and notes that according to OECD TG 234 para 55 it is stated that: "*Secondary sexual characteristics are under endocrine control in species like the Japanese medaka; therefore, observations of physical appearance of the fish should if possible be made at the end of the exposure. In the Japanese medaka, the papillary formation on the posterior part of the anal fin in females is androgen sensitive. OECD TG 230 (38) provides relevant photographs of male secondary sex characteristics and androgenised females.*" Thus, OECD TG 234 as well as OECD TG 229 refer to secondary sex characteristics as a valid endpoint that can underline an endocrine mode of action even if gonadal histopathology remains inconclusive. Hence, this endpoint is requested in this decision to minimise the risk of inconclusive results of the requested study.

Specification of the assay protocol
The test must be started according to the protocol laid down in OECD TG 229 (21 days) with adult fish covering all standard endpoints and additionally those described in the section below. This test section will provide data useful to conclude on reproductive effects like fecundity. Additionally, the endpoint VTG can provide insight into underlying endocrine modes of action.

The first part of the test (OECD TG 229) must be conducted according to the specifications in the OECD TG 229: two replicates (or vessels) must be used for zebrafish (each vessel containing five males and five females). Four replicates or vessels per treatment must be used for fathead minnow (each vessel containing two males and four females). This is to accommodate the territorial behaviour of male fathead minnow while maintaining sufficient power of the assay. Four vessels or replicates per treatment are used for medaka (each vessel containing three males and three females). The second part of the test (OECD TG 234) must be conducted with four replicates. Therefore, to match the requirements of the OECD TG 234 protocol, using zebrafish, the two replicates used for the first part of the test must be divided to have four replicates for the second part of the test (OECD TG 234). The species-specific requirements according to the OECD TG 229 for the number of replicates were pointed out in a PfA. This was therefore included in this decision as this is also in accordance with both OECD TGs 229 and 234. You did not agree to extend the number of replicates above two for the OECD TG 229 part of the study. Both parts of the test (OECD TG 229 as well as OECD TG 234) are relevant for identification of the ED properties of the substance. Therefore, using medaka or fathead minnow would require four replicates according to OECD TG 229.

After the 21 days the eggs of all exposure replicates and the control are collected and used to start an assay protocol following the OECD TG 234 (63 days). The eggs of each OECD TG 229 replicate are divided to set up two replicates in the OECD TG 234 test.

If there are not enough eggs available at day 21, the start of the OECD TG 234 can be postponed for 1 or 2 days.

Note that in this interim period, the fish are to be further exposed. The collected eggs for the use in the OECD TG 234 test should be stored immediately in petri dishes. The fertilised eggs in cell stadium 4 or 8 should directly be selected via binocular and exposure must start immediately afterwards, using the same exposure concentrations, as in the OECD TG 229 test. All standard endpoints must be covered (in addition to those described in the section below). This section of the assay will provide data on endpoints related to sexual development like sex ratio and gonad histology, and also covers the early life stages of the fish.

Hence, the requested assay design allows to conclude on reproductive effects and on the impact on sexual development.

Parameters to be measured in addition to those already included in OECD TG 234 and OECD TG 229
The additional endpoints furthermore allow to conclude on the underlying endocrine modes of action and consider effects owing to unspecific liver toxicity.

- For the OECD TG 229 study, the fertility (the number of fertilised and viable eggs) after 21 days must be documented.
- Histopathology of gonads (evaluation and staging of oocytes and spermatogenic cells) must be conducted at the end of the OECD TG 234 in all concentrations and the control. Histopathology of gonads must be conducted at the end of the OECD TG 229 when there is an impact seen on fecundity and fertility in F0 fish, unless VTG or secondary sex characteristics are clearly impacted.
- Histopathology of the liver must be included at the end of both parts (OECD TG 229 and OECD TG 234) of the test in all concentrations and the control to detect effects on hormone levels and synthesis caused by specific target organ toxicity of the Substance. This information is necessary to distinguish specific endocrine-mediated effects from effects owing to unspecific liver toxicity.

In your comments to the PfAs received for the draft decision, you disagree to conduct the histopathology of gonads. You argue that this investigation is "*going clearly beyond the OECD TG […] as no rational is provided why this additional parameter is needed for this specific test substance. […]".* Furthermore, you argue that it is not clear when the additional histopathology of gonads is to be performed.

ECHA considers that it was implied by the wording of the MSCA PfA that additional histopathology of gonads should be performed at the end of the OECD TG 229 study. OECD TG 229 explicitly states that the gonads should be preserved for optional histopathology examinations. Moreover, although OECD TG 229 considers histopathology of gonads as an optional investigation, it acknowledges that "*authorities may require this additional endpoint […] in cases where vitellogenin and secondary sex characteristics did not respond to the chemical exposure*".

In this specific case, the analysis of gonadal histopathology at the end of the OECD TG 229 part reduces the risk for an inconclusive test result, which would trigger further testing in the case that the results from the OECD TG 234 part remain inconclusive. Gonadal histopathology at the end of OECD TG 229 part is only requested here if (i.) effects on fertility/fecundity are observed and (ii.) no conclusive effects on VTG levels or secondary sex characteristics are found. Hence, even if not mandatory in OECD TG 229,

histopathology of the gonads in the above-described scenario is proportionate compared to the risk of inconclusive results.

To address the missing information identified in section 1, the combination of OECD TG 229 and OECD TG 234 will provide relevant information both on reproductive effects as well as on effects on sexual development and their underlying modes of action which are required to conclude on the endocrine disrupting properties of the Substance in the environment.

Request for the full study report
You must submit the full study report which includes:
- a complete rationale of test design and
- interpretation of the results
- access to all information available in the full study report, such as implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.

This will enable the eMSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the potential hazard of Endocrine disruption for the environment by the Substance.

c) Alternative approaches and how the request is appropriate to meet its objective

- The request is appropriate because it will provide information which will clarify potential adverse effects of the Substance on fish sexual development and reproduction due to an (anti)oestrogenic, (anti)androgenic activity and interference with steroidogenesis in one study. This will enable the eMSCA to conclude on potential ED properties, and to confirm whether the Substance is an endocrine disruptor according to the WHO/ IPCS criteria.

- The request is the least onerous measure because, beside the presumed effects on sexual development, effects on egg counts were observed for the structural analogue OMC and therefore effects on reproduction must be evaluated for the Substance. Both would not be possible using only a basic FSDT study (OECD TG 234). The combination of OECD TG 229 with OECD TG 234 makes it possible to evaluate effects of the Substance on reproduction (OECD TG 229) as well as including sensitive juvenile life stages of fish and the evaluation of effects on sexual development (OECD TG 234). The whole combined assay has a test duration of 84 days compared to an e.g., Medaka extended One Generation Reproductive Toxicity Study (MEOGRTS, OECD TG 240) that lasts for 133 days.

  Possible alternatives covering the same endpoints would be fish full life cycle studies at the level 5 of the OECD Conceptual framework (CF) (OECD, 2018) such as a Medaka or Zebrafish Extended One Generation Reproduction Test (MEOGRT/ZEOGRT, OECD TG 240). A fish full life cycle or multi-generation test would include all sensitive life stages and would be robust enough to conclude on the environmental ED concern. However, regarding the presumed (anti)oestrogenic, or (anti)androgenic mode of action ECHA considers that there is scientific evidence that sexual development is the sensitive endpoint and that transgenerational effects. i.e., from F1 to F2, are of minor importance in this case. Hence, as the MEOGRT/ZEOGRT also includes part of the F2 generation, this would be disproportionately time and resource consuming in comparison with the requested combination of OECD TG 229 and OECD TG 234

(duration 84 days). The MEOGRT lasts 133 days. The number of fishes is lower in the combination of OECD TGs 229 and 234 (700 fishes in F0 and F1 for four concentrations) than in the MEOGRT (924 fishes in F0 and F1). However, both fish numbers are *de facto* lower, because the number of eggs is reduced after hatching.

Consequently, there is no other experimental study available at this stage which will generate the necessary information and does not require the testing of vertebrate animals.

In your comments to the draft decision, you noted "*that the proposed study design is not in line with animal welfare provisions of REACH as no tests requiring no or less animals are either discussed or considered, nor is a rational given why such tests listed/recommend in the OECD 150 guideline were not considered suitable to address the concern in a sequential/tiered testing for an ED assessment*", furthermore stating: *"In addition, as ECHA highlighted on page 10 (2.2 b) and page 12 (2.2.c) the OECD 229 investigates a sensitive endpoint (based on data noted for OMC), allows the discrimination between systemic toxicity and endocrine related effects and will also help to determine the right concentration range to be covered in a follow up OECD 234, if further testing is needed. ECHA does not consider at all such an approach, but argues in section 2.2.c that the only alternative would be a level 5 test (e.g., an OECD 240). However, no rational is given why such a test would be required as currently not even a level 3 test is available and the level 4 test with OMC revealed no effects up to toxic concentrations considering OMC being not an ED."* Furthermore, you proposed an alternative sequential testing approach, starting with a level 3 test (such as an OECD TG 229 study) and, following assessment of the results, potentially a level 4 test.

ECHA states that the available data raise sufficient concern for the Substance to request already a level 4 study design to be able to conclude on its ED properties. Hence, the tired approach using first only a level 3 study according to OECD TG 229 is not needed. The available data point to effects of the Substance on reproduction and sexual development. Hence, an assay is needed that can assess both endpoints. This can either be a level 5 fish assay or the requested combination of OECD TGs 229 and 234. Regarding overall resources and test duration, the requested study design is the most appropriate to yield conclusive results.

Furthermore, you considered the requested study as scientifically not correct and not in line with animal welfare provision of REACH and disproportionate. You stated that not only water solubility, but also the toxicity triggers the test concentration chosen for the FSDT and noted that that systemic toxicity appeared at the highest test concentration (i.e., the water solubility) in the FSDT with OMC.

ECHA notes that systemic toxicity effects seen in the FSDT with OMC were in most cases minimal. Only in some cases mild and in two cases moderate. Severe effects in the liver did not appear. Furthermore, these effects might be caused by baseline toxicity, owing to the high lipophilicity of OMC (log Kow > 6). The Substance is expected to show baseline toxicity at higher concentrations based on its lower lipophilicity (log Kow 4.78).

Furthermore, you stated that the combined test has no advantage compared to a sequential testing in line with OECD GD 150 regarding the number of animals but raises several concerns. There are some technical challenges as it is no standard procedure for which an OECD test guideline is available. No study plans and no historical control data exist and the CROs have practically no experience with such a test. As no study plan exist the costs will increase (special demand) and due to the lack of practical experience and

historical control data the uncertainty as well. Finally, the CROs see several technical challenges/ problems with the proposal. Based on these statements you considered that the legal certainty and final acceptance of such a study for regulatory purposes is a real concern. *"In addition, as practically no data for fish toxicity after prolonged repeated exposure are at hand (being part of the OECD 229) the study design bears the risk that the concentration range used will not be adequate for the OECD 234 part and parts if not the entire study might have to be repeated, besides of analytical challenges that can be worked on in the OECD 229."*

ECHA notes that technical challenges should be minimal since the requested study is a combination of the established OECD TG 229 and 234 protocols without any additional endpoints or measurements. Additionally, the controls, study quality parameters and the statistical power of the combined assay are directly comparable to those of OECD TGs 229 and 234 and hence regulatory acceptance of the study outcome is not considered a concern. ECHA further notes that under substance evaluation also from a legal point of view non-standard tests can be requested and used for further regulatory decision making.

Regarding the risk that the concentration range used will not be adequate to continue with the OECD TG 234 part ECHA notes that if in the highest test concentration at the end of the first part of the study (OECD TG 229) there are not enough eggs to start the second part of the study (OECD TG 234), it still would be possible to proceed with remaining three test concentrations required as a minimum in the respective OECD 234 test guideline and to yield conclusive results for the ED properties of the Substance.

You commented that there is an inconsistency requesting four test concentrations but accepting three as indicated above.

ECHA clarifies that as requested in the decision the aim is to test four concentrations, for the reason provided. However, ECHA acknowledged in the phrase above that in certain situations, even if the first test is correctly started with four test concentrations, the second part cannot be continued with four concentrations. Therefore, starting the combined testing with four concentrations would ensure that if some problems occur in one of the test concentrations, the second part of the study is left with an acceptable number of test concentrations, although continuing the study with the four initial test concentrations is preferable. If the test would be started with three test concentrations, and something happens to one of those, the remaining two test concentrations would create problems for the interpretation of the study and would lead to the need to repeat the study if inconclusive.

As the combined study starts with the OECD TG 229 part, the study plans and historical data can be used similar to other OECD TG 229 tests. Besides of this, ECHA considers that historical data are not a prerequisite to reach a conclusion on the test outcome and that the design of the test provides sufficient statistical power and controls to be accepted as reliable if documented according to scientific standards and the requirements laid down in OECD TGs 229 and 234.

ECHA considers the requested combination of both assays as the most appropriate test design to cover adverse effects on reproduction and sexual development. In contrast to performing an OECD TG 229 and OECD TG 234 assay as sequential assays, the requested combination is expected to be much more sensitive. This is because the combination allows for starting the OECD TG 234 assay with eggs from fish already treated with the Substance. Hence, effects transferred from parental fish to their F1 can also be detected in this setting.

d) Consideration of time needed to perform the requested study

In your comments to the draft decision, you requested an extension of the timeline from 24 months to 30 months, highlighting the current low capacity of Contract Research Organisations (CROs) to perform the requested study. You considered that 24 months are sufficient to perform an OECD TG 229 test however, a standard OECD TG 234 test would require 30 months. Finally, you considered that 'an even longer time window has to be assumed' if a combined OECD TG 229 and OECD TG 234 test is required.

ECHA has exceptionally extended the deadline by an additional 12 months, to take into account the current longer lead times in CROs.

Therefore, ECHA has granted the request and set the deadline to 36 months.

2.3 References relevant to the requests (which are not included in the registration dossier)

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Appendix B: Procedure

This decision does not imply that the information you submitted in your registration dossier(s) are in compliance with the REACH requirements. ECHA may still initiate a compliance check on your dossier.

12-month evaluation

Due to initial grounds of concern for Endocrine disruption and for wide dispersive use, the Member State Committee agreed to include the Substance IPMC (EC No 275-702-5, CAS RN 71617-10-2) in the Community rolling action plan (CoRAP) to be evaluated in 2016. The United Kingdom (UK) was the competent authority ('the evaluating MSCA') appointed to carry out the evaluation in 2016.

In accordance with Article 46(1) of the REACH Regulation, a substance evaluation decision was issued on 6 April 2018 requesting further information. You submitted information on 8 October 2020.

Following the withdrawal of the UK from the European Union, the competent authority of Germany was appointed as the eMSCA to continue the evaluation.

In accordance with Article 45(4) of REACH, the eMSCA carried out its evaluation based on the information in the registration dossier(s) you submitted on the Substance and on other relevant and available information.

The eMSCA completed its evaluation considering that further information is required to clarify the following concerns: Endocrine disruption.

Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 6 October 2021.

Decision-making

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

The decision making followed the procedure of Articles 50 and 52 of REACH as described below.

(i) Registrant(s)' commenting phase
ECHA received your comments and forwarded them to the evaluating MSCA.

The evaluating MSCA took your comments into account (see Appendix A). The request(s) and the deadline (as explained in Section 2.2.d) were amended.

(ii) Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee
The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision and modified the draft decision (see Appendix A).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendment(s).

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

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-80 written procedure and ECHA took the decision according to Article 52(2) and Article 51(6) of REACH.

After the deadline set in this decision has passed, the evaluating MSCA will review the information you will have submitted and will evaluate whether further information is still needed to clarify the potential risk, according to Article 46(3) of REACH. Therefore, a subsequent evaluation of the Substance may still be initiated after the present substance evaluation is concluded.

Appendix C: Technical Guidance to follow when conducting new tests for REACH purposes

Test methods, GLP requirements and reporting

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.

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.

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].

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*
   a) 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.
   b) The reported composition must include all constituents of each Test Material and their concentration values.

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 "How to prepare registration and PPORD dossiers"[4].

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