Helsinki, 13 February 2024

Addressees
Registrant(s) of Substance 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylindeno[5,6-c]pyran
EC number: 214-946-9, CAS number: 1222-05-5

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

A.1 The Larval Amphibian Growth and Development Assay (LAGDA, test method: OECD TG 241, 2015) with the Substance and the following specifications (see also Appendix A):

- A dose range-finding test must be conducted to determine the appropriate test concentrations and the optimal experimental set-up considering the properties of the Substance;
- At least four test concentrations must be used and the number of replicates must be doubled (8 replicates) for controls compared to each test concentration (4 replicates);
- The test must be performed under flow-through exposure conditions;
- Concentration of the Substance must be monitored at least twice a week, for at least one replicate in each treatment group rotating between replicates of the same treatment group. It must be demonstrated that the concentration of the Substance is stable throughout the test (within 80-120% of nominal concentrations);
- Histopathology of the thyroid gland must be performed also at test termination;
- Histopathology of gonad glands at test termination must include gonad staging;
- Measurements of thyroid hormones TSH, free T3, total T3, free T4 and total T4 in the plasma must be performed at NF62 stage and time to reach this stage must be accurately reported;
- Measurements in the plasma of Vitellogenin (VTG) (liver), 17-beta oestradiol (E2) and Dihydrotestosterone (DHT) must be performed at test termination.

Deadlines

The information must be submitted by **18 August 2026**.

Conditions to comply with the information requested

To comply with this decision, you must submit the information in an updated registration dossier, by the deadlines 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/ies 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.

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

1. Potential risk

1.1 Potential hazard of the Substance

Following its assessment of the available relevant information on the Substance, the evaluating MSCA has identified the following potential hazard which must be clarified: Potential endocrine disruptor properties.

a) Potential endocrine disrupting properties

According to IPCS/WHO (2002) "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations". This definition has been now added to the amended CLP Regulation (Commission Delegated Regulation (EU) 2023/707 of 19 December 2022).

Based on this definition, the substance is an endocrine disruptor (ED) if the following conditions are met:

- it shows adverse effects(s) in (an intact) organism, or its progeny, or (sub)population;
- it shows endocrine activity, i.e. it has the potential to alter the function(s) of the endocrine system; and
- there is a biologically plausible link between the adverse effects and the endocrine activity, i.e. the Substance has an endocrine disrupting mode of action (ED MoA)

Information from registration dossier as well as publicly available data from *in vitro* and *in vivo* tests raise a concern for endocrine effects in fish, amphibian and mammals mediated via the HPG (Hypothalamic–pituitary–gonadal) and HPT (Hypothalamic–pituitary–thyroid) axes.

Evidence of endocrine activity of the Substance based on in vitro studies (OECD CF Level 2[2])

- (anti)estrogenic activity:

Six *in vitro* studies have conducted *in vitro* tests using various human cell lines (HEK293, HEK237, U2OS, HepG2, MCF-7, HELN and MELN), transfected or not with human ESR1 (hERα) or ESR2 (hERß), to evaluate the effect of the Substance on the activation of these receptors (Seinen et al. 1999, Schreurs et al. 2002, Schreurs et al. 2004, Gomez et al. 2005, Cavanagh et al. 2018, Schreurs et al. 2005). All these studies consistently show:

- a marginal agonist effect towards hERα (LOEC ~ 10 µM),
- no detectable agonist effect towards hERß,
- an ability to antagonize the effect of estrogens on both hERα and hERß (LOEC from 0.1 to 1 µM).

Using MCF7 expressing hERα, Bitsch et al. (2002) did not observed a proliferative effect of 10 µM, while Evans et al. (2012) observed a weak estrogenic activity (EC10 ~ 4 µM).

[2] OECD Conceptual Framework on Endocrine Disrupters Testing and Assessment. Presented in OECD (2018), Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, OECD Publishing, Paris. Published on 3 September 2018 https://doi.org/10.1787/9789264304741-en

Using HEK293 cells transiently transfected (TT) with zebrafish ER (zfER) α, ß1 or ß2, Schreurs et al. (2004) showed that the Substance lacked agonist activity toward zfERα but induced an anti-estrogenic activity on zfERß1 and zfERß2 transcriptional activity.

- (anti)androgen activity:

An antagonist effect on the activation of human androgen receptor (hAR) was observed for high concentrations of the Substance (IC 50 = 1 µM) by using stably transfected U2OS cells (Schreurs et al. 2005). A significant antagonist effect on R1881-activated hAR was also observed in PALM cells (IC25 = 5.15 µM) (Cavanagh et al. 2018). An antagonist effect was also identified on dihydrotestosterone-activated hAR in MDA-kb2 cells (IC50 = 11.5 µM) (Kortenkamp et al. (2014)). In the same way, Mori et al. (2007) observed an AR antagonist activity by using transfected Chinese hamster ovary cells.

- Thyroid activity:

Two studies investigated the activity of the Substance on thyroid receptors. No agonist activity was detected on TRα or TRß in the stably transfected Chinese hamster ovary cells (Mori et al. 2007) and in the T4-TTR assay (Cavanagh et al. 2018).

- Steroidogenesis:

In an adrenal human cell line, the addition of 2.5 or 25 µM of the Substance in the culture medium decreases the amount of hormonal secretions and differencially modulates the mRNA expression of various steroidogenic enzymes (Li et al. 2013). In another study, using subcellular fractions from the gonads of Carp (*Cyprinus carpio*), the Substance decreased the activities of various steroidogenic enzymes (IC50 equal to 68 to 1000 µM) (Schnell et al. 2009).

- Other *in vitro* mechanistic pathways:

In human osteoblastic U2OS cell line, an antagonist activity of the Substance was observed on hPR (Progesteron receptor)(Schreurs et al. 2005).

Evidence of endocrine activity and adversity of the Substance based on in vivo studies in vertebrates

- In vivo test in fish (OECD CF Level 3)

E modality

Schreurs et al. (2004) conducted a test with 4- to 5-week old transgenic zebrafish transfected with an ERE-LUC plasmid. At the end of the 96h-exposure period, the results showed a consistent antagonistic activity of the Substance *in vivo*. Luciferase activity was reduced to 70% and 20% of the E2 positive control at 0.1 and 1 µM, respectively. This study indicates an estrogenic antagonistic activity of the Substance in juvenile transgenic zebrafish and supports the anti-estrogenic activity observed *in vitro* on zebrafish ERβs transactivation in the same study.

In your comments to the draft decision, you note that the effects observed in Schreurs et al. (2004) at 1 µM (258 µg/L) are above the NOEC for mortality of 68 µg/L reported in [redacted] 1997. Therefore, you consider that the effets can be linked to overall toxicity and not specifically to an endocrine disruption effect.

ECHA notes that the NOEC for mortality of 68 µg/L (mean measured concentration) was calculated from a 36-day early-life-stage test (OECD TG 210) on *Pimephales promelas* while Schreurs et al. (2004) conducted an acute test with 4 to 5-week old fish, i.e. much older fish than in the OECD TG 210. Thus, the sensitivity in terms of toxicity are not expected to be the same in both tests that cannot be compared. In addition, only nominal concentrations are reported in Schreurs et al. (2004) and considering the properties of the Substance (volatility and adsorption), it is likely that the actual exposure concentrations were lower. Despite the uncertainties about the actual exposure concentrations, the results of Schreurs et al. (2004) provide indications of anti-estrogenic MoA even at very low concentrations of the Substance.

Yamauchi et al. (2008) assessed the estrogenic effects of the Substance in 4 month-old adult male medaka (*Oryzias latipes*) by measuring both the vitellogenin (VTG) expression by ELISA and the transcription level of selected genes by qPCR in the liver. The medium was renewed daily during the 72h of exposure. The results showed an increase in VTG protein levels in the fish exposed to 500 µg/L, corresponding to 14% of the E2-induced level (1 nM). This induction was also observed at the transcriptional level with an increase in vgt I (at 500 µg/L) and vtg II mRNA (from 50 µg/L). Out of the genes investigated in the liver, only ERα transcription was induced at 500 µg/L. Changes in ERβ, PXR, cyp3a transcript levels were not significant. Although the measured concentrations decreased during the overall exposure period, the results of the study indicate an estrogenic activity of the Substance in male medaka.

In your comments to the draft decision, you disagree with the conclusion drawn from the study of Yamauchi et al. (2008). You consider that:

(i) the effect at 500 µg/L (nominal) can be regarded as toxicity-related effects and are not specific to endocrine disruption because the concentration is near to the median LC50 of 950 µg/L calculated by the authors.

ECHA notes that the LC50 of 950 µg/L was measured in 24h-old larvae while the estrogenic effects were investigated in 4-month old adult medaka. Thus, the sensitivity in terms of toxicity are not expected to be the same in both experiments. Furthermore, the authors indicate that the concentrations used in the main experiments were based on the results from the 96-h acute toxicity, i.e. the concentration of 500 ug/L corresponds to half of that of the LC50. As expected, no overt signs of toxicity are reported in the experiment at 500 µg/L. Thus, ECHA considers that the effet observed in the study can be related to endocrine disruption.

(ii) the ca. 50-fold increase in vtg II gene expression at 50 µg/L is an incidental finding because no effects are reported for hepatic vtg concentration and mRNA expression levels for ERα, ERβ, and VTG I at that concentration.

ECHA agrees that at 50 µg/L (nominal), only vtg II was significantly up-regulated. However, ECHA is of the view that vtg II up-regulation may indicate that early changes on estrogen-related genes start to operate. Indeed, at 500 µg/L (nominal), the increase of hepatic vtg level fits with the statistically significant up-regulation of ERα, vtg I and vtg II gene expression level, although not ERβ. Despite some uncertainties related the actual exposure concentrations, this study provides the evidence that the Substance is potentially estrogenic in fish.

- *In vivo test in fish (OECD CF Level 3)*

T modality

Chae et al. (2023) studied the thyroid disruption and neurotoxicity potential of the Substance in early-life stage of zebrafish (*Danio rerio*). At the end of the 5-day exposure period, a significant decrease in T4 hormone level at all the concentrations tested was observed (measured concentrations from 0.13 to 3.10 µg/L). Furthermore, significant changes in the expression of thyroid hormone related genes were observed. Indeed, *crhβ* gene expression showed a significant up-regulating trend while *tshβ*, *dio1*, and *ttr* gene expressions were significant down-regulated. Regarding neurodevelopment-related genes, *mbp* and *gap43* expression was significantly down-regulated, suggesting potential adverse effects in neuronal development in fish larvae. Regarding the behavioral assessment of fish by light stimulation, a significant decreasing trend on the total distance moved was observed, suggesting a potential hypoactivity to the fish larvae following exposure to the Substance. No effets on thigmotaxis were observed. Although the measured concentrations were much lower than the nominal concentrations, the results of this study reveal that the Substance can disrupt thyroid hormone synthesis and can exert effects on the neurodevelopment of zebrafish in early life stage.

In your comments to the draft decision, you argue that:

(i) the decrease of T4 hormone level at all tested concentrations did not show any dose-dependent relationship indicating an abnormal high control T4 value.

T4 hormone levels in controls can vary between experiments, they will depend of experimental conditions and the stage of the organisms, which emphasize the importance to use as reference the concurrent controls of the study. In addition, ECHA notes that three differents experiments with differents musks under similar conditions were reported in the publication, the controls levels were similar in the three experiments. In ECHA's views there is no justification to consider as abnormal the T4 levels measured in the control. Moreover, ECHA agrees that a dose-response relationship was not observed. The text of the decision was modified accordingly. However, the absence of dose-response relationship does not dismiss the observed significant decrease of T4 hormone levels at all tested concentrations. Furthermore, ECHA considers that the effects reported in this study occur at very low concentrations and after short term exposure, thus, there is a strong concern for thyroid disruption after longer term exposure. According to the ECHA/EFSA guidance (2018), hormonal changes like reduction in T4 levels should be a trigger for further studies.

(ii) none of the expressed genes demonstrated a dose-dependent relationship (a significant down regulation of UGT1AB, TTR and KLF9 gene expression was not observed for all the concentrations tested while no modulation was observed on the other 6 genes: tshβ, nis, tg, dio1, dio and sult1st5), therefore they are most likely incidental findings

ECHA notes that the significant decrease ($p < 0.05$ according to Dunnet test) of T4 in all the concentrations tested fit with the statistically significant ($p < 0.05$ according to Dunnet test) up regulation of crhβ at all concentrations with the exception of the test concentration of 0.34 µg/L (increase not statistically significant). This finding can be interpreted as compensatory efforts in reaction to reduced T4 concentrations in the larval fish and further support that the observed significant decrease in T4 is not incidental. The overall genes investigated in the study may involve different molecular processes related to the thyroid hormone regulation. Absence of effects on some of these genes resulting in uncertainties on the exact mechanism but does not exclude the relevance of the findings. In addition, ECHA clarifies that according to the results of the study, a dose-dependent down-regulation of *tshβ* and *dio1* genes (p for trend <0.05) was observed. These findings together with the significant up regulation of crhβ support the observation on T4 levels.

(iii) The effect on behaviour is an incidental finding because there was no dose-dependance demonstrated and none of the related neuronal genes showed a significant change in mRNA expression

In ECHA's view there is not justification to consider the changes on behaviour as incidental findings as a statistically significant effect compared to the control was observed at 0.96 µg/L and a significant decrease trend (p for trend <0.05) of the total distance moved was determinated. ECHA agrees that no significant changes on neuronal-related genes expressions compared to the control were reported in the study. However, a significant down-regulation trend (p for trend <0.05) was observed for mbp, gap43, and syn2a genes.. As the effect reported in this study occur at very low concentrations and after short-term exposure, uncertainties remain about the potential effects after longer-term exposure.

- *In vivo* test in amphibian (OECD CF Level 3)

T modality

Histological alteration on thyroid gland of *Xenopus laevis,* after dietary exposition to HHCB is reported in the study of Pablos et al. (2016). In this study, the authors used a protocol adapted from the OECD TG 231. Premetamorphic tadpoles were exposed to food, spiked with concentrations of the Substance: 0.05 mg/kg; 0.5 mg/kg; 5 mg/kg and 50 mg/kg, until completing metamorphosis (NF 66). A transient developmental acceleration for the group exposed at 50 mg/kg at day 14 was observed, which was not the case at day 23. Histological parameters of the thyroid gland were investigated at day 23 (tadpoles) and at the end of metamorphosis. At both stages, thinner follicle cell epithelia were seen for the 5 and 50 mg/kg exposed groups and papillary projections were reported at the day 23 for the two highest doses. In the control group, the authors also noted hyperplasia and cellular hypertrophy in less 20% of follicles. No statistical analysis of histopathology and no thyroid hormone measurements were conducted in the study. Furthermore, some shortcomings are noted in the study such as the lack of information about the internal Substance measurements done in total froglets and the lack of explanation about the Substance levels found in the control froglets. Although this study does not allow to conclude on the ED properties of the Substance, the results provide indications of a thyroid-related effect on *Xenopus laevis* that needs further clarification.

In your comments to the draft decision, you disagree that the study of Pablos et al. (2016) demonstrates an *in vivo* effect of concern on thyroid related modality on *Xenopus laevis*. You argue that:

(i) the histopathological effects observed at 50 mg/kg HHCB occurred in presence of too high toxicity as supported by the mortality rate of 10.66 ± 6.1%

(ii) the histopathological changes observed at 5 mg/kg in 33.4% of the animals are only mild. According to OECD TG 231, for a result to be considered positive, the effect on the thyroid should be remarkable, but mild changes cannot be considered as 'remarkable' changes. You conclude that this study should therefore be considered as negative.

ECHA notes that the study does not provide raw data on survival of tapdoles per replicate. However, it is indicated that the mortality rate generally did not exceed 10% in any replicates in the controls and in the treatment groups. Only the group treated with the highest dose (50 mg HHCB/kg food) showed a mortality rate that slightly exceeded 10%

(10.66±6.1%) and none of the replicates were compromised Thus, it is not possible to attribute the effects of the highest dose on thyroid gland to a general toxicity of the Substance. Moreover, ECHA remarks that histological changes were already observed at 5 mg/kg HHCB. In that respect, the OECD TG 231 indicates that advanced development or remarkable histological effects are indications that the chemical can be considered to be "thyroid active". Considering that histological changes were observed at the two highest doses with progressive intensity together with the fact that a transient acceleration of development was observed at day 14 at the highest dose, ECHA considers that the study provides relevant *in vivo* mechanistic indications for potential thyroid effects.

(iii) a robust fish early life stage study similar to OECD TG 210 ([redacted] 1997) showed no effect on hatching at 140 µg/L

The fish early life stage toxicity test (OECD TG 210) is designed to define lethal and sub-lethal effects of chemicals on fish early life stage. According to the ECHA/EFSA guidance (2018), the test does not measure 'EATS-mediated' parameters. Parameters as hatchability and development are considered as 'sensitive to, but not diagnostic of, EATS'. The ECHA/EFSA guidance also indicates that there is limited evidence to suggest that some thyroid endocrine disruptors are able to interfere with the metamorphosis of fish embryo to the larvae. Thus, in ECHA's view, the results of the OECD TG 210 ([redacted] 1997) cannot demonstrate that a T-mediated adverse effect does not occur and cannot dismiss results observed in Pablos et al. (2016).

(iv) Overall, you consider that the studies in the assessment did not provide clear *in vivo* mechanistic information as well as any *in vivo* effects and that it cannot be concluded that HHCB can disrupt thyroid hormone synthesis in fish or in amphibians.

Based on what has been described above, ECHA considers that data on fish and amphibian together with the findings on mammals (see below) provide evidence that the Substance is active on the HPT axis.

- *In vivo* test in mammals (OECD CF Level 3 to 5)

Mammals are part of environmental organisms and their endocrine system, in particular the HPG- and HPT-axes, is highly conserved within vertebrates. Therefore, the available mammalian data can be used to support the analysis of ED properties in the environment.

EAS modalities

A weak estrogenic activity was observed in an uterotrophic assay (OECD CF level 3) after oral exposition of 0.6 and 40 mg /kg /bw with a non-significant but dose-related increase of the relative uterus weight, associated with a significant increase of the liver weight at the highest dose (Seinen et al. 1999).

In your comments to the draft decision, you suggest that the relative increase in uterine weight can be considered an incidental finding for the following reasons:

(i) It is difficult to draw conclusion with certainty about dose-related effect with only two data points;

ECHA agrees with this observation as none of the results were statistically significant. However, the uterus weight increased with the doses (0.63, 0,71 at 50 and 300 ppm compared to the control 0.56 g) and at the high dose the result tended towards the positive

control, which in itself is significant (0.90 g, * P<0.05). An increase of 30% in uterine weight at the highest dose led to a warning about the estrogenic modality.

(ii) There is no evidence that the increase in the relative uterus weight is outside the historical control data range;

ECHA reminds that the study dates back to 1999. At that time, historical controls were not a requirement and were not provided.

(iii) the results of the uterotrophic assay could not be reproduced in the OECD TG 443 study.

The uterotrophic assay is intended to allow the detection of weak and strong estrogens as well as anti-estrogens. An effect consistent with a weak estrogenic activity was observed in the study (Seinen et al. 1999). ECHA considers, that comparison of this study with the OECD TG 443 is difficult, since different species, animals with different sexual maturation (immature rat in the uterotrophic assay) and different exposure times were used. The exposure time in study OECD TG 443 is much longer and other interactions/compensation mechanisms may take place. Therefore, in ECHA's view, the absence of effect on the uterus in the OECD TG 443 study cannot dismiss that the effect observed in the uterotrophic assay is an indication of a estrogenic activity.

Indications of adverse effets related to EAS (Estrogenic, Androgenic, Steroidogenesis) modalities has been observed in an Extended One Generation Reproductive Toxicity Study (OECD TG 443, unpublished study report, 2021) available in the registration dossier (OECD CF level 5). A dose-related decrease in the relative anogenital distance in F2 pups was observed from the mid-dose (75 and 150 mg/kg bw/day) in males and females, respectively. Moreover, in males in cohort 1B (parental animals of the F2 pups), there were also statistically significant increases in relative prostate weight and in the seminal vesicles weight at the top dose (150 mg/kg bw/day) and a statistically significant increase in relative testis weight at the highest doses (75 and 150 mg/kg bw/day).

A proposal for amendment (PfA) was submitted by a MSCA to add that *in addition it is acknowledged that it is more relevant to analyze testis and epididymis weights as absolute weights since these organs are less dependent on body weight than other organs. The absolute testis weights were statistically significantly reduced in top dose (150 mg/kg bw/day) males both in cohort 1A and cohort 1B, and absolute epididymis weights were statistically significantly and dose-dependently reduced in all dose groups in cohort 1A and 1B males (up to 13-14% at top dose). In the top dose F0 males absolute epididymis weights were also statistically significantly reduced (ca. 7%) compared to control males.*

The study provides indications that the Substance could have anti-androgenic and/or estrogenic activity.

In your comments to the draft decision, you mention that:

(i) the decrease in AGD in the OECD TG 443 was considered as an incidental finding in the report as values from the mid- and high-dose were within the historical control data range, similar effects could not be found in the F1 pups and the control values in this study were high compared to the historical control data.

ECHA highlights that the results in F2 pups show a dose-related decrease in the anogenital distance compared to the concurrent control. In addition the eMSCA has clarified that the differences with controls were statistically significant for the two highest doses (75 and

150 mg/kg bw/day). In addition, the absence of effects in F1 pups may result from the difference in exposure patterns between parents of F1 and F2 as F0 were not exposed in utero in contrast to F1. Moreover, the historical controls allow to verify that the measurement is more or less in the same range as previous studies. However, they cannot be used as a reference to refute a result that is dose-dependent and significant at the two highest doses. Therefore, the presented (limited) historical data should not be used to overrule the specific concurrent negative control data to discard the effects seen the study.

(ii) although Cohort 1A is similarly exposed compared to Cohort 1B, no effect on prostate weightis observed in Cohort 1A and no significant effect on seminal vesicle weight in 1B, so it is not a reproducible effect. Furthermore, the increased relative prostate weight did not have histopathologic correlation and was not dose-related.

ECHA notes that the duration of exposure in the OECD TG 443 study is different between the two cohorts (13 weeks exposure for Cohort 1A and 19 weeks exposure for cohort 1B). In ECHA's view this could explain why the effects are more visible in cohort 1B than cohort 1A. For prostate, there is a statistically significant increase in relative weight at the highest dose in cohort 1B (whereas no change in cohort 1A at the same dose). For seminal vesicles, although not statistically significant, the increase in relative seminal vesicle weight reached 10% in cohort 1B and is noticeable. In addition, the statistically significant increase in the relative weight of prostate and non-statistically significant increased relative seminal vesicle weights cannot have any histopathologic correlation as no histopathological was performed in cohort 1B. Moreover, although not statically significant, the increase in the relative seminal vesicle weight reached 10%.

(iii) absolute testis weights were decreased while relative testis weights were increased and these effects are due to restriction in food consumption as shown in an article from Carney et al., 2004.

ECHA notes that in the article of Carney et al., 2004, the observed effects in organ weights were observed from 30% of feed restriction in dams during *in utero* and postnatal development in rats which corresponds to the gestation period until PND22. In the OECD TG 443 study, there were no decrease in food consumption in females in F0-generation to explain the effects observed in males in F1-generation. There is no significant effect in food consumption in males in F1-generations at PND22 (+0.85%, -3.6% and -0.71% at low, mid and high doses, respectively, at PND22). Therefore, in ECHA's view, the effects observed on testis weight cannot be attributed to reduction in food consumption.

T modality

In relation to thyroid pathway, effects were also observed in the OECD TG 443 study (unpublished study report, 2021). Exposure to the Substance was associated with significant increases in thyroid weight in the parental generation (P0) and the F1 generation for both sexes. Hypertrophy of the thyroid gland was also observed in the parental generation and F1 generation for both sexes at the mid and top doses (75 and 150 mg/kg bw/day). Additionally, statistically significant decreases in the level of T4 were reported in P0 and F1 males. A significant increase in TSH was observed in F1 females at the top dose.

In the registration dossier, an increased thyroid weight was observed in males in the repeated dose 90-day study (OECD TG 408, OECD CF level 4, 1996) in absence of histological changes. In the dose-range finding study (based on OECD TG 421, OECD CF level 4) for the EOGRTS (unpublished study report, 2021), an increased thyroid weight

was also observed in males and females (histology not performed). Hormone measurements were not performed in either of these studies.

In your comments to the draft decision, you postulate that these findings do not indicate an ED effect but are a result of the increase in liver weight due to induction and activation of hepatic metabolic enzymes needed for the metabolism of the Substance. This increase in hepatic metabolism causes breakdown of T4 thyroid hormone and triggers the pituitary gland to induce thyroid stimulating hormone (TSH) levels, which results in cellular hypertrophy and higher thyroid weight to compensate.

ECHA notes that in the OECD 443, significant thyroid effects (increased organ weight, histopathological and hormones changes) were observed for males in F1-generation at all doses while liver effects were observed only at the top dose (increase relative liver weight and histopathological changes). For females in F1-generation, no effect on liver were observed but there were significant thyroid effects (organ weight and histopathological changes) at the top dose. Therefore, based on the available data, a temporal relationship cannot be demonstrated to support your claim that the toxicity observed in the thyroid is secondary to the liver enzyme induction and activation. Moreover, in the absence of substance-specific data which provide proof of the contrary, humans and rodents are considered to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance) (ECHA/EFSA, 2018).
In ECHA's view the findings on the thyroid are considered treatment-related and not consecutive to an unspecific secondary effect.

Conclusion on potential endocrine disruptor properties

In summary, the available data raise a concern for endocrine effects on vertebrates mediated via the HPG and/or HPT axes.

Antagonist effects of the Substance on the activation of ER and AR receptors as well as marginal estrogenic activity, alteration of the activity of steroidogenic enzymes and antagonist activity on the progesterone receptor have been identified in several OECD CF level 2 *in vitro* studies. They are supported by OECD CF level 3 mechanistic *in vivo* studies with fish that confirmed that the Substance can have an antagonist activity towards ER (Schreurs, 2004) but can also induce an estrogenic activity (Yamauchi et al., 2008). In an EOGRTS study, significant reduction of the anogenital distance and decreased weigth in some male reproductive organs have been evidenced in rodents. These effects could be related to E, A or S modalities. However, the available data do not provide information on adverse effects that may result from an (anti)estrogenic or (anti)androgenic activity in fish or amphibian and are not sufficient to conclude on the ED potential for the environment for EAS modalities.

Regarding T modality, there are indications that the Substance is active on the HPT axis due to findings in mammals (effects on thyroid weight, changes in thyroid histology and decrease levels of T4 in rats), in fish exposed during early life (decreased level of T4 and changes in thyroid related genes) and in amphibian (histological changes of the thyroid gland). However, none of the available studies provides information on endocrine activity and apical adverse effects specific to the T-modality that are sufficient to establish a plausible link.

The available information is not sufficient to draw a final conclusion on the potential hazard for the environment, i.e. to assess whether the Substance meets the criteria that define

an endocrine disruptor for the environment. Therefore, further data which examines EAS and T modalities on vertebrates is needed.

In your comments to the draft decision, you claim that based on the available information on fish and amphibians, it can be concluded that the Substance is not an endocrine disruptor for the environment. You argue that the *in vivo* results for E, A, S and T modalities demonstrate no *in vivo* effect of concern, as well as no adverse effect at the organism level. You further explaine that ECHA/EFSA guidance (section 3.5.1, figure 6) indicates that OECD CF Level 3 studies are considered as intermediate events and according to the results of the studies used in this assessment, you considered that there are no intermediate events for E, S and T. You support your conclusion by the lack of adverse effects in the fish early life stage study (OECD TG 210) performed by [redacted] (1997). In addition, you consider that the results of the OECD TG 443 test do not support the need for further evaluation regarding ED properties in the environment.

ECHA notes that all the studies were used in a WoE approach. As explained and clarified in the sections above, the studies on fish and amphibians provide *in vivo* mechanistic information. Furthermore, because of the high level of conservation of the endocrine system across taxonomic groups, the mammalian data may also be relevant for other vertebrates (ECHA/EFSA, 2018). The information on fish and amphibians together with the findings on rodents give sufficient indications that the substance may act via one or more modalities (EAS and T) to establish a concern that requires clarification. The lack of adverse effects in the OECD TG 210 is not an indication that potential endocrine effects cannot occur because the test does not have endpoints that specifically respond to endocrine disruptor chemicals.

1.2 Potential exposure

According to the information you submitted in all registration dossiers, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of [redacted] per year.

Furthermore, you reported that among other uses, the Substance is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing and at industrial sites.

The Substance is used in the following products: washing & cleaning products, biocides (e.g. disinfectants, pest control products), air care products, polishes and waxes, perfumes and fragrances and cosmetics and personal care products.

The Substance can be released to the environment from industrial and professional facilities using the Substance and from consumer uses leading to emissions to municipal waste water treatment plants as well as by direct emissions.

The Registration dossiers provide exposure scenarios and estimations of exposure for consumers, workers and for the environment. Based on this information, exposure to general population and the environment exists.

It is further supported by monitoring data. Indeed, the Substance has been frequently detected in surface waters, sediments, as well as sludge and effluents from the wastewater treatment plants (Norman database, Lange et al., 2015, Zeng et al., 2005, Sumner et al., 2010). The Norman database reports maximum concentrations of the Substance across Europe in freshwater (32 µg/L), marine water (0.02 µg/L),

freshwater sediments (240 µg/Kg) and groundwater (0.184 µg/L). Measured concentrations of the Substance have been also reported in urban, rural and indoor air (Peck et al., 2006, Sofuoglu et al., 2010)

The substance has been also detected and measured in groundwater, in biota, including Apex predators, and in humans tissues (breast milk,maternal blood and umbilical cord):

- Data from the EU LIFE APEX Project shows both high frequency of detection and high concentrations of the Substance in top predators (otter, seals, buzzards and fish across the EU);
- The Substance has been detected and quantified in marine mammals (Nakata, 2005, Kannan et al. 2005, Nakata et al. 2007, Moon et al. 2012) such as finless porpoises, stripped dolphin, grey seal, pymy sperm whale, mink whales, etc. Besides, Nakata, 2005 revealed a transplacental transfer of the Substance to the fetus during pregnancy of finless porpoise, both mother and fetus contained levels of the Substance;
- The Substance has been also detected and quantified in breast milk, maternal blood and umbilical cord blood samples collected from women living in Korea, China, USA, Switzerland, Sweden, Denmark (Duedahl-Olesen et al., 2005, Lignell et al., 2008, Lee et al. 2015, Zhou et al., 2012, Kang et al., 2010, Zhang et al., 2015, Schlumpf et al., 2010)

Therefore, exposure to general population and the environment exists.

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 and information from published literature demonstrate an exposure of the general population and the environment.

Based on this 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 potential hazard and in particular on endocrine disruptor properties. Consequently further data is needed to clarify the potential risk related to endocrine disrupting properties.

1.4 Further risk management measures

If the properties of the Substance are confirmed, the evaluating MSCA will analyse the options to manage the risk. New regulatory risk management measures could be:

- Identification as substance of very high concern (SVHC) for ED properties under Article 57f of REACH;
- a subsequent authorisation or a restrictions of the use of the substance for ED properties. This would result in stricter risk management measures, such as minimisation of emissions.
- ECHA notes that a new Commission Regulation has just been adopted that introduces

a new hazard class for endocrine disruptors to the CLP Regulation 1272/2008. Harmonised classification as an endocrine disruptor to the environment and to human health is therefore an additional regulatory risk management measure which could be taken for the Substance.

2. How to clarify the potential risk

2.1 Development of the testing strategy

Based on the data described in section 1.1, a concern for ED properties relevant for human health and for the environment is identified.

This decision is focused on clarifying the potential risk related to Endocrine Disruption properties of the Substance for the environment. The data available raise a concern for endocrine effects in vertebrates mediated via the HPG and/or HPT axis but are not sufficient to conclude. A test is therefore required to investigate ED properties related to both HPG and HPT axes in vertebrates.

The potential ED properties for human health may be addressed at a later stage.

2.2 Request A.1: The Larval Amphibian Growth and Development Assay (LAGDA)

a) Aim of the study

The requested OECD TG 241-Larval Amphibian Growth and Development Assay (LAGDA) will allow to evaluate the effects of the Substance on the endocrine system of amphibian species, covering multiple life-stages, beginning with early embryos and ending two months after completion of metamorphosis. The requested test is the only test that includes E, A and T-mediated parameters and parameters sensitive to S modality as summarized in table 16 of the ECHA/EFSA guidance (2018) and in table A.2 of the OECD guidance document 150 (OECD, 2018). Indeed, the test provides information on disruption of both the HPT axis at metamorphic climax (NF62) and of the HPG axis at test termination, when gonads are fully differentiated. Therefore, it is the only test that provide relevant information on both the thyroid and the EAS modes of action and informs about the population relevance of the effects (development, growth and reproductive development).

The OECD TG 241 is a Level 4 test according to the OECD Conceptual Framework on Endocrine Disrupters Testing and Assessment (OECD, 2018), i.e. an *in vivo* assay providing data on adverse effects on endocrine-relevant endpoints, in particular for the endpoints described above.

b) Specification of the requested study

The study must be performed according to the OECD TG 241 (OECD, 2015). All quality criteria must be respected.

The Substance is considered difficult to test because it can volatilise from water to air (Henry's Law constant of 11.4 Pa·m³/mol at 25°C) and has adsorptive properties (log K_{OC} 4.16). For difficult to test substances, you must consider the approach described in the OECD Guidance Document n°23 (OECD, 2019).

In order to optimise the test capacity as regard to the investigation of the potential endocrine disruptor properties of the Substance, the OECD TG 241 is requested with some additional specific technical requirements as well additional parameters to be measured

during the conduct of the test. They are described in the sections below.

Route of exposure

The Substance is moderately soluble in water (1.65 mg/L). Therefore, the exposure must take place as described in OECD TG 241 via testing in water. Use of a solvent must be avoided. The test must be performed under flow-through condition in order to maintain stable exposure concentration in the system.

Test material and concentration

The test material is the Substance. The test material must be representative for the registered Substance, in particular with respect to the concentrations of constituents and impurities, as specified in Appendix C, and with the highest purity in order to avoid confounding effects of impurity(ies).

At least four test concentrations must be used and the number of replicates must be doubled (8 replicates) for controls compared to each test concentration (4 replicates) in order to give adequate statistical power for the test. Generally, a concentration separation (spacing factor) not exceeding 3.2 is recommended.

Considering the properties of the Substance, the concentrations of the Substance are to be determined at appropriate intervals during the exposure period. The exposure concentrations of the Substance must be determined at least once a week for all replicates in each treatment group. As it is indicated in the OECD TG 241, it must be demonstrated that the concentration of the Substance is stable throughout the test (within 80-120% of nominal concentration).

Besides, the iodide content of water used in the study needs to be measured in order to comply with the iodide levels commonly found in freshwater system to ensure the quality and robustness of the assay (generally comprised between 0.004 - 0.158 µM). The iodine content and supplementation of the test water must be measured and reported to comply with the paragraph 17 of the OECD TG 241. Additionally, as indicated in paragraph 17 of the OECD TG 241, iodine content may also be measured in food in addition to test water as freshwater vertebrates cover their main iodine demand via the food.

Conduct of a dose range-finding test

As the Substance is volatile and adsorptive, a dose range finding test must be performed in order to determine the appropriate test concentrations and optimal experimental set-up to reduce technical challenges and increase the robustness and quality of the data obtained in the main study.

Results from existing studies must be considered in determining the highest test concentration so as to avoid concentrations that are overtly toxic and induce excessive mortality.

The dose range-finding test will not only help to decide the concentrations tested in the main study, but will also allow to adjust some experimental conditions, such as the optimal flow rates or any other parameter or condition that could compromise the quality and validity of the test. As in the main study, the dose-range finding study must be performed under flow-through condition. A flow rate of at least 5 tank turnovers per day must be applied in order to avoid chemical concentration declines due to metabolism by both the test organisms and aquatic microorganisms present in the aquaria or by dissipation

(volatilization, sorption) of the Substance. The dose range finding will help to determinate if the flow rate needs to be adapted in the main study.

Additional mandatory parameters to be measured

In order to conclude on the ED properties of the Substance as mentioned in section 1.1 (a) of the Appendix A, it is necessary to collect information on endocrine activity as well as on apical adverse effects to establish a biological plausible link between them.

Based on the available data detailed in Section 1.1. of the Appendix A, both HPG and HPT axes are under investigation for the Substance. Additional parameters related to endocrine activity and apical adverse effects for each modality (EAS and T) must be measured to clarify the concern and allow to confirm or dismiss the ED properties of the Substance for the environment.

Table 1 lists the parameters in the OECD TG 241 and the timepoint when they must be measured. The table also lists additional mandatory parameters that must be measured, as explained below:

- Histopathology of the thyroid gland at test termination;
- Histopathology of gonads gland at test termination must include gonad staging 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 sexual development can be detecte;
- Measurement of thyroid hormones TSH , free T3, total T3, free T4 and total T4 in plasma must be performed at the interim sampling NF62 stage (this stage corresponds to the metamorphosis climax during which T3 and T4 reach maximum levels);
- Measurements in plasma if needed in order to obtain a sufficient volume to perform the analysis (generally 200 µL of plasma).

As explained in Section 1.1. of the Appendix A, there are indications that the Substance is active on the HPT axis due to findings in mammals (effects on thyroid weight, changes in thyroid histology and decrease levels of T4 in rats), in fish exposed during early life (decreased level of T4 and changes in thyroid related genes) and in amphibian (histological changes of the thyroid gland). However, none of the available studies provides information on endocrine activity and apical adverse effects specific to the T-modality that are sufficient to establish a plausible link.

The EFSA/ECHA guideline No 1107/2009 recommends that changes in hormone levels should be evaluated in conjunction with any changes in thyroid gland weight and histopathology, as well as neurological or other developmental adverse effects.The measurements of these parameters are not included in the OECD TG 241. However, methods to measure thyroid hormones are easily adaptable from established assays (e.g. for human or rat hormones) and are increasingly performed in assessing the potential endocrine adverse effect of chemicals in literature and validated protocol and techniques are available (see Fini et al., 2017, Mughal et al., 2018, Spirhanzlova et al., 2019, Martínez-Guitarte et al., 2021). This information will allow to clarify the suspected thyroid mode of action which leads to potential adverse effets in the organisms.

The sexual steroids E2 and DHT are involved in the regulation of sexual differentiation, development, and maintenance of sexual functions by production spermatozoa and oocytes. E2 and DHT are traditionally associated to female and male reproductive development and function, respectively. As explained in the section 1.1, the Substance is shown to interact with EAS pathways in *in vitro* and *in* vivo studies. Thus, measurement of VTG, E2 and DHT concomitantly to E and A-mediated parameters (sex ratio, histopathology of gonads gland and reproductive ducts at the test termination) must be performed at test termination in order to investigate whether the potential adverse observed effects correlate with sexual steroid hormone disruption (see Table 1). Together, they are necessary to clarify the suspected (anti)estrogenic and/or (anti)androgenic and potential steroidogenesis mode of action of the Substance. The measurements of these parameters are not included in the OECD TG 241. However, methods to measure them are available in established assays e.g. for fish OECD TG 229, the validation LAGDA report (U.S EPA, 2013) as well as in the literature (see Martins et al. 2020).

To avoid bias, sampling for thyroid and sexual steroid hormones must be performed at the same time (e.g. same hours in the morning or in the evening) for all animals. If it cannot be done, the distribution of time collection must be evenly distributed across groups (not all individuals of one group sampled concomitantly and all individuals of another group at a later time point).

Table 1: Summary of mandatory parameters and sampling time requirements of the requested OECD TG 241

*Endpoints**	*Daily*	*Interim Sampling (Larval sampling NF62 stage)*	*Test Termination (Juvenile sampling)*
Mortality and abnormalities	X		
Time to NF stage 62		X	
Histo(patho)logy (thyroid gland)		X	X
Morphometrics (growth in weight and length)		X	X
Liver-somatic index (LSI)			X
Genetic/phenotypic sex ratios			X
Histopathology (gonads, reproductive ducts, kidney and liver)			X
Vitellogenin (VTG)			X
17 B estradiol (E2)			X
DHT (Dihydrotestosterone)			X
TSH		X	
Free T3		X	
Total T3		X	
Free T4		X	
Total T4		X	

* All endpoints are analyzed statistically

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, such as implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation.

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

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

The request for The Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241) is:

- Appropriate, because it will provide information which will clarify the ED concern for the environment. The OECD TG 241 is the only level 4 test of the OECD Conceptual framework (CF) (OECD 2018) that is validated for assessing E, A, S and T modalities in environmental species. A tiered testing strategy starting with a CF level 3 study is not appropriate as there are already *in vitro* and *in vivo* data available which show endocrine activity in vertebrates. Therefore, a CF level 4 test that provides information on apical/adverse effects is needed. With the addition of mechanistic parameters described above, the requested test covers in a single study parameters related to endocrine activity and an adverse effect and will contribute establishing a plausible link between the two elements. This will enable the evaluating MSCA to conclude on the ED properties of the Substance for the environment and to set appropriate regulatory risk management measures, e.g. SVHC identification if needed.

- The least onerous measure, because there is no equally suitable alternative method available to obtain the information that would allow to clarify E, A, S and T modalities in one test.

- Another option would be to request a fish sexual development test (FSDT, OECD TG 234, OECD, 2011) FSDT is a level 4 test of the OECD CF that assesses early life stage effects in fish and potential adverse consequences of endocrine-disrupting chemicals (e.g. estrogens, androgens and steroidogenesis inhibitors) on sexual development. However, it does not assess parameters related to thyroid disruption. If the results of the test do not allow to confirm the ED properties of the Substance based on EAS modalities investigated, another Level 4 test such as the OECD TG 241 would have to be requested to clarify the ED properties due to the remaining concern via thyroid modality. The available data indicates that the Substance is active on both HPG and HPT axis, both are investigated in the OECD TG 241. Conducting a tier approach may require double testing and a higher number of animals to be tested. Therefore, the tiered approach was not considered as the better option.

- Another option would be to request an Amphibian Metamorphosis Assay (AMA, OECD TG 231, OECD, 2009). AMA is a level 3 test of the OECD CF (i.e. screening test) that covers endpoints related to thyroid function and HPT axis (thyroid histopathology and time to metamorphosis/develomental phases). However, , the AMA test does not investigate endpoints related to EAS modalities such as ED effects mediated by the HPG axis and may not necessarily allow a definitive conclusion on T modality. Therefore, the OECD TG 241 is needed to generate these data and to definitively conclude on the ED hazard on the Substance for the environment.

In addition, as explained in the sections above, the Subtance is manufactured in and/or imported to the European Economic Area at high tonnage, exhibiting widespread uses. The Substance is considered as an ubiquitous contaminant, it has been frequently detected specially on surface waters, sediment, sludges, effluent as well as in biota including Apex predators. Wildlife, which may include vulnerable and/or endangered species are currently exposed to the Substance. Requesting an OECD TG 241, with the specifications described in section 2.2, is justified because it will save time to regulate the Substance if the ED properties are demonstrated by any of the modalities covered by the test.

In your comments to the draft decision, you note that the OECD TG 241 may not be relevant, because, according to the OECD guidance document 150 (p.60), there is a lack of knowledge about whether anti-estrogenic, anti-androgenic and steroidogenesis-related activities can be measured. Instead, you suggeste to request a Medaka Extended One-Generation Reproduction Test (MEOGRT, OECD TG 240) as this study covers anti-estrogenic, anti-androgenic, thyroid and steroidogenesis-related activities.

ECHA clarifies that the OECD guidance document 150 (OECD, 2018) further indicates that a LAGDA could be used when there are some data available about the possible thyroid disrupting properties of a chemical, or if the chemical is suspected of having (anti)estrogenic or (anti)androgenic properties (p.292, paragraph 426). The measurement of additional parameters requested in this decision (sex steroids E2 and DHT, VTG, gonadal histopathology, including gonadal staging together with the sex ratio determination) will enhance the capacity to detect the (anti)estrogenic and/or (anti)androgenic properties and potential steroidogenic mode of action of the Substance.

The Medaka Extended One-Generation Reproduction Test (MEOGRT, OECD TG 240) includes various EAS-mediated apical endpoints and mechanistics parameters, however, the thyroid endpoints are only sensitive to and not diagnostic of the thyroid modality, which makes their interpretation more difficult (e.g. compared with the LAGDA, no histopathology of the thyroid gland is performed in the MEOGRTS). Furthermore, considering the properties of the Substance (volatility and adsorption) and the exposure duration of the MEOGRT (OECD TG 240), which is considerably longer than that of OECD TG 241, it might be more difficult to maintain the substance stable over the exposure duration, increasing the likelihood of obtaining inconclusive results. In addition, a higher number of vertebrates is used to perform an OECD TG 240, which, together with the technical challenges due to the properties of the Substance, would make the request of such a test disproportionate. Therefore, the OECD TG 241 with the additional specifications, as requested, is relevant and suitable to clarify the ED concern for the environnement.

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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 dossiers.

12-month evaluation

Due to initial grounds of concern for PBT/vPvB and for endocrine disruption, the Member State Committee agreed to include the Substance in the Community rolling action plan (CoRAP) to be evaluated in 2022. France 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.

Decision-making

ECHA notified you of the draft decision and invited you to provide comments. ECHA received your comments and forwarded them to the evaluating MSCA. The evaluating MSCA took your comments into account. The request(s) were not amended.

Amendment of the deadline(s)

Following your request, ECHA has exceptionally extended the standard deadline by 12 months to consider currently longer lead times in contract research organisations. On this basis, ECHA has extended the deadline to 27 months.

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 one proposal for amendment to the draft decision and made modifications to the draft decision consequently (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). You did not provide any comments on the proposal for amendment.

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-85 written procedure and ECHA took the decision according to Article 52(2) and Article 51(6) of REACH.

Follow-up evaluation

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*

   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