Helsinki, 9 December 2021

Addressees
Registrant(s) of 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one
EC number: 244-240-6 and 216-133-4
CAS number: 21145-77-7 and 1506-02-1

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. Fish sexual development test (FSDT, OECD TG 234), using the Japanese medaka (*Oryzias latipes*) or zebrafish (*Danio rerio*) and with the Substance, with at least four test concentrations, and including the following additional endpoints:
   - histopathology of the gonads;
   - histopathology of the liver;

   The exposure must take place as described in OECD TG 234 via testing water. Use of a solvent must be avoided.

Deadline

The information must be submitted by **15 January 2024** from the date of the decision.

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 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] under the authority of 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 (MSCA) 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 are necessary and appropriate.

ECHA
EUROPEAN CHEMICALS AGENCY

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

1. Potential risk

1.1 Potential hazard of the Substance: Potential endocrine disrupting properties

Following its assessment of the available relevant information on the Substance, the evaluating MSCA (eMSCA) and ECHA have identified the following potential hazards which must be clarified: endocrine disruptor (ED) for the environment.

Publicly available as well as registration data from *in vitro* and *in vivo* tests raise a concern for endocrine effects in fish mediated via the HPG (Hypothalamic–pituitary–gonadal) and HPT (Hypothalamic–pituitary–thyroid) axes.
Amongst others, the *in vivo* studies in fish of ([redacted], 1999), [redacted], 1997) and ([redacted], 1996) are contained in the registration dossier. The *in vitro* studies of (Schreurs et al., 2004) and (Schreurs et al., 2002) are mentioned in the registration dossier. All *in vivo* studies except the three studies specified above and all *in vitro* studies are publicly available.

a) EU Risk Assessment

In your comments to the draft decision, you claimed that there is already a EU Risk Assessment Report (RAR, 2008, by NL) and that the RAR and its conclusion is not cited in the decision. The RAR conclusion regarding ED properties is cited here: "*The criterion for endocrine disrupting effects for PBT substances is evidence of ED potential, e.g. listed in the Community Strategy for Endocrine Disrupters. There is no evidence of ED potential; AHTN is not listed in the Community Strategy for Endocrine Disrupters (COM(2001)262final) as a substance with suspected or proven ED potentia*l." Furthermore you noted that "*many of the studies concluded in the RAR to provide no evidence for endocrine activity are now interpreted by ECHA to indicate the opposite*".

There are two main instances where a different conclusion was drawn in the current substance evaluation in comparison to the evaluation contained in the EU RAR:

- In chapter 3.2.6.1, the EU RAR authors stated that no activity was noted on vitellogenin production in carp hepatocytes with reference to a publication of Seinen et al., 1999. In this publication (Seinen et al., 1999, page 166), it is stated that AHTN [the Substance] and HHCB did not affect the VTG production in carp hepatocytes with reference to a publication by Smeets et al., 1999. However, in this publication (Smeets et al., 1999) it is not specified whether the Substance was tested at all.
- the *in vivo* test of Schreurs et al. 2004, was described in the EU RAR similarly as in the actual evaluation. In section 3.2.6.1, a statement of Schreurs et al. 2004 is cited: "*The actual concentrations at which we observe anti-oestrogenic effects are around or below the no-observed-effect levels from these studies* [FELS-studies, ([redacted] (1997), ([redacted], 1999)]. *That is, no developmental disorders were or will be observed at the concentrations used in our transgenic zebrafish assay*." This sentence served as a closing sentence for the chapter 3.2.6.1 "Endocrine interactions" in the RAR.
  However, in ECHA's opinion, this sentence cannot serve as indication that there is no concern for endocrine effects of the Substance. This transgenic fish assay (Schreurs et al. 2004) provides in spite of the short duration of 96 h at the concentrations 6.9 and 84.6 µg/L indication for an antioestrogenic mode of action (MoA).
  For more explanation regarding the effect concentrations in the short term test by Schreurs et al. 2004 and the FELS tests ([redacted] (1997), ([redacted]

[redacted], 1999) see section 1.1.b. It is concluded that the indicative effect in the study by Schreurs et al. 2004 occurs at concentrations below and around the derived NOECs form long-term fish studies (FELS). There is a strong concern that these ED indicative effects are not secondary to other systemic toxic effects but point to a specific ED mode of action of the Substance.

In summary, there are mainly two differences between the evaluation in the RAR and the actual evaluation regarding endocrine properties, mostly because the evaluation in the RAR was focussed mainly on PBT/vPvB properties and human health, and there are new studies available now.

Therefore, ECHA concludes the assessment and database contained in the EU RAR is insufficient to clarify the concern for ED properties in the environment.

b) *In vitro* tests

Oestrogenic, antioestrogenic, antiandrogenic as well as antiprogestagenic properties were observed for the Substance in reporter gene assays in several cell types (Schreurs et al., 2005a; Schreurs et al., 2005b); (van der Burg et al., 2008); (Schreurs et al., 2004); (Schreurs et al., 2002); (Mori et al., 2007); (Seinen et al., 1999). In two published E-Screens not contained in the registration dossier, the Substance caused enhanced cell proliferation (Bitsch et al., 2002; van Meeuwen et al., 2008). Furthermore, effects on steroidogenesis were seen (Li et al., 2013).

- Oestrogenic and antioestrogenic *in vitro* effects of the Substance

Antioestrogenic effects were consistently seen in four reporter gene assays (Schreurs et al., 2004; Schreurs et al., 2002; Schreurs et al., 2005a; Schreurs et al., 2005b; van der Burg et al., 2008) with the oestrogen receptors hERß (human Estrogen Receptor ß), zfERß and zfERγ (zebrafish Estrogenic Receptor ß and γ) and several cell types (HEK293 (Human embryonic kidney), U2OS cells, HepG2 cells) and IC_{50} values between 1.9 and 2.51 µM (Schreurs et al., 2005a; Schreurs et al., 2005b; van der Burg et al., 2008) and LOEC values between 0.1 and 10 µM (Schreurs et al., 2004; Schreurs et al., 2002).

Oestrogenic effects were seen in four reporter gene assays with CHO-K1 cells (EC_{10} of 0.36 µM) (Mori et al., 2007) and HepG2 cells, HEK293 cells, U2-OS cells (Schreurs et al., 2004; Schreurs et al., 2002; Seinen et al., 1999) although no effects in the latter cells were observed in other tests (Schreurs et al., 2005a; Schreurs et al., 2005b; van der Burg et al., 2008). Oestrogenic effects were limited to the receptor hERα.

In addition, two E-screens showed dose-dependent cell proliferation and thus oestrogenic effects with a relative potency of 1E-5 and 2.5E-5 (Bitsch et al., 2002; van Meeuwen et al., 2008). In both assays an oestrogen-antagonist could reverse the oestrogenic effect of the Substance. Hence the observed oestrogenic effect is confirmed to be caused by activation of the oestrogen receptor (ER).

- Antiandrogenic *in vitro* effects of the Substance

There are three studies using reporter gene assays where antiandrogenic effects of the Substance were seen in U2-2 cells and CHO-K1 cells with IC_{50} values between 0.15 and 3.16 µM (Mori et al., 2007; Schreurs et al., 2005a; Schreurs et al., 2005b; van der Burg et al., 2008). No androgenic agonism was observed.

- Effects of the Substance on steroidogenesis

Li et al. observerd in H295R cells a decreased production of progesterone (at 0.25 µM), estradiol (E2) and testosterone (at 25 µM). The gene expression of CYP19 was

downregulated at 2.5 µM and 25 µM. CYP17 gene expression was upregulated at 25 µM. The enzyme activity of 17α-hydroxylase activity (a part of CYP17) was significantly induced at 0.25 µM and higher concentrations. The other part of CYP17, the 17,20-lyase activity was almost completely inhibited at 25 µM (Li et al., 2013).

In ovarian microsomes of carp, CYP19 was significantly inhibited at 1mM (Schnell et al., 2009).

Testosterone production of H295R cells was reduced at 0.01 and 0.1 nM (0.84 and 0.73-fold resp.) of the Substance (Ling, 2009), but not at higher concentrations. Production of E2 was reduced at 0.01 and 1 nM (0.71 and 0.8-fold resp.).

- Antiprogestogenic effects of the Substance

Antiprogestogenic properties were seen in two reporter gene assays (CALUX bioassay) with IC_{50} values between 4.9 pM and 25.1 nM (Schreurs et al., 2005b; van der Burg et al., 2008). No progestagenic effects of the Substance were seen.

In your comments to the draft decision you argued that many of the assays did not properly control for cytotoxicity. ECHA states that not in all, but in many tests cytotoxicity was examined. From these test results where cytotoxicity was examined it can be concluded that the Substance does not exert overt cytotoxicity also for the remaining studies where it was not examined. Moreover all studies together are evaluated in a weight of evidence (WoE) approach.

Further, you noted that *in vitro* screening studies are generally more prone to false positive and artefactual results than are the standard regulatory studies. ECHA submits that the results are not taken in isolation in the WoE approach. Positive *in vitro* results trigger a concern, and therefore a higher-tier test is needed to confirm the concern or not.

In your comments to the draft decision you noted that the *in vitro* studies of Schreurs et al. (2005a, 2005b) showed both oestrogenic and antioestrogenic effects and that the *in vivo* study of Scheurs et al, 2004 showed no oestrogenic effects. In your view this would indicate that the *in vitro* data were not reliable and not predictive for the whole animal.

However, the available *in vitro* data show consistently antioestrogenic effects on the hERß, whereas on the ERα no oestrogen antagonistic effects at the same, or only at higher concentrations than on ERß were seen ((Schreurs et al., 2005a; Schreurs et al., 2005b), (van der Burg et al., 2008), (Schreurs et al., 2004), (Schreurs et al., 2002)). This indicates that ERß is more sensitive against the antioestrogenic impact of the Substance than ERα. In comparison to ERß, on ERα rather oestrogenic effects were seen (if a comparison of both receptors in the same study was made). In none of the studies the Substance caused oestrogen agonistic effects on ERß. On the zebrafish oestrogen receptor gamma (zfERγ) strong oestrogen antagonistic effect were seen, as well as on the hERß in the same *in vitro* study (Schreurs et al., 2004). Schreurs et al., 2004 assumed that the antioestrogenic effects seen in the *in vivo* transgenic zebrafish assay were probably mainly mediated by the zfERγ.
In summary, ECHA considers that different oestrogen receptors may evoke different effects which may also vary across cell types. In general, in the oestrogen receptor assays the antioestrogenic effects were more pronounced, aside from the assay by Mori et al., 2007, where only ERα was tested, showing no effect on antioestrogenicity . Therefore, the *in vitro* tests are not contradicting the results of the *in vivo* test (Schreurs et al., 2004). The antioestrogenic or oestrogenic MoA of the Substance was also stated by Schreurs et al., 2002, which indicated that the Substance is a selective estrogen receptor modulator (SERM).

SERMs "have the ability to act as both agonists and antagonists, depending on the cellular and promoter context and the ER subtype targeted" (Schreurs et al., 2002).
You further stated that "*the publication of Schreurs, 2005 is included in the RAR of 2008 and was presumably not discussed because it raised no concern.*" ECHA notes that both studies (Schreurs et al., 2005a; Schreurs et al., 2005b) were evaluated in the RAR where the same results were depicted as in the actual evaluation. It was stated that oestrogen antagonistic effects were seen on the hERß, as well as antiandrogenic and antiprogestagenic effects. Probably from the effects seen, no concern was raised since the *in vivo* study in mice (Seinen et al., 1999) did not show any oestrogenic effects and was deemed to be negative ("no uterotrophic activity of AHTN and HHCB was noted at relatively high dietary exposure levels up to 50 and 300 ppm", Seinen et al., 1999). However, no antioestrogenic effect was evaluated in this study. In addition, as stated above, the focus of the RAR (2008) did not lay on the endocrine disruption evaluation.

c) *In vivo* tests

- *In vivo* tests in fish

A short-term test (duration 96 h) with 4 to 5 weeks old transgenic juveniles of *Danio rerio* was conducted (Schreurs et al., 2004). Fish were fed once daily with live brine shrimp. 5 to 6 fish were used per concentration. The test design was semi-static, half of the water was renewed daily, the temperature was 26-27 °C. The Substance concentrations used were nominal 25.8 and 258 µg/L and measured 6.9 and 84.6 µg/L (solvent DMSO). For measurement of antioestrogenicity, the fish were co-exposed to E2 (10 nM). E2 treatment caused an increase of luciferase activity in the transgenic fish. Antioestrogenic effects were seen as the Substance inhibited the activity of the endogenous oestrogen receptor at low µg/L range (6.9 µg/L) as evidenced by reduced luciferase activity. The concentration of nominal 10 µM (2 584 µg/L) was toxic to the fish and not used for the juvenile transgenic zebrafish assay. It was not specified to which extent the 10 µM was toxic to fish. No information on the toxic effects for the Substance at 0.1 and 1 µM or about mortality in control is given. The antioestrogenic effects (6.9 and 84.6 µg/L) occurred at concentrations below the solubility limit of the Substance of 1.25 mg/L. This effect is of relevance for the endocrine evaluation of the Substance since the effect seen fit to the effects which were observed in other studies in a WoE approach. Moreover, according to ECHA, the study is well conducted. In your comments to the draft decision, you argued that the authors did not include adequate controls to demonstrate whether the effects observed where due to exposure to the Substance. ECHA considers that the antioestrogenic effects seen were statistically different compared to E2 treatment (10 nM) without AHTN (see figure 3 in the publication). This treatment serves as negative control although it was not specified in the text. The acute toxicity on fish of E2 is LC_{50} (96 h) > 0.5 mg/L (from ECHA dissemination site). 10 % mortality was observed at 0.5 mg/L E2. Therefore no toxic effects are to be expected at the used concentration of 10 nM E2 (2.7 µg/L). The test of Schreurs et al., 2004 is used in a WoE approach together with the other tests.

You further argued that the result of the evaluation had another result than the RAR, 2008. In the RAR, a sentence from the publication by Schreurs et al. 2004 served as closing sentence for the chapter 3.2.6.1. "Endocrine interactions" and declared that the concentrations were antioestrogenic effects were observed layed at or below the NOECs in the two FELS studies mentioned below.
The NOECs in the two FELS tests with the Substance were 35 µg/L each for malformation and the LOECs were 67 µg/L and 50 µg/L, respectively ([redacted], 1997), ([redacted], 1999).
The NOEC in the Fish prolonged toxicity test (in compliance to OECD TG 204) by ([redacted], 1996a) is 89 µg/L. However, this test had only a duration of 21 days and did not begin with sensitive live stage (fish embryos) and is therefore less meaningful.

The study by Schreurs et al. 2004 shows that at concentrations below and around the NOECs from long term fish tests (FELS) there is an diagnostic effect (antioestrogenic MoA), which was not examined in the FELS. Since these indicative effects occur at concentrations below the derived NOECs form long-term fish studies there is a strong concern that these ED indicative effects are not secondary to other systemic toxic effects, but point to a specific ED mode of action of the Substance that needs to be clarified further.

You noted that Scheurs et al., 2004 stated that AHTN bioaccumulates in fish but that the data included in Figure 4 of the respective publication showed that the internal concentration of AHTN in the fish did not increase with time. In ECHA's opinion the comparison of test concentrations in vessels with and without fish indicate that the rapid disappearance of the Substance during the test was caused by an uptake of the Substance or adherance to fish skin or test vessel. The concentration of the Substance was significantly increased on day 3 (difference between day 1 and the following days). In fish exposed to 0.1 µM of the Substance, about 61 µM was found and in fish exposed to 1 µM, about 554 µM found. From this short period of time it is not possible to give relevant information about bioaccumulation, the bioaccumulation of the Substance with reference to the RAR, 2008 is <2000 (the B-criterion). In any case, also a substance with a lower bioaccumulation is of relevance for the environment if it exerts endocrine disrupting effects.

Another transgenic short term fish test with duration of 48 h employing 10 to 12 days old marine medaka (*Oryzias melastigma)* is available. The fish were adapted to artificial seawater with a salinity of 3% at 28°C and fed with both brine shrimp and hormone-free flake food. There were 7 to 8 larvae per treatment. The Substance concentrations were in the range of 0.2584 to 2 584 µg/L (0.001 to 10 µM, nominal, the concentrations were not measured). The solvent was 0.1% DMSO. For examination of antioestrogenicity, the larvae were co-exposed to 0.04 µM E2. Green fluorescent protein (GFP) expression in the liver was observed. Antioestrogenic activity was detected by decreased GFP signal intensity, but at higher concentrations (nominal 1292 µg/L, 5 µM). At 2584 µg/L (10 µM) 100% mortality occurred. No information about control mortality is given (Ling, 2009).

Furthermore, a short-term test with male *Oryzias latipes* was conducted using the Substance. The fish were four-month old post hatching and the exposure lasted 72 hr. The fish received as food brine shrimp twice daily. Three fish per concentration were tested for hepatic vitellogenin (VTG) analysis and six fish per concentration for gene expression analysis. The test solutions were changed daily, the temperature was 25 °C. Nominal concentrations were 5, 50 and 500 µg/L.

Concentrations were only measured at t = 0 and t = 24 h. Calculation of measured concentrations using geometric mean: for nominal 500 µg/L: 27.5 µg/L, for 50 µg/L: 2.7 µg/L; using Time weighted average (TWA): for nominal 500 µg/L: 95 µg/L, for 50 µg/L: 9.9 µg/L (solvent DMSO 0.01 %). At measured concentrations of the Substance at 27.5 µg/L (geometric mean), respective 95 µg/L (TWA) VTG protein was increased. The genes expressing VTG I and VTG II and the gene for ERα were upregulated at 27.5 µg/L (geometric mean), (95 µg/L, TWA), (Yamauchi et al., 2008).

In your comments to the draft decision you argued that the effect appeared at 500 µg/L (nominal) that is near the EC_{50}. ECHA interprets that, in this statement, the LC_{50}, instead of the EC_{50}, value was meant. However, the LC_{50} is 1 mg/L (Yamauchi et al., 2008, acute fish assay with larvae of medaka, most probably nominal) or 1.49 mg/L (initial measured) in a Fish, prolonged toxicity test with Bluegill sunfish ([redacted], 1996). In a WoE approach this study can be used to give supporting indications. You mentioned that only two concentrations were tested. This would not have been accepted in a requested test, however in a WoE approach this test can give valuable indications.

Furthermore, you argued that the results are contradicting because in the study by Yamauchi et al., 2008 an estrogen effect was seen (increased VTG), whereas in the study by Schreurs 2004 an antiestrogenic effect was seen. ECHA notes that these varying effects are possible as the Substance can act as an selective estrogen receptor modulator (SERM). This was also stated by Schreurs regarding the results of the *in vitro* tests (Schreurs et al., 2002).
Additionally, the two studies were differently designed and utilized different species. However, both studies show a concern that the Substance can act via the HPG axis with potentially multiple modes of endocrine action.

You further argued that in the study by (Yamauchi et al., 2008) no clear concentration-response for VTG gene expression was seen and that the effect on VTG gene expression was only observed at 500 µg/L, and that the effect was lower than for the positive control. For the gene expression of VTGI and VTGII a statistical significant effect was seen only at 500 µ/L (nominal). As only the highest concentration was significantly changed nothing can be said about concentration-response relationship. It is a normal observation that the positive control E2 (here 1 nM) has more pronounced effects than the treatments with xenobiotics.

You further stated that Yamauchi, 2008 reported "*no statistically significant increases in the levels of liver CYP mRNA, but in fact similar increase patterns were observed, so the possibility that the effects on VTG were caused by increased liver metabolism is clear*." ECHAconsiders that Yamauchi et al. 2008 examined the effects on CYP3A38 and CYP3a40 which are responsible for metabolism of xenobiotics. However, no statistical significant effects were seen as depicted in Fig. 5 of the publication. Therefore this endpoint is considered not to be of relevance. Moreover, it is unclear why an increase in liver VTG could be explained by an enhanced liver metabolism. Measurement of VTG as oestrogen or antioestrogen biomarker is a specific effect than can only be seen after activation/inhibition of the oestrogen receptor by an oestrogen/antioestrogen acting substance. Yamauchi et al., 2008 suggests that the Substance induces "hepatic ERα and VTG mRNAs and VTG protein in male medaka, although the toxicological consequences of this response remain unclear". In ECHA's opinion the upregulation of VTG I and II gene expression fit to the upregulation of ERα gene and to the hepatic VTG increasement seen in this study and to the estrogenic effects on ERα seen in the *in vitro* tests above. The effects in this study are oestrogenic and they appeared consistently at 500 µg/L (nominal). The absence of effect on gene expression of ERß fits to the occurrence of these oestrogenic effects, as *in vitro* studies above only antioestrogenic effects on ERß were seen. The concern needs to be clarified via the new data required in this decision.

An embryonal fish assay was conducted with *Danio rerio* and duration of 48 hr, the concentrations were 1-1000 µg/L. At least 20 eggs were used per exposure group (only eggs that reached at least the four-cell stage). Exposure to the Substance did not result in reduced larval survival time up to 100 µg/L (observed in a second test) (Carlsson and Norrgren, 2004); (Carlsson, 2007).
The embryos exposed at 33 µg/L (= LOEC) of the Substance exhibited a reduced heart rate. In your comments to the draft decision you stated that in the EU RAR of 2008 it was concluded the effect was minimal and no concentration-response relation existed, that only at 1000 µg/L the line has started to descent. This also makes the ecological relevance of these effects unclear.
ECHA states that also a small change of this parameter could lead to a significant effect, because the normal heart rate deviation was very small. Therefore, this effect could be of biological relevance, but it is not clear. You stated that *"it is noted that during the development of the OECD 236 FET study that changes in heart beat and curved spine were commonly associated with many chemicals and considered to demonstrate toxicity, not*

endocrine disruption ([redacted],2002;and [redacted],2009)." ECHA states that the consideration about the connection between the decreased heart beat rate and thyroid effects is not followed anymore in this amended decision due to insufficiently available supporting information on this from several scientific publications.

A fish early-life stage (FELS) test according to OECD TG 210 was conducted with *Pimephales promelas* ([redacted], 1997). The measured concentrations were 8.1, 18, 35, 67 and 140 µg/L. Another fish early-life stage test according to OECD TG 210 ([redacted], 1999) used the nominal concentrations of 10, 20, 35, 50, 75 µg/L (confirmed by measurement).
In both tests malformations in form of missing caudal fin were seen at 67 µg/L [redacted], 1997) and at 50 µg/L ([redacted], 1999) exposure to the Substance, respectively. In addition in ([redacted], 1999), curled or curved bodies were observed at 50 µg/L exposure to the Substance.

In your comments to the draft decision you claimed that "*the missing caudal fins were observed in a very narrow dose window between no effect and marked toxicity and/or lethality. Therefore, the explanation is much more likely to be generalized toxicity and stunted growth with a threshold than an ED effect.*"

In the FELS study by [redacted], 1999, the LOEC (zebrafish) for malformation is 50 µg/L (NOEC = 35 µg/L, missing tail fin, curved body), whereas no effects on hatch and survival were seen at the highest concentration of 75 µg/L (= NOEC). However, at this NOEC, all fish had missing tail fins and most of them showed a curved or curled body. In the other FELS study the LOEC (fathead minnow) for malformation is 67 µg/L (NOEC = 35 µg/L), whereas the LC50 is 100 µg/L: at 140 µg/L 82% of fish were dead and at 67 µg/L the mortality was 6 %. Therefore, it is visible that there is a clear gap between the Substance concentrations that caused malformations or general toxicity. Furthermore, the Substance caused total absence of caudal fins, not merely a shortening in the majority of fish exposed to 67 µg/L and in all fish at 140 µg/L ([redacted], 1997). For the nine fish exposed to 67 µg/L that had tail fins, no significant difference in the relative tail-fin length was seen compared to control. Hence, where the caudal fin did exist, it was not shortened. If this was caused by generalized toxicity, observation of a shortening rather than a total absence of caudal fins would have been more likely. Therefore, ECHA considers that the malformations were not the result of generalized toxicity.

While providing your comments to ECHA's and MSCAs' proposals for amendment, you also mentioned a new fish in vivo study (Hodkovicova et al., 2020) where male juvenile rainbow trout (total length 32.2 cm, weight 516.8 g) were exposed over the diet to 854 µg/kg and 8699 µg Substance/kg feed (twice daily at 1 % of body weight) for 6 weeks. VTG levels were measured in the plasma and histopathology of gonads was examined. You stated that VTG levels were not increased and histopathology of gonads was negative. You noted that the study provides strong evidence that the Substance does not induce endocrine-disrupting effects in fish similar to those that could be seen in the requested FSDT.
ECHA considers this study as not reliable with regard to estrogenic effects as, also in the male control fish, increased VTG levels were seen (range 303 to 985 ng/mL), potentially caused by an estrogen acting impurity. The VTG levels in the lower exposure group were between 173 and 945 ng/mL. In the higher concentration the range was between 362 and 609 ng/mL. On gonad histopathology no effects were seen in the control and the treatments.
The diagram (Fig. 1) of the publication identifies that the mean plasma VTG values for both exposure groups were lower than the control (however not statistically significantly). However, this would fit to an anti-estrogenic MoA, as outlined above. Due to the enhanced VTG value in the control the study by Hodkovicova et al., 2020 is not considered as reliable

regarding VTG measurement. Hence, the comparison of the treated fish groups with the control fish groups is unreliable and the results are questionable. Another difference to the other studies is that the exposure for this study was performed using the diet. Hence the Substance could be metabolised in the liver, while no exposure via gills uptake is possible. The appearance of effects on the gonads (histopathology) might be influenced by the exposure route via diet.
Due to the differing exposure routes, direct comparison of the data from this study with the others is not possible.
Therefore, the study cannot be used to argue an absence of the concern for endocrine disruption of the environment caused by the Substance. Taking into account all available data, there still remains a concern for endocrine disruption which needs to be clarified.

From the effects observed in *in vivo* tests in fish, ECHA considers that the Substance may exert an influence on the HPG axis. *In vivo* short term tests showed antioestrogenic (inhibition of E2-induced luciferase in transgenic *D. rerio*) and oestrogenic (induction of VTG in *O. latipes*) effects.

- *In vivo* tests in mammals

Mammals are environmental organisms and the endocrine system, in particular the HPG and HPT-axes, is conserved within vertebrates. Therefore, ECHA notes that the available mammalian data can be used to support the concern for ED properties in the environment even if they are not, on their own, sufficient to conclude on the concern.

The majority of studies in rats provide no indications for oestrogenicity, androgenicity, steroidogenesis (EAS) or an antiprogestogenic related toxicity of the Substance. Nonetheless, some EAS sensitive parameters were influenced by the Substance in an Extended One Generation Reproductive Toxicity study (EOGRTS , OECD TG 443) in rats ([redacted], 2020): in the high dose, anogenital index (AGI) in female offspring was increased in the F1, but not in the F2 generation.
You claimed that since this finding was not observed in the F2 generation, it may be incidental and that the reduction in bodyweight in the high dose group was caused by reduced food consumption, in turn caused by the negative impact on palatability of the diet by the Substance.

However, ECHA notes that a similar trend for increased AGI was observed in F1 males. In addition, the dose-range finder study (based on OECD TG 421) for the EOGRTS ([redacted], 2018) showed a dose-dependent increase in AGI in both sexes. Statistical significance was not achieved likely due to the low number of animals (N = 5 per group/sex). With regard to the palatability, this issue was as well assessed in the dedicated study (up to 1200 ppm) with no effects on food consumption observed at the 300 ppm treated group ([redacted], 2018). ECHA notes that the study authors of the palatability study concluded that the diet was palatable. In summary, although the toxicological relevance of an increased AGI is not clear, these findings nevertheless show a change in an EAS-sensitive parameter, adding to the evidence for triggering additional investigations with regard to the concern for the environment.

In the EOGRTS, balano-preputial separation (BPS) was delayed dose-dependently in F1 male offspring. You commented that the delay in BPS in the high dose group could be due to a lower pre-weaning body weight. However, ECHA considers that in the high dose, the lower pre-weaning body weight (about -16% at PND 21) cannot fully explain the substancial delay of 5.5 days for BPS. As other developmental landmarks (e.g. tooth eruption and eye opening) were not affected, delayed BPS rather indicates a specific effect on an EAS-sensitive parameter, adding to the evidence for triggering additional studies

with regard to the environment.

In contrast to the rats studies, the Substance displayed clear reproductive toxicity in an OECD TG 414 study with rabbits without significant maternal toxicity ([redacted], 2019). Reprotoxic findings comprised reduced gravid uterus weight, increased post-implantation loss and total number of resorptions. These parameters are sensitive to, but not diagnostic for, EAS-mediated activity and can be influenced by the antiprogestagenic activity of the Substance observed *in vitro* as well. However, further diagnostic parameters which might allow to conclude on the specific MoA are not part of the study protocol when using rabbits. The study director applied the same NOAEL values to maternal and fetal toxicity, indicating their opinion that the maternal toxicity was significant. Furthermore, food consumption and bodyweight gain were reduced in the high dose group, as overall 22.7% less food was consumed compared to the control group. However, corrected body weight gain and corrected body weight (-0.9%, -3.7%, and +1.9% in the low, mid, and high dose compared to controls respectively) were not significantly different. Also, although pregnancy in rabbits is known to be sensitive towards feed restriction, based on literature data, feed restriction by 80-90% of control values is required to induce an increase in the number of abortions and/or resorptions (Nitzsche, 2017). Therefore, ECHA considers the unspecific toxicity as mild and the findings of reduced gravid uterus weight, increased post-implantation loss and total number of resorptions as specific effects on development induced by the Substance. The affected parameters are sensitive to endocrine modes of action justifying additional studies with regard to the concern for the environment.

An OECD TG 421 study ([redacted], 2018) in rats demonstrated increased thyroid weight after exposure to the Substance in Postnatal day (PND) 14 pups (no thyroid histology or hormone measurement were performed). Effects of the Substance on the HPT-axis were also demonstrated in an OCED TG 443 study ([redacted], 2020). Exposure to the Substance was associated with increases in thyroid weight and hypertrophy/hyperplasia of the thyroid gland in parental animals and offspring. Additionally, increases in the levels of T3, T4, and Thyroid Stimulating Hormone (TSH) in F1 females were reported. However, the underlying mode of action remains unknown. Although effects on the liver (e.g. weight increase and hepatocyte hypertrophy) were observed, the increase of thyroid hormone levels indicate an effect pattern rather different from typical inducers of hepatic enzymes leading to increased thyroid hormone excretion. In fact, after short-term treatment in rats, the Substance did not induce hepatic peroxisome proliferation, and in contrast to 3-methylcholanthrene and phenobarbital , did not induce ethoxyresorufin-O-deethylase (EROD) or RPOD[2] activity, respectively (Steinberg et al., 1999). You argued that the thyroid findings are influenced by general stress and are clearly due to an indirect mechanism not indicative of an endocrine disruption MoA. Furthermore, you noted that the HPT axis can be influenced by liver enzyme induction associated with hepatocyte hypertrophy, but that induction of UDP-GT by the Substance was not measured in any study. ECHA notes that the EOGRTS shows a clear dose-dependent increase in the incidence of thyroid hypertrophy. Furthermore, increases in thyroid weight and increased TSH, T3, and T4 levels were reported in F1 females. Increased thyroid weight was also observed in the dose-range finder study (based on OECD TG 421) for the EOGRTS.

Together, these findings are considered treatment-related rather than unspecific side-effects of other toxicity. The increase of TSH and thyroid hormones in F1 females indicate an effect pattern rather different from the typical inducers of hepatic enzymes leading to increased thyroid hormone excretion. Even if the liver plays a role, in the absence of data demonstrating rat-specificity of the findings, the thyroid effects are considered relevant

[2] https://www.sciencedirect.com/science/article/pii/S0378427499001769?via%3Dihub

for vertebrates in general, triggering additional investigations to clarify the concern for the environment.

In summary, taking into account the effects observed in mammals and fish *in vivo*, ECHA considers that:

- There is a possible influence of the Substance on the HPG axis due to findings in fish (induction of VTG in male medaka; inhibition of E2-induced luciferase in transgenic zebrafish), and in mammals (i.e. repro-toxicity in rabbits; increased AGI and delayed BPS in rats).
- There are indications for an influence of the Substance on the HPT axis due to findings in mammals (effects on thyroid weight, thyroid histology, and levels of TSH, T3, and T4 in rats). In addition, the (fin) malformations in *Pimephales* might also be related to disruption of the thyroid axis. However, this concern is not followed with the testing strategy described in this decision.

Therefore, the available and current information show that the Substance interacts with the endocrine system of fish. There are indications that the Substance also interacts with the endocrine system of mammals. In order to clarify if the Substance is an ED for the environment, further data on population relevant adverse effects in fish with a clear link to the endocrine system is needed and thus the available information is not sufficient to draw a conclusion on the hazard. Further information is needed on the endocrine properties for the environment.

ECHA notes that, although the studies referenced in this decision were not performed according to GLP or OECD guidelines and without inter-laboratory validation, all studies were used by ECHA in a WoE approach. ECHA concludes that these studies give sufficient indication for a concern for endocrine disrupting properties of the Substance in the environment which need to be clarified with a more comprehensive additional test.

1.2 Potential exposure

According to the information you submitted in the registration dossier and chemical safety report, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 1 000–10 000 tonnes per year. In your comments to the draft decision, you submitted that the tonnage is lower and on a decreasing trend. However, ECHA finds that this decreasing trend in tonnage is not apparent across all registrations and that the aggregated tonnage of the Substance estimated in the dossiers is still above 1 000 tonnes per year, thereby contributing to the overall potential risk of the Substance for the environment. Even taking into account your informal statements not reflected in the dossiers, the aggregated tonnage would amount to significantly over 100 tonnes per year. This tonnage would still be sufficient to raise a concern for the environment arising from potential non-threshold effects of the Substance and its use pattern.

The 2008 European Union Risk Assessment Report (EU-RAR, 2008) reports that among other uses, the Substance is used as an ingredient in fragrance oils. Uses of these fragrance oils are reported in consumer products such as perfumes, cosmetics, soaps, shampoos, detergents, fabric conditioners, household cleaning products, air fresheners, etc. Furthermore, perfume oils are used in the professional sector in detergents, cleaning agents and biocides (e.g. disinfectants, pesticides). Thus, the Substance has a wide dispersive use.

The information you provided on manufacture and uses demonstrate a potential for exposure of the environment. Monitoring studies show the presence of the Substance in

various environmentally relevant compartments: fresh water and marine water, sediment and biota (Tumova et al., 2017), (Lange et al., 2015), (Schmid et al., 2007), (Subedi et al., 2012), (Trabalon et al., 2015), (Sumner et al., 2010), (Xie et al., 2007).

Therefore, exposure to the environment has been confirmed for the Substance.

In your comments to the draft decision, you considered most of these studies as 'very old'. ECHA does not consider these studies which have been conducted less than 15 years or, in the case of Trabalon et al., 2015 and Lange et al., 2015, around six years ago, as old or outdated to support the concern for the Substance arising from environmental exposure.
In addition, in a proposal for amendment, ECHA mentioned a more recent study from Tumova et al., 2017 that shows the occurrence of the Substance in Czech rivers and biota.

You furthermore submitted in the comments to the draft decision that in the study of Trabalon et al. (2015), the Substance was found in levels in the environment below those at which effects were found in *in vivo* studies in fish. ECHA considers endocrine disruption in the environment as an effect for which a threshold can be derived only with difficulty, if at all, due to the potentially large difference in sensitivity across environmental species. Hence, the environmental exposure levels measured in the studies still raise a concern for the environment in ECHA's view even if they are below the NOEC identified in toxicity studies or a threshold derived for human health as stated in the Trabalon study. Finally, the conclusion regarding the effects of the Substance on human health is not the aim of the information requirement contained in this decision.

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 in the environment according to the WHO/IPCS definition.

The information you provided on manufacture and uses as well as available monitoring data demonstrate a potential for exposure of the environment.

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

As explained in Section 1.1 above, the available information is not sufficient to conclude on the hazard and in particular the endocrine disruptive properties. Consequently, further data is needed to clarify the potential risk related to endocrine properties.

1.4 Further risk management measures

If the properties of the Substance are confirmed, the eMSCA will analyse the options to manage the risks. New regulatory risk management measures can be identification as a substance of very high concern (SVHC) according to Article 57(f) of REACH and, as a result, to be listed in REACH Annex XIV with possible further risk management measures, such as an authorisation and/or restriction process in order to substitute the Substance and/or minimise environmental exposure. These measures 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, an SVHC identification would trigger additional information duties of producers and importers to ECHA according to Article 7(2) of REACH and 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

This decision is focused on clarifying the potential ED properties of the Substance in fish. The data already available raise a concern for endocrine effects in fish mediated via the HPG and HPT axis.

However, if the FSDT requested in the current decision is inconclusive for the EAS pathways, or if no EAS effects are observed, the T pathway which is currently still uncertain, could be followed up by a second decision asking for amphibian data, e.g. requesting an Amphibian Metamorphosis Assay (AMA, OECD TG 231) or a Larval Amphibian Growth and Development Assay (LAGDA , OECD TG 241).

2.2 Fish sexual development test (FSDT, OECD TG 234)

As detailed in Section 1.1, information on adverse effects related to the endocrine activity of the Substance are required to conclude on the potential hazard.

The FSDT must be conducted to examine the ED properties of the Substance since this assay can inform both on the adversity and the endocrine activity in fish and thus, enable establishing the mode of action (MoA) for the suspected endocrine disruption. Documentation of a plausible, endocrine-mediated MoA for the observed adverse effects is crucial for the identification of the Substance as an ED.

a) Specification of the requested study

The conduct of the FSDT is requested with some additional endpoints, such as histopathology of gonads (evaluation and staging of oocytes and spermatogenic cells) and of the liver which must be performed.

- Test material and concentration

At least four test concentrations must be used (instead of the minimum number of test concentrations referred to in the OECD TG 234).

This will allow to:

- derive a robust concentration-response curve for the endocrine disruption endpoints. A full concentration-response relationship helps to distinguish the potential endocrine related effects from systemic toxic effects. The highest test concentration should cause clear systemic (i.e. non endocrine-specific) toxicity.
- reduce the risk for the test to be inconclusive with respect to regulatory decision making, and to avoid that further tests need to be requested as a follow up to this decision.

The concentration range and spacing must cover effects as seen in the available short-term test (Schreurs et al., 2004) and also to include effects seen on malformations of tail fin and body at higher concentrations (Croudace, 1997, and Hooftmann and Borst, 1999).

- Route of exposure

The Substance is moderately soluble in water (1.25 mg/L). Therefore, the exposure must take place as described in OECD TG 234 via testing water. Use of a solvent must be avoided.

- Parameters to be measured

To fully take advantage of the fish test, the following parameters must be measured in addition to the parameters described in the OECD TG 234:

i. Histopathology of gonads (gonad staging) must be conducted in order to examine endocrine related effects on development of gonads. By evaluation and staging of oocytes and spermatogenic cells, effects on e.g. delay of development can be seen.

ii. Histopathology of the liver must be included to detect effects on hormone levels and synthesis based on specific target organ toxicity of the Substance. This information is necessary to distinguish primarily endocrine-mediated effects from secondary effects, resulting from the liver toxic effects due to other potential MoA.

- 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 for Endocrine disruption for the environment for the Substance.

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

The request is:

- Appropriate, because the FSDT (OECD TG 234, at the level 4 of the OECD Conceptual framework (CF) (OECD 2018)) is validated for examining the oestrogen/antioestrogen, androgen/antiandrogen and steroidogenic modalities. Therefore, the FSDT is appropriate to examine indicative and adverse endocrine effects.
- The least onerous measure; indeed another way to examine indicative and adverse endocrine effects would be to request an amphibian test, AMA (Amphibian Metamorphosis Assay, OECD TG 231) or LAGDA (Larval Amphibian Growth and Development Assay, OECD TG 241), for investigation of the thyroid properties and the FSDT for the EAS properties in parallel. However, the two tests require a higher number of animals to be tested. Therefore, it was decided to first request the FSDT alone, which may already be sufficient to conclude on the ED properties of the Substance for the environment. The available mechanistic data point to HPG and HPT axis mediated effects which can be covered by the test as specified above.
- Proportionate, because another potential request instead of the FSDT would be the MEOGRT (Medaka Extended One Generation Reproduction Test, OECD TG 240). However, conducting a MEOGRT would mean a higher economical effort. Additionally testing at the level 5 of the OECD CF seems disproportionate at this stage of evaluation. As it is presumably possible to examine the expected results using the FSDT, the request of an MEOGRT would be disproportionate.
- Adequate, because requesting a Fish Short Term Reproduction Assay (OECD TG 229), i.e. a screening study (level 3 of CF) would not allow to examine further the effects seen in the available tests. There are already enough mechanistic data

available to directly proceed with a Level 4 test (FSDT) according to the OECD CF to conclude on the endocrine disrupting properties of the Substance.

- Necessary, because further information requests at OECD CF Level 2 or 3 would be insufficient to further clarify the concern for the environment. Based on the available information, a test is necessary which is capable of showing a MoA, adverse effect and the plausible link between the two elements. This information would be necessary to conclude on the ED properties and the further regulatory risk management measure, e.g. SVHC identification. A tiered testing strategy is not sensible as there are in vitro and in vivo data available which show mechanistic effects, and there are indications from studies with mammals on EAS (e.g. increased AGI, diagnostic effects). Therefore, a level 4 test is needed that give information on indicative and adverse effects in this test.

In your comments to the draft decision you argued that the modified OECD TG 234 assay proposed by ECHA is not validated to detect antioestrogenic, antiandrogenic, and antisteroidogenic effects, see Table 1 of OECD TG 234. However, in the guideline for the FSDT (OECD TG 234), Article 11 states that: *"The in vivo FSDT is intended to detect chemicals with androgenic and oestrogenic properties as well as anti-androgenic, anti-oestrogenic and steroidogenesis inhibiting properties."* In the table 1 in the OECD TG 234 clear effects on sex ratio are specified for antiestrogenic, antiandrogenic, and antisteroidogenic chemicals. Hence, the request of the FSDT is reasonable. The validation for these endpoints you mentioned is also confirmed in the OECD Revised Guidance Document 150 (OECD, 2018). Hence, the OECD TG 234 assay requested in this decision is necessary to clarify the identified concern and will allow the assessment of the (anti)-oestrogenic, antiandrogenic or steroidogenic parameters.

In addition, although dealing with non-standard endpoints, the study protocol must follow the GLP criteria as far as possible, with regards to documentation and reporting criteria. Missing historical control data with respect to specific endpoints does not constitute a basis for withdrawing the study as requested in this decision.

d) Consideration of time needed to perform the requested studies

In your comments to the draft decision, you stated that the timeline of 15 months is not possible, even for the standard FSDT assay. You requested another extension of the deadline of 4 months during the commenting of a proposal for amendment.

ECHA has reviewed the information you provided as a justification and extended the original timeline by 7 months and set the deadline to 22 months.

Appendix B - References relevant to the request (which are not included in the registration dossier)

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Appendix C - 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 dossiers.

12-month evaluation

Due to initial grounds of concern for Endocrine disruption, the Member State Committee agreed to include the Substance (EC numbers 216-133-4 and 244-240-6, CAS RN 1506-02-1 and 21145-77-7) in the Community rolling action plan (CoRAP) to be evaluated in 2020. Germany is the competent authority ('the evaluating MSCA') appointed to carry out the evaluation.

In accordance with Article 45(4) of REACH, the evaluating MSCA 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 evaluating MSCA 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 11 March 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. For the purpose of the decision-making, this decision does not take into account any updates of your registration dossier after the end of the 12-month evaluation period.

(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).

(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 and B).

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

ECHA invited you to comment on the proposed amendment(s). You provided comments on the draft decision. Your comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 52(2) and Article 51(5).

In your comments during the proposal for amendment consultation, you mentioned a new *in vivo* study in fish. The eMSCA assessed this study and reflected its assessment in the

draft decision.

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-76 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 D - 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 and other parameters relevant for the property to be tested, in this case endocrine disruption.

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