Helsinki, 12 March 2021

Addressees
Registrant(s) of Resorcinol listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: Resorcinol
EC number: 203-585-2
CAS number: 108-46-3

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. The Larval Amphibian Growth and Development Assay (LAGDA, test method: OECD TG 241 (OECD, 2015)) (Request A.1), on the Substance, specified as follows:
   - Concentrations 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. Concentrations must be expressed as measured and nominal;
   - Measurement of TSH, free T3, Total T3, free T4, Total T4 in the plasma must be performed at NF62[1] (and time to reach this stage must be accurately reported);
   - Histo(pathology) of thyroid gland must be performed at NF62 and at the end of exposure;
   - When relevant, data on assay performances, quality criteria and validations (limits of detection, quantifications, coefficient of variations, specificity) must be reported;

Deadline

The information must be submitted by ***19 September 2022*** *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 corresponding study in the corresponding endpoint of IUCLID.

[1]NF62: Nieuwkoop and Faber stage 62

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[2] by Christel Schilliger-Musset, Director of Hazard Assessment

[2] 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

1. Potential risk

1.1 Potential hazard of the Substance

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

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".*

Based on this definition, the Substance may be 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).

The available information shows that the Substance may have endocrine disruption properties relevant for the environment, having adverse effects on the thyroid gland and thyroid hormones, potentially leading to developmental toxicity and effects at the population level.

Evidence of endocrine activity on the thyroid system based on in vitro *studies*

The Substance was tested in a number of *in vitro* tests investigating potential inhibition of Thyroid Peroxidase (TPO) from porcine (Coval & Taurog, 1967, Taurog 1970, Cooksey *et al.,* 1985, Lindsay *et al.*, 1992, Jomaa *et al.*, 2015), rat tissues (Paul *et al.*, 2014, Paul Friedman *et al.*, 2016) and human cell lines (Paul Friedman *et al.*, 2016, Jomaa *et al.*, 2015). Several studies were performed to investigate effects of the Substance on TPO using different substrates (tyrosine, guaiacol, BSA, fluorescent Amplex Ultrared, luminol). Inhibition of TPO was consistently identified in these studies independently of the test system. Known TPO inhibitors were used as controls in several studies and confirm the sensitivity and adequacy of the corresponding test systems.
In your comment, you claimed that the TPO inhibition potency of the Substance is low. The level of potency of the Substance can be approached by comparison with known potent TPO inhibitors such as propylthiouracyl (PTU) and methimazole (MMI) that have been used in humans as drugs for this property. Except in Paul et al. 2014, the Substance was found of higher or intermediate potency between MMI and PTU and can therefore be considered as a potent TPO inhibitor *in vitro***.**

No effect of the Substance on the NIS transporter is reported (Waring *et al.*, 2012).

Contradictory results are reported on the effect on iodine uptake by thyroid, but consistently with TPO inhibition, the Substance is observed to inhibit the incorporation of iodine into thyroid hormones and its precursors using porcine thyroid tissues (Berthezene *et al.*, 1979, Cooksey *et al.*, 1985, Lindsay *et al.*, 1992).

The Substance did not alter effects mediated through Thyroid-stimulating hormone (TSH) receptor (Santini *et al.*, 2003), but an agonist effect to thyroid hormones was shown *in vitro* in two T-screen assays in rat pituitary tumour cell line GH3 (Ghisari *et al*., 2009, Waring *et al.,* 2012).

A thyroid-disrupting mode of action of the Substance is therefore supported by *in vitro* studies reporting TPO inhibition as TPO is an essential enzyme in the synthesis of thyroid hormones. Due to conservation of hormonal regulation among vertebrates, the Substance is likely to interact with thyroid systems of any vertebrate species of the environment. Indeed, the conservatism of thyroid systems and the effect of TPO inhibition are described in fish (fish early life stage adverse outcome pathway (AOP) 271 (not adopted yet), Nelson *et al.,* 2014) and in amphibians (Optiz *et al.*, 2005 and 2006).

Evidence of endocrine activity/effects relevant for the environment based on in vivo *studies*

According to the Revised OECD Guidance Document 150, certain effects observed in *in vivo* studies can be linked with a potential endocrine activity.[3]

One screening level study on fish embryos has been identified in the literature (Thienpont *et al*., 2011). In addition, one exploratory screening study on mutant fish embryos (Jarque *et al*., 2018), two embryotoxicity studies with fish embryos (Van Leeuwen 1990) and one reproduction study with invertebrates (*Daphnia*) are available to assess potential chronic effects of the Substance in the environment (IUCLID Dossier).

In a short-term screening test zebrafish eleutheroembryos (48 hours post-fertilisation) were exposed for three days to freshly prepared test solutions of 25 test substances, including the Substance, under semistatic conditions (Thienpont *et al*., 2011) (Klimisch score 2 and ToxRtool 2). Thyroid gland functionality was evaluated as a decrease in the intrafollicular T4 content (IT4C). In the first set of the experiment, the IT4C in embryos exposed to the Substance at the maximum tolerated concentration of 200 mg/L was significantly decreased in comparison to controls ($p < 0.05$) and therefore the Substance was regarded as Thyroid Gland Function Disruptors (TGFD) and TPO inhibitor on zebrafish eleuthoembryos. In the second set of the experiment, 5 to 8 test substance concentrations were tested for concentration-response curves. EC10 and EC50 values were determined to describe the thyroid disrupting potency. The thyroid disrupting index (TDI, LC50/EC50) was used as a descriptor of thyroid disrupting hazard. An EC50 value of 82 ± 37 µM (ca. 9.02 mg/L) and an EC10 value of 2 ± 4 µM (ca. 0.22 mg/L) were reported for the thyroid disrupting potency of the Substance. A NOEC value of 10 µM (1.1 mg/L) was obtained from experimental data by performing one-way ANOVA analysis. Systemic toxicity, expressed as LC50, was 5003 ± 100 µM (ca. 550 mg/L) for the Substance, resulting in a TDI of 61.

In your comment, you contested the reliability of Thienpont *et al*., 2011, based on the use of a non-standard test method. You also question the variability in the responses observed in terms of the EC50 and EC10 measures (shape of the curve and linear regression calculated), contesting particularly the reliability of the dose-response relationship between the Substance and thyroxine (T4) content. You commented that the iodide content of the embryo water used in the study (0.005 µM) was at the lower end of the range of the levels commonly found in freshwater systems (0.004 - 0.158 µM) (Tukes, 2017), and that there was uncertainties in the calculated TDI. Moreover, the supporting data do not contain data on up-regulation of mRNA and protective effects by iodine for the

[3] Revised OECD GD 150 Section B

Substance in the study.

However, you scored the Thienpont *et al.* (2011) study as Klimisch 2 in your CSR and you further indicated in your comments that the study data are reliable with restriction, which was agreed by the eMSCA and by Finland during a previous assessment, so the results of this study are indicative of a potential hazard.

The fact that the Thienpont study was using a non-standard determination of the thyroid effect of the Substance is not per se an issue. Indeed, as highlighted in the OECD Conceptual Framework (CF), "*The OECD Conceptual Framework lists the OECD TGs and standardised test methods available, under development or proposed, that can be used to evaluate chemicals for endocrine disruption. It is not an exhaustive list and will be updated as new assays are developed. Assays other than those described in the list may also be valuable for assessing chemicals for endocrine disruption and could be assigned to a level based on the level descriptors. The CF is intended to provide a guide to the tests available which can provide information on assessment of endocrine disruption, but is not intended to be a testing strategy. Furthermore, the CF, as revised in 2017, does not include evaluation of exposure as it is intended for hazard identification/characterisation".*
The linear regression calculated for the Substance, beside being the lowest of all tested chemicals, is of good quality and is acceptable ($r^2 = 0.88$). A variation comprised between 20-45% of the control value for the IT4C can not be considered similar to the control, as expressed by the authors and confirmed of being significantly different to the control by the ANOVA and student t-test on the entire regression. Despite the fact that standard error for the EC10 are important, the variation is sufficiently important to be significantly different from the control response and to be considered positive and thus indicative of an effect on thyroid gland function.

In this assay, the iodide content was indeed at the lower range of the levels commonly found in water, but it is in the range of iodide content recommended by OECD.

Indeed, the OECD TG 241 (LAGDA) states that "*Based on previous work, successful performance of the assay has been demonstrated when dilution water iodide (I-) concentrations range between 0.5 and 10 µg/L. Ideally, the minimum iodide concentration in the dilution water throughout the test should be 0.5 µg/L*". As being of 0.005 µM (0.63 µg/L), the iodide content is sufficient to ensure a proper thyroid gland function and a proper development of organisms.
There is indeed uncertainties with the magnitude of the calculated TDI as T4 hormone content and systemic toxicity were assessed in two different series of experiments. Nevertheless, the biological response seems coherent and is indicative of a TPO inhibition arising at a lower concentration than systemic toxicity.

This effect triggers the request to generate more data to confirm the effect observed on thyroid gland function.

Supportive data are not available for the Substance on up-regulation of mRNA and protective effects by iodine, but are available for MMI. MMI presents the same mode of action as the Substance, namely it is a TPO inhibitor. Thienpont *et al.* (2011) showed that the addition of iodine up to 4000 µM were unable to rescue the thyroid gland function. It seems possible that increasing the iodide content of water will not lead to protective effect of the thyroid gland function against the effects of the Substance.
It seems unlikely that systemic toxicity is the only driver of IT4C reduction in zebrafish as the LC10 was determined to be 4164 µm (458 mg/L) and that the test for IT4C was performed at a MTC of 200 mg/L in order to avoid these lethal effects.
Based on a data set of 25 substances, concentration of IT4C, was shown to be sensitive

in reflecting a direct effect on the thyroid gland function, such as TPO inhibition.

The exploratory screening study was a short-term screening test on zebrafish eleutheroembryos (48h post fertilisation, 30 embryos per concentration and replicate) exposed for three days to 5 concentrations (and a control) in semi-static conditions (Jarque *et al.*, 2018, ToxRtool score of 2). The study utilised the transgenic (Tg) zebrafish line Tg(tg:mCherry) in which the reporter gene mCherry (encoding a membrane-bound red fluorescent protein) is under the control of the thyroglobulin (tg) promotor. Therefore, the fluorescent protein mCherry is expressed specifically in the thyroid and is correlated with the expression of thyroglobulin. An increase of fluorescence is indicative of an increase in the synthesis of thyroglobulin in response to increased TSH stimulation of the gland (thyroglobulin is upregulated by TSH) due to the repression of thyroid hormone synthesis. At 120 hpf, embryos were analysed by fluorescence microscope. Induction of fluorescence by the Substance was observed, with a maximum fold induction of 2.1 in comparison to negative control. At a concentration up to 100 µM, the repression of fluorescence or weaker induction of the fluorescence was observed due to a potential interfering or secondary toxic effect. An EC50 value of 3.4 ± 1.6 µM (ca. 0.37 mg/L) for fluorescence induction and a LC50 value of 5197 µM (ca. 572.2 mg/L) was observed, resulting in a thyroid disrupting index (TDI; LC50/EC50) of 1529. For the other TPO inhibitors tested, EC50 ranged from 279 µM for MMI, 366 µM for ethylenethiourea to 1096 µM for phloroglucinol. A BMD20 (concentration at which a 20% increase of the *tg*:mCherry fluorescence was observed) value of 0.663 µM (ca. 0.073 mg/L) was determined for the Substance. This study therefore provides an indication that the Substance alters thyroid hormone synthesis *in vivo* in fish.

Regarding this study, you commented that the response curve is different from the other chemicals tested and that no information was provided on the iodide content of the water used. However, the eMSCA considers that the presence of general toxicity at the highest dose explains the shape of the curve but does not contradict the existence of an effect. Regarding iodide content, the test was performed according to the OECD TG 236 (Fish Embryo Acute Toxicity test), for which indication of iodine content is not requested, which explains why this information is not available.

The other available aquatic toxicity test results (*daphnia* reproduction, fish embryo/early life stage toxicity) are not specific to detect endocrine disrupting effects, but the fish early life stage toxicity test measures parameters which are considered "potentially sensitive to, but not diagnostic of, EATS modalities" (*i.e.* estrogen/ androgen/ thyroid/ steroidogenesis modalities) as some thyroid active chemicals may interfere with embryonic development and metamorphosis (OECD GD 150, 2012).

A *Daphnia magna* 21-day reproduction test was available and was performed following the OECD TG 211 (IUCLID dossier). At the end of the exposure, the Substance did not show any adverse effects at the highest measured test concentration (172 µg/L) on survival, growth or reproduction of *Daphnia magna*. Moreover, no male neonate generation were observed during the assay, indicating that no hormone related activity occurred.

One study (Van Leeuwen 1990, ToxRtool score of 2) reported results of two long-term fish early life stage data performed with zebrafish (*Brachydanio rerio*, not duplicate) and rainbow trout (*Salmo gairdneri*, duplicate), respectively. The test method was performed according to the fish early life stage (FELS) draft guideline available at that time (OECD TG 210). The endpoints were total embryotoxicity (teratogenicity) and mortality. The Substance induced teratogenic effects both in zebrafish and rainbow trout. For zebrafish, the 7-day EC50 for total embryotoxicity (lethality and malformations) was 54.8 mg/L, 7-day LC50 was 262 mg/L, and LOEC range from 100 to 320 mg/L. In the 60-day study

with rainbow trout the EC50 of total embryotoxicity (lethality and malformations) was 260 mg/L, and LOEC range from 32 to 320 mg/L. The observed effect may be indicative, but not diagnostic of thyroid disruption and can be induced by various modes of action (mediated or not mediated via endocrine disruption). This information therefore does not constitute an evidence of an apical effect related to thyroid disruption.

The current substance evaluation process focuses on the endocrine disrupting properties for environment. However, the database in rodents, although not consistent in every data point, show a pattern of effects with increased thyroid weight, histopathological findings (hyperplasia and decrease in colloid), slight increase in TSH and triiodothyronine (T3), and slight decrease in T4 (ECHA, 2020). Moreover, induction of goitre and severe hypothyroidism was reported in several human cases, for which the Substance was administered dermally on a damaged skin on a (sub)chronic basis.

In your comments, you disagreed with the conclusion that rodent data show a clear pattern of effect, in particular in the two-generation study and you highlighted that no effect was detected in a developmental neurotoxicity (DNT) study. The data set from mammals has been thoroughly discussed in the context of the proposal to identify the substance as a Substance of Very High Concern (SVHC) due to its endocrine properties relevant for human health. ECHA agrees that the mammalian data is not consistent in every data point and study. However, the data support the concern that the Substance may be a thyroid disruptor in the environment.
The study referred to as a DNT study by the Resorcinol Task Force (RTF) was indeed a preliminary study to the two-generation study and included limited investigations. In this study, statistically significant effects were observed on the locomotor activity of the male offsprings.
Besides, in its opinion adopted on 12 June 2020, the Member State Committee (MSC) overall concluded on the experimental data that "*findings consistent with the mode of action (MoA) of thyroid disruption via thyroperoxidase (TPO) inhibition are also reported in several experimental studies via drinking water. Similar findings reported in studies conducted by subcutaneous, dietary and inhalation routes provide supportive evidence. In particular histopathological changes in the thyroid and changes in the circulating levels of T3 (triiodothyronine) or T4 (thyroxine), are considered as adverse effects.*" Although a final decision on the SVHC identification for human health has not been taken yet, the minutes of MSC-70 meeting report that "MSC unanimously acknowledged that there is scientific evidence that resorcinol is an endocrine disruptor [for human health] as defined by the WHO/IPCS (2002)"[4].

These data support that the Substance may be a thyroid disruptor for vertebrates in the environment.

Taking the available information together in a weight of evidence approach, there is sufficient evidence to consider that the Substance has ED properties impacting the thyroid gland function, especially the thyroid hormones. It was reflected by the decrease of IT4C in zebrafish eleutheroembryos (Thienpont *et al.*, 2011) and recently highlighted again by data from the screening study (Jarque *et al.*, 2018), indicating that the Substance induces fluorescence in thyroid gland of specifically genetically modified zebrafish eleutheroembryos for detection of ED compound. These data highlighted the individual effects of the Substance on thyroid gland function, but do not provide indication on potential effects at the population level.

[4] Minutes of the 70th Meeting of the Member State Committee (MSC-70). 10-12 June 2020 web conference. Adopted on 7 September 2020 https://echa.europa.eu/documents/10162/28685870/MinutesofMSC-70_adopted-1.pdf/2972d2e5-6a5b-67ce-efc8-1a67a8e025a9

However, none of the available studies provide information on apical and adverse effects in consequence of the capacity of the Substance to affect thyroid regulation. The information currently available is therefore not sufficient to draw a final conclusion on the potential hazard, *i.e.* to meet the conditions that define an endocrine disruptor for the environment. In order to conclude on the potential ED properties, further information is needed for the Substance.

Regarding the above-mentioned environmental studies, we agree with your comments that they have limitations and are therefore not sufficient to identify the Substance as an ED for environment. However, these studies provide evidence that the Substance possibly can be an ED for the environment and justify to request a further study to clarify this potential risk.
In your comment, you argued that *in vitro* TPO inhibition in itself does not demonstrate a hazard as the Substance must enter the thyroid gland at sufficient levels and you mentioned that "*it is highly unlikely that resorcinol may induce thyroid function disruption in living aquatic organisms at environmentally relevant concentrations*". This conclusion is however based on your assessment of a low TPO inhibition potency of the Substance that is not sound as explained above (see 1.1 a) and on the efficiency of the metabolic pathways in rodents. Quick metabolisation is acknowledged, but it has to be considered together with the high potency of the Substance to inhibit TPO *in vitro.* Low systemic concentrations of the Substance may therefore induce effects. Toxicokinetic data in rodents also show that the free Substance can be present systemically after oral exposure. Besides, data on metabolism for the other taxa than mammals are lacking. Therefore, the current database is insufficient to exclude that the Substance can induce thyroid function disruption in living aquatic organisms at environmentally relevant concentrations. As you mentioned, TPO inhibition *in vitro* is not sufficient to demonstrate a hazard, but a hazard cannot be excluded based on toxicokinetic data. For environment, the available data provide additional indications that justify the need to clarify the hazard for the environment. The requested study is a prerequisite to elucidate the effect of thyroid disruption and to characterise the effective concentrations for these effects in aquatic organisms.

1.2 Potential exposure

According to the information you submitted in the chemical safety reports, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 10 000 – 100 000 tonnes per year.

You reported that among other uses, the Substance is used by industrial workers, professionals and consumers in cosmetics and personal care products (e.g. pH regulators and water treatment products, laboratory chemicals in health services and scientific research and development, adhesives and sealants, pharmaceuticals and polymers), in the manufacture of chemicals, rubber products, plastic products, fabricated metal products and machinery and vehicles. The available information indicates wide dispersive use. In your comments you refer to a monitoring exercise, stating that there has not been changes in the use patterns that would result in greater risk than during the monitoring exercise. Further you claim that local and regional concentrations of the Substance are low. More precisely, ECHA notes that the monitoring data provided and the corresponding exposure scenarios show that there is some discharge of the Substance in the environment resulting from its use. As detailed in the summary of the targeted monitoring exercise on the Substance, 4 types of downstream user sector could be source of the Substance for the environment. Tyre manufacturing, phenolic resin production, wood adhesives and flame retardant production.

For tyre manufacturing plants (4 sites out of 28 no longer operating in 2020):

- a large monitoring exercise was performed but some uncertainties remain. 1/3 of the samples (6 out of 18) were not available for analyses beside being planned to be incorporated in the exercise which provided a less complete picture of the effluents of the Substance in tyre manufacturing plants. On one site, a high value (117 µg/L vs 21µg/L) was quantified and was dismissed without more details than "value for one site (117µg/L) was considered atypical". Without a more detailed justification, this value can be used as a worst case scenario as you had previously done in your report. So a $PEC_{localwater}$ of 11.83 µg/L can be considered.
- No samples have been obtained during the monitoring exercise from the Scenario 3 plant, where the wastewater potentially containing the Substance or the Substance-based resins is discharged to sewer untreated. This may lead to direct release of water containing the Substance or the Substance-based resin directly to the environment. You actually informed us that this discharge scenario is no longer operating, but local release may have occurred in the past leading to environmental exposure.
- For Scenario 4, release in the environment occured (after waste water treatment and tankered and dilution) leading to local concentration in water of 6.44µg/L.

In 2020, 13 new sites were operating, among which two operate scenario 2 (wastewater potentially containing the Substance and/or the Substance-based resins treated on site and discharged to sewer).

For phenolic resin production:

- In worst case estimation, the influent had a highest concentration of 300 µg/L, with an elimination rate of 89.7%, which led to a final release of water with 30.1 µg/L, contributing to increase the local concentration in the Substance (concentration > PNEC).

For flame retardant production plants:

- You estimated that release of the Substance occurred, with local water concentration of 3.36 µg/L (with background concentration).

Moreover, in the different exposure scenarios developed in the CSR, the life cycle stage of articles was never analysed and considered. The sole justification is that there is no relevant subsequent service life for the different usage. Nevertheless, this assertion seems incomplete and overused. Indeed, the Substance is used as wood adhesive and sealant, in coatings, and resins and as flame retardant. These uses are considered as dispersive due to the widespread of the articles containing the Substance. Thus, you have not provided evidence that the release from these products is negligible or that no release from polymers would occur. Exposure of the environment through service life of articles is therefore likely.

Continuous discharge into the environment could lead to raise in concentrations e.g in the aquatic environment, leading to potential adverse effects.

Therefore, an exposure of 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.

The information you provided on manufacture and uses demonstrates an exposure of the environment.

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

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

1.4 Further risk management measures

An adverse effect, plausibly linked to endocrine activity needs to be established for a substance to fulfil the definition of an endocrine disruptor. The guidance document regarding ED identification recommends to demonstrate "the biological plausibility of the link between the adverse effects and the endocrine activity" (ECHA/EFSA, 2018).

The LAGDA study, with the additional mechanistic parameters requested in this decision, will investigate apical endpoints to detect an adverse effect on the thyroid and provide additional evidence to support that any adverse effects observed are plausibly linked to endocrine activity via the thyroid pathway (*i.e.* the Substance has a thyroid disruptive mode of action), as required for identification of the Substance as an SVHC for its endocrine disrupting properties.

If the required information confirms that the Substance is an endocrine disruptor in amphibians, there will be a possibility to propose its identification as a substance having endocrine disrupting properties whose effects to the environment give rise to an equivalent level of concern according to Article 57(f) of REACH.

The Substance is already proposed to be identified as a substance having endocrine disrupting properties whose effects to the human health give rise to an equivalent level of concern according to Article 57(f) of REACH[5]. The MSC did not unanimously agree that it presents an equivalent level of concern, necessary for an SVHC identification according to article 57(f) and further discussion will take place in the REACH Committee.

If the Substance is included in Annex XIV of REACH due to its ED properties, the scope of the authorisation process depends on whether it is identified as SVHC due to its ED properties for human health or for environment, or both. If identified as an SVHC due to ED properties for the environment, an assessment of risk for the environment would be added to the scope for authorisation, according to Article 62(4)(d) of REACH. Consequently the exemptions specified in Article 56(5) of REACH and relevant with regard to uses of the Substance (use in cosmetics in particular), would not apply.
Therefore, an SVHC identification due ED properties for the environment would result in stricter risk management measures compared to those currently in place or those required

[5] https://echa.europa.eu/documents/10162/53d2eb0e-b0e8-fabb-b4b0-a56c246cb0a3

based on ED properties for human health, such as improved measures at manufacturing sites, better waste management and revised instructions on safe use, if appropriate.

In your comments, you considered that the realistic possibility that the information requested would allow improved risk management measures is not established and that the current risk management measures are already more than adequate to minimise risk to the environment. However, an absence of risks cannot be concluded if a hazard, and in particular a hazard related to ED properties, is not elucidated. In addition, the identification of ED properties for the environment will open the way to SVHC identification and possibly to authorisation that is a risk management measure heading towards substitution of the uses of the substance.

2. How to clarify the potential risk

As mentioned above, several screening studies *in vitro* and in fish describe endocrine disruption potential of the Substance. By inhibiting TPO, the Substance modifies thyroid hormones synthesis. Nevertheless, according to the Revised OECD GD 150 on standardised test guidelines for evaluating chemicals for endocrine disruption, no apical endpoints data have been identified on the Substance yet to demonstrate that disruption of thyroid function leads to environmental adversity of the Substance relevant at population level. Moreover, the existing AOP 271 in fish early life stage regarding TPO inhibition is not yet validated. Therefore, this concern therefore needs to be further clarified.

In your comments you did not contest the inhibition of TPO by the Substance, but you argue its inability to lead to adversity.
ECHA concludes that it is possible to gain such information by performing the requested Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241) that includes apical as well as mechanistic endpoints linked to disruption of thyroid hormones and would allow to confirm or dismiss the concern for the ED properties of the Substance for the environment. The Revised OECD GD 150 has been recently updated (September 2018) recognising that the LAGDA serves as a higher tier test with an amphibian, included at Level 4 of the OECD CF on Endocrine Disrupters Testing and Assessment, where *in vivo* assays also provide data on adverse effects on endocrine relevant endpoints.

Moreover, the necessity to obtain such data to conclude on the potential ED effect is correlated to the high tonnage and the dispersive uses of the Substance that may impact large area and populations if apical adverse effects occurred at population level.

2.1 Request A.1: The Larval Amphibian Growth and Development Assay (LAGDA, test method: OECD TG 241; OECD 2015)

a) Aim of the study

As detailed in Section 1.1 above, information on adverse effects related to the endocrine activity of the Substance is required to conclude on the potential hazard. The test guideline of the Larval Amphibian Growth and Development Assay (LAGDA) describes a toxicity test with an amphibian species that considers growth and development from fertilisation through the early juvenile period. It is an assay that assesses early development, metamorphosis, survival, growth, and partial reproductive maturation. It also enables measurement of a suite of other endpoints that allows for diagnostic evaluation of suspected endocrine disrupting chemicals (EDCs) or other types of developmental and reproductive toxicants. The requested study will allow evaluation of the effects of the substance on the endocrine system and especially on thyroid and thyroid gland function. The requested study is the most sensitive developmental toxicity study with exposure

through the environment which enables to detect adverse effects on thyroid as it includes many parameters related to endocrine activity/mode of action. It is the only OECD guideline that can inform both on adversity and endocrine activity related to thyroid and thus, enable establishing the ED MoA.
The OECD TG 241 is not a standard information requirement, but the information requested aims at clarifying the potential risk that the Substance poses. Therefore, it is requested under the current substance evaluation.

b) Specification of the requested study

To address the missing information identified above, the OECD TG 241 study will allow to identify information both on thyroid effects and developmenal effects, which are required to conclude on the endocrine disrupting properties, and to confirm whether the observed thyroid mode of action is of concern for the Substance.

The requested study must be performed according to the OECD TG 241 (OECD, 2015). All quality criteria must be respected. Below the specifications of the test design to be followed are explained, as well as the mandatory and recommended parameters.

Test material and concentration

The test material should be the Substance, as specified in Appendix C, and with the highest purity in order to avoid confounding effects of impurity(ies).

The iodide content of water used in the study needs to be checked 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 checked and reported to comply with the recommendation of the paragraph 17 of the OECD TG 241 to ensure the success of the assay. This remark is reinforced based on your comments on this issue. Additionally, as indicated in paragraph 17 of the OECD TG 241, you may monitor iodine content in food as fresh water vertebrates cover their main iodine demand via the food.

For the purposes of this test, results from existing studies (fish tests such as OECD TG 229, TG 234 and TG 236) must be considered in determining the highest test concentration so as to avoid concentrations that are overtly toxic. If there are no relevant data to be used for concentration level setting, it is recommended that results of a range-finding study are reported with the main study. It is recommended to use a minimum of four chemical concentrations and appropriate controls (including solvent controls, if necessary). Generally, a concentration separation (spacing factor) not exceeding 3.2 is recommended.

Route of exposure

The assay must be performed under flow-through conditions in order to maintain stable exposure concentration in the system and to avoid decline in concentration over time. This has to be verified during the assay by measuring concentrations during the study. In your comments, you specify that you might encounter difficulties in maintaining the concentration of the Substance. Based on your comment, regular analytical monitoring of the Substance is therefore required to know its exact concentration during the experiment and to evaluate its disappearance should it occur. In this aim, the exposure concentrations of the Substance must be determined at least twice a week for at least one replicate in each treatment group, rotating between replicates of the same treatment group. As indicated in the OECD TG 241, the flow rate to each tank should be constant in

consideration of both the maintenance of biological conditions and chemical exposure and is recommanded to operate at least 5 tank turnovers per day. You can also follow the recommendation in the OECD Guidance Document (GD) 23, on Aqueous Phase Aquatic Toxicity Testing of Difficult Test Chemicals which provides indications to limit biodegradation of a test substance during assessment.

Control group

Based on your comment, it is anticipated that the historical control data available for the LAGDA may be limited. We consider that historical data are not a prerequisite to reach a conclusion on the test outcome and that the design of the OECD TG 241 provides sufficient statistical power. According to the OECD TG 241, the number of replicates is doubled (8 replicates) for controls compared to each test concentration (4 replicates) in order to give adequate statistical power for the test.

Parameters to be measured or recommended

At NF stage 62, a larval sub-sample (up to 5 animals per replicate) is collected and various endpoints are examined (Table 1) and the remaining animals continue exposure until 10 weeks after the median time to NF stage 62 in the control. At test termination (juvenile sampling) additional measurements are made. Table 1 lists the parameters in the OECD TG 241 and the timepoint when they must be measured. The table also lists additional parameters, some of which are mandatory (i), while others (ii) are recommended as explained below.

(i) <u>Additional mandatory parameters to be measured</u>

The following parameters must be measured in addition to the parameters requested in the guideline:

- Histopathology of the thyroid gland at both stages.
- Measurement of TSH, free T3, Total T3, free T4, Total T4 in the plasma must be performed at NF62 (and time to reach this stage must be accurately reported).
- When relevant, data on assay performances, quality criteria and validations (limits of detection, quantifications, coefficient of variations, specificity) must be reported.

In order to avoid bias, sampling for thyroid 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).

The list of parameters (requested or recommended) to be provided results from the analysis of the data currently available on the Substance (as detailed below), and which is specific to the suspected mode of action of the Substance for environmental species. Based on the available data, a thyroid-disrupting mode of action via TPO inhibition is suspected. As detailed above in section 1.1, TPO inhibition have been observed in a number of *in vitro* tests from mammals. In fish, a decrease in intrafollicular T4 content was observed in Thienpont *et al.* (2011) and an increase of fluorescence indicative of an increase in the synthesis of thyroglobulin in response to increased TSH stimulation of the thyroid gland was observed in Jarque *et al.* (2018). Data on mammals (rodents and humans) also show a pattern of effects consistent with TPO inhibition with consequences on the thyroid hormones concentrations (ECHA, 2020). The measurement of thyroid

hormones as an additional parameter is requested in order to provide supporting information on this MoA (TPO inhibition) in an environmental species (amphibians). In this specific case, these additional measurements are requested to inform whether the potential adverse effects occurring on development and time to metamorphosis results from an impact of the substance on the thyroid gland histology/thyroid gland function and whether they do not occur through general systemic toxicity..

As noted in section 1.4. above, an adverse effect, plausibly linked to endocrine activity need to be established for a substance to fulfil the definition of an endocrine disruptor.

Mechanistic data informative of the mode of action leading to the effects are therefore important in a possible future SVHC identification of the Substance as an endocrine disruptor for the environment in the aim to link the apical effects with endocrine activity in the suspected mode of action.

Considering the intended risk management measure and the information available on the suspected mode of action of the Substance, mechanistic parameters specific of thyroid-disruption and of TPO inhibition (as specified above) must therefore be provided by the LAGDA for the Substance.

Two MSCAs questioned in their respective proposals for amendement (PfA) the justification to request as mandatory the additional parameters related to hormones measurements. Following discussions, the MSC agreed that adding measurements of TSH, free T3, Total T3, free T4, Total T4 is well justified. This is for the reasons, as detailed above, referring to the suspected mode of action of the Substance and the intended risk management measure, *i.e.* to potentially conclude that the substance fulfils the definition of an endocrine disruptor for the environment and the criteria for an SVHC identification.

The MSC has however considered that it is not necessary to add some of the originally requested parameters. Consequently the request for TSHβ gene expression, MIT and DIT at any stage, and the request for TSH, free T3, Total T3, free T4, Total T4 at the end of exposure were removed from the decision.

(ii) <u>Measure of vitellogenin (VTG) at NF62 and at the end of exposure as a recommendation</u>

Measurement of VTG concomitantly to phenotypic/genetic sex could be performed in order to ensure that the potential observed effects are arising from thyroid effects and not other endocrine effects. It will also serve as control parameters allowing to conclude if effects are more linked to general toxicity or arising from ED effects.

Two MSCAs questioned in their respective Proposal for Amendment the justification to request as mandatory the measure of VTG for the substance. However, measurement of VTG provides mechanistic information on other endocrine modes of action than thyroid-disruption, in particular (anti)estrogenic and (anti)androgenic modes of actions, as well as possible teratogenic effects. Consequently, it can allow to discriminate on the endocrine mode of action of the adverse effects potentially induced by the substance. It cannot be excluded that the substance may also act via endocrine disturbance not related to thyroid. However, as available data primarily raise a concern for thyroid disruption, it has been agreed thatthe measure of VTG is only recommended as an additional measure, but it is not mandatory.

Table 1: Summary of endpoints to be measured or recommended

*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	
Liver-somatic index (LSI)			X
Genetic/phenotypic sex ratios			X
Histopathology (gonads, reproductive ducts, kidney and liver)			X
Vitellogenin (VTG) (recommended)		X	X
TSH		X	
Free T3		X	
Total T3		X	
Free T4		X	
Total T4		X	

Request for the full study report

You must submit the full study report which includes:

- a complete rationale of test design and
- doses of the Substance (reference and batch number) along the experiment
- all detailed images for metamorphosis change and identification
- 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 evaluating MSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the potential hazard for the Endocrine disruption for 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, test method: OECD TG 241) is:

- Appropriate, because it will provide information which will clarify adverse effects potentially caused by endocrine activity of the Substance and especially effect on thyroid on wildlife species. This will enable the evaluating MSCA to conclude on

potential ED properties of the Substance for the environment. In 2017, within its conclusion document, Tukes (The Finnish Competent Authority) noted "*In this case the added value could be the proof of adverse apical effects resulting from thyroid disrupting activity.*" (Tukes, 2017). At the time Tukes wrote the conclusions, the LAGDA was not recognised as providing such apical information. However, since that statement, the situation has changed and it is now agreed that the LAGDA is the only test available, and validated, in the OECD Conceptual framework (CF) that includes apical as well as mechanistic endpoints linked to disruption of thyroid hormones. It is therefore the only test allowing to confirm or dismiss ED properties regarding thyroid effects of the Substance for the environment. There are only two OECD-validated assays sensitive to thyroid effects that can provide such useful information on adverse apical effects resulting from thyroid disrupting activity. The Amphibian Metamorphosis Assay (AMA, OECD TG 231) and the OECD TG 241 (LAGDA). Anti-thyroidal diagnostic criteria include thyroid gland hypertrophy/atrophy, follicular cell hypertrophy, follicular cell hyperplasia, and as additional qualitative criteria: follicular lumen area, colloid quality and follicular cell height/shape. According to the Revised OECD GD 150, the AMA test is a screening test (placed at the level 3 of the OECD CF - "*in vivo assays providing data about selective endocrine mechanism(s)/pathway(s)*") and its use for definitive conclusion for adverse effects on populations is uncertain. The LAGDA test is a level 4 test of the OECD CF *("in vivo assays providing data on adverse effects on endocrine relevant endpoints")*, with several more endpoints for thyroid activity (thyroid histopathology and time to metamorphosis).

- The least onerous measure because there is no equally suitable alternative method available to obtain the information that would clarify the potential hazard, and this is the only OECD TG with such extensive parameters for endocrine activity and endocrine mediated effects on thyroid gland and hormones levels in level 4 test for environment according to the Revised OECD GD 150. In particular, the AMA (OECD TG 231) also describes some specific thyroid function endpoints related to interaction with the HPT axis (thyroid histopathology and time to metamorphosis/develomental phases) (OECD, 2009). However, the AMA test would not allow a definitive conclusion on thyroid disrupting properties of the Substance, due to the lack of apical endpoints in the test method that would indicate clear adverse population effects mediated by the HPT axis. Indeed, the AMA is not considered as a level 4 tests in the OECD CF, and it is placed only at the level 3 of the OECD CF .
- The LAGDA allows the comparison of the effects occurring on development from thyroid gland and hormones impairment and effects arising from estrogenic effects by measuring phenotypic and genetic sex of organisms.
- As detailed in the OECD GD 150, the performance of an AMA and the "*observations of delayed development (metamorphosis) in OECD TG 231 may require long-term data obtainable from the Larval Amphibian Growth and Development Assay (LAGDA; OECD TG 241) before a more definitive conclusion can be drawn about endocrine disruption.*" Moreover, the OECD 150 details that the LAGDA could "*be used at any stage in the hazard assessment process, the most likely use scenario will be when there are some data available about the possible thyroid disrupting properties of a chemical*" indicating that this would not lead to a level 3 data gap as you claim in your comments. In order to avoid the necessity of another assay at a later step of the evaluation process, if an AMA would be requested first, and to reach a definitive conclusion on the concern investigated, the LAGDA is the best option, the least onerous and the most informative. Indeed, duration of the test is

longer (21 days vs 112 days) providing a better assessment of toxicity, other histopathological determination can be made to check for toxicity before and after metamorphosis, a clearer and more accurate observation of delay in metamorphosis, more doses are used to generate a more accurate estimation of concentration-dose effects.

- When comparing the AMA to the LAGDA for proportionality of the requested test, the minimum amount of tadpoles needed in the AMA (320 larvae for three test concentrations plus the control) is lower compared to the LAGDA (480 larvae for four test concentrations plus control). However, as more (i.e. four or five) test concentrations are recommended for the AMA, the only significant difference between the tests is the number of replicates in the control: four for the AMA and eight for the LAGDA. However, in the LAGDA the higher number of replicates in the control addresses the variability better and, therefore, helps to ensure appropriate statistical power of the test. Moreover, if the AMA results are positive or inconclusive, a follow-up test may be required in the form of the LAGDA.

In your comments to the draft decision, you considered that the LAGDA test guideline is insufficiently validated and testing laboratories do not have enough experience with the test leading to an invalid study. You argue that this will lead to a high risk of false positive results or huge uncertainties. ECHA does not agree with your comments as the LAGDA is a validated test method and, therefore, should be considered fit to provide the information needed. Taking into account the fact that the AMA (OECD TG 231) has been the only test available on amphibians for a long time, ECHA agrees that the laboratories likely have more experience conducting the AMA compared to the LAGDA. However, the lack of experience gained on the test method should not be used as a reason to request an alternative older test method when more recent state of the arts techniques are validated. Similarly, the lack of historical control data cannot be used as a reason for not conducting a state of the art technique.

From the PfAs addressing the design of the request, you concluded that they provide indications that the design of the LAGDA is not sufficiently well-developed and validated. Your interpretation is not correct. Indeed, a general support to conduct a LAGDA have been expressed in the PfAs. The points raised in the PfAs relate to the additional parameters investigating the thyroid function and in particular the TPO function. They are additional parameters added in the request to the LAGDA standard design. These additional elements aim to adapt the design of the request to the specific case of the Substance. Therefore, the validity of the design of the LAGDA itself is not challenged by those PfAs. Moreover, you did not question the relevance of these additional elements in your initial comments.
In your comment you contest the validity of the LAGDA especially because its validation comprised a limited number of laboratories, testing only one substance with a direct thyroid mode of action, with mixed results and expressing several inconsistencies. However the LAGDA assay presents a validated status at the OECD, meaning that this assay is considered sufficiently robust and reproducible to be used for regulatory purpose..

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

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Appendix B: Procedure

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

12-month evaluation

- Due to initial grounds of concern for Endocrine disruption, the Member State Committee agreed to include the Substance (EC No 203-585-2, CAS RN 108-46-3) in the Community rolling action plan (CoRAP) to be evaluated in 2019. 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 on 18 March 2020.

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. The request and its justification was slightly amended. However, all the comments related to the re-inclusion of the substance on CoRAP or to the SVHC identification were not taken into account as they are out of the scope of the present decision. In particular, the basis for re-inclusion of the Substance is addressed in the corresponding justification document[6].

(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.

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

[6] https://echa.europa.eu/documents/10162/a4fc9130-88d7-0b52-d076-4841e0fa1794

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

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

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision as modified during its MSC-73 meeting and ECHA took the decision according to Article 52(2) and 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.

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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[7].

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.

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"[8].

[7] https://echa.europa.eu/practical-guides
[8] https://echa.europa.eu/manuals