Helsinki, 08 August 2022

Addressees
Registrant(s) of Bis(2-propylheptyl) phthalate listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: Bis(2-propylheptyl) phthalate (DPHP)
EC number: 258-469-4
CAS number: 53306-54-0

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. An amphibian metamorphosis assay (AMA) (test method: OECD TG 231), using the Substance and the following specifications (see also Appendix A):
   - The test material must be representative for the Substance, in particular with respect to the concentrations of isomers, constituents and impurities.
   - Because of the low water solubility (2 ng/L) of the Substance, the AMA must be conducted with dietary exposure, including the following non-standard adaptations of the OECD TG 231:
     - The Substance must be dissolved in acetone, afterwards mixed with dry Sera Micron® and evaporated with filtered air to dryness again. Caution should be taken to ensure that the Substance does not crystallise as the solvent is removed;
     - A negative control with Sera Micron® similarly treated with acetone without the Substance must be prepared;
     - The test must be conducted under flow-through conditions to ensure an acceptable water quality;
     - The spiked Sera Micron® must be fed as a suspension prepared with dilution water. This solution must be prepared shortly before the beginning of the test and divided into individual aliquots, e.g. in scintillation vials, so each aliquot holds enough food for an entire treatment for a single day;
     - Before the beginning of the test, the intended concentration of the Substance in the spiked diet must be verified by analytical measurements; To check the concentration, triplicate samples of the dosed food must be extracted with a suitable extraction method and the Substance concentration in the extracts must be measured by an appropriate analytical method;
     - A dose range-finding test must be performed in order to reduce technical challenges and increase the robustness and quality of the data obtained in the main study.
     - At least five concentration levels with four replicates must be tested in the main study to obtain a full dose-response relationship to derive a sound

LOEC/NOEC.

- In addition, the concentration of the Substance and the two metabolites: mono-(2-propylheptyl) phthalate (MPHP) and mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP) in the animals, must be analytically measured. Analytical measurement of the total body burden of the Substance, MPHP and OH-MPHP must be performed in full body homogenate at the end of the test. From each treatment group at least three animals must be pooled and analysed using an adequate sample preparation and analytical set up.
- Liver histopathology and assessment of the hepatosomatic index must be performed.

Deadline

The information must be submitted by **13 February 2025**.[1]

Conditions to comply with the information requested

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

You must update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.
You will find the justifications for the requests in this decision in the Appendix A entitled 'Reasons to request information to clarify the potential risk related to Endocrine disruption'.

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] under the authority of Mike Rasenberg, Director of Hazard Assessment

[1] The final deadline includes the 90-day period addressed in Article 53(1) of REACH and the seven-day period addressed in point 9(d) of the terms and conditions of REACH-IT.
[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 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 and ECHA have identified the following potential hazards which must be clarified.

a) Potential endocrine disrupting properties

The available information in the registration dossier shows *in vivo* thyroid and pituitary effects in mammals, and raises concern that the Substance might act as an endocrine disruptor (ED) in the environment.

Studies in rats provide clear evidence that the Substance interacts with the thyroid system. Thyroid hypertrophy/hyperplasia was evident in both sexes with dose-dependent incidence in a 90-day repeated dose toxicity (RDT) study ([redacted], 1995) and in the F1 generation of a two-generation study, where also increased thyroid weight was observed ([redacted], 2009). In addition to thyroid hypertrophy/hyperplasia, histology of the pituitary gland revealed an increased number of basophilic cells (thyreotropes) in males in the RDT study (3/10 and 8/10 at the mid and high dose, respectively) and in F1 males in the two-generation study (7/25 animals at the high dose). These findings indicate an increased production of thyroid stimulating hormone (TSH) in the pituitary, presumably via feedback response of the hypothalamo-pituitary-thyroid (HPT) axis due to decreased circulating thyroid hormone levels. However, neither information is available on TSH and thyroid hormone levels, nor are there any dedicated studies providing mechanistic information.

Thyroid hypertrophy/hyperplasia occurred at lower doses than liver hypertrophy in the RDT study and at similar doses in the 2-generation study in F1 animals. Thyroid weight in males was the most sensitive parameter in the 2-generation study. The authors of these two key studies suspect that, regarding the thyroid modality, peroxisome proliferators can cause an induction of metabolic enzymes in the liver (e.g. UDP-GT) leading to a increased excretion of thyroid hormones (see e.g. Hinton et al., 1986). Indeed, observations in several studies indicate that the Substance may be a peroxisome proliferator. These findings include increased liver weights, hepatocellular (centrolobular) hypertrophy with cytoplasmatic eosinophilia, increased hepatic cyanide-insensitive palmitoyl-CoA activity (Pal-CoA), as well as reduced serum cholesterol and triglyceride levels.

However, other possible modes of action for thyroid disruption have been described for certain phthalates (see e.g. Boas et al., 2012), and there is neither any specific study testing the influence of the Substance on hepatic thyroid hormone metabolism nor any other mechanistic information. Therefore, a range of presumed molecular initiating events (MIE) can be suggested which all could lead to the same pattern of effects (thyroid and pituitary histopathology findings). In the absence of data confirming an indirect rat-specific mode of action, and since the thyroid system is highly conserved, the findings in rats are considered relevant for vertebrates in general.

With respect to the environment, there are no studies available investigating endocrine-mediated modes of action and/or adverse and population relevant effects of the Substance in fish or amphibians. The mammalian data available are concluded not to be of population relevance, since no direct impacts in reproduction, growth or survival are observed. Thus, with respect to the environment neither estrogen, androgen and

steroidogenic (EAS) nor thyroid (T) modalities have been sufficiently investigated to draw a conclusion on the endocrine disrupting properties of the Substance.

However, as the thyroid system is highly conserved within vertebrates, the available mammalian data presented above raise a substantial concern as to whether the Substance may act as an endocrine disruptor in the environment. Hence, further data are needed to clarify this concern.

1.2 Potential exposure

According to the information you submitted in the registration dossier and chemical safety report, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 100,000-500,000 tonnes per year.

The Substance is used as a plasticizer mainly in polyvinyl chloride (PVC) articles like car interiors, cables, building materials, medical devices but also for adhesives and sealants, paints, and building/construction materials. The SPIN database (2018) indicates a "potential exposure" with an "intermediate range of applications" (SE) and a "very probable use in article productions" (DK, NO, SE). Since the Substance is not classified only limited information is available. In a 2021 study the Substance was included in a list of chemicals of concern found in plastic toys (Aurisano et al., 2021).

Consequently, there are wide spread and wide dispersive consumer uses in which the Substance is applied as an additive. Emissions of the Substance can occur during various stages of the service life of products and after disposal and hence an exposure to the environment cannot be excluded.

In your comments to the proposals for amendment, you brought forward additional references from literature which detailed findings of the Substance in environmental media ([redacted] 2012; Nagorka and Koschorreck, 2020). In particular, you argued that these findings, in combination with the low water solubility and ready biodegradability of the Substance, make it very unlikely that relevant environmental concentrations are reached which could lead to a potential risk for organisms in the environment. Although your comments appeared not directly related to any proposal for amendment, ECHA responds to provide further clarity on the concern that needs to be investigated. ECHA disagrees with your conclusion, as at least one of the cited studies (Nagorka and Koschorreck, 2020) points towards the presence and potential for accumulation of the Substance in organic matter. Additionally, ECHA considers that thresholds for endocrine-mediated effects in the environment are difficult to determine and setting a safe concentration that would be sufficiently protective is not possible based on current knowledge. Hence, even the comparatively low concentrations, measured in the references you cited, show that exposure of the Substance to the environment occurs and poses a risk if the Substance acts as an endocrine disruptor. Hence, the concern for ED properties needs to be clarified.

1.3 Identification of the potential risk to be clarified

Based on two regulatory studies conducted according to OECD TGs and GLP contained in the registration dossier and disseminated in the ECHA database as well as two supporting studies from the published literature, the Substance may be an endocrine disruptor in the environment.

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

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

As explained in Section 1.1 above, the currently available information is not sufficient to conclude on the ED properties of the Substance in the environment. Consequently, further data is needed to clarify the potential risk related to the ED properties.

1.4 Further risk management measures

If the ED properties of the Substance in the environment are confirmed, the evaluating MSCA will analyse the options to manage this risk. Further regulatory risk management measures can be an identification of the Substance as a substance of very high concern (SVHC) according to Art. 57(f) of REACH. A SVHC identification would trigger additional information duties of producers and importers to ECHA according to Article 7(2) of REACH and information duties in the supply chain and for consumers according to Article 33 of REACH.

This regulatory risk management measure could potentially be followed by an authorisation and/or restriction process to substitute the use of the Substance and/or to minimise environmental exposure.

2. How to clarify the potential risk

2.1 Amphibian metamorphosis assay (OECD TG 231) with dietary exposure

a) Aim of the study

As detailed in Section 1.1, information is required to conclude on the potential ED properties of the Substance in the environment. The mammalian data available indicate that, although the mode of action remains unknown, the thyroid system is most likely the main target of an ED activity of the Substance. In your comments to the draft decision, you noted that some observations in rats point towards peroxisome proliferation and hepatic enzyme induction underlying the thyroid effects. However, ECHA points out that other modes of actions along the HPT axis cannot be excluded. Even if hepatic enzyme induction would be the only mode of action, it may also be relevant for species other than rats, e.g. amphibians. Therefore, a study is required that investigates the interference of the Substance with the normal function of the HPT axis in environmental vertebrates.

The requested amphibian metamorphosis assay (AMA) will provide basic mechanistic information on the interaction of the Substance with the thyroid system of vertebrates. As further detailed in Section 2.1.c), the requested study is concluded to be the most appropriate assay, since it can be combined with a feeding protocol and yields data which will be essential to further clarify the environmental ED concern. Furthermore, if adverse effects on metamorphosis are observed, they may be used to conclude on population relevance and hence whether the Substance fulfils the WHO/IPCS definition of an endocrine disruptor in the environment.

In your comment to the draft decision you indicated that the AMA is designed as a screen for thyroid activity in amphibians, and does not provide information on adversity or endocrine activity for use in assessing the environmental risks of an individual chemical. ECHA concurs that the AMA is recognised as a critical assay of the OECD Conceptual Framework (CF) (OECD, 2018) because amphibian metamorphosis provides a well-studied, thyroid-dependent process which responds to substances active within the HPT axis (OECD TG 231, 2009).

A proposal for amendment raised that the EFSA/ECHA guidance on Endocrine Disruptors

(ECHA/EFSA, 2018) states that "*in the case of amphibians, changes in thyroid histopathology should be considered adverse at the population level only when observed together with effects on development (i.e. delay or acceleration). This is due to the fact that thyroid histopathology often represents compensation to thyroid insufficiency (Marty et al., 2017). Nevertheless, changes in development in amphibians even if observed in the absence of investigation of thyroid histopathology are considered population relevant effects. However, the degree of delay or acceleration in the development that can be considered adverse at population level is uncertain (Marty et al., 2017).*" The evaluating MSCA considers the AMA as more than a screening test based on the potentially observed effects on metamorphic development which can be regarded to be of population relevance unless available information demonstrates the contrary. Thus, the requested AMA study can be conclusive with respect to the ED properties of the Substance.

You also considered the AMA requested here as technically challenging and that guidance is lacking on how the results of the proposed study design will be interpreted. However, ECHA notes that the dose-range finding study requested may provide an opportunity to reduce these technical challenges, in particular on the dietary exposure.

You noted in your comments to the draft decision that uncertainties will remain with regards to bioavailability and toxicokinetics of the Substance in amphibians.

You also noted that, in mammalian organisms, phthalate diesters are rapidly metabolised to their respective monoesters whereas studies cited in Boas et al. (2012) used phthalate diesters mainly in *in vitro* systems with no, or at least questionable, metabolic capacity. Based on these comments, a proposal for amendment to the draft decision was received to also include the measuring of the metabolites of the Substance in the study to yield a more complete picture of the internal body burden of the Substance in the exposed test animals. ECHA expanded the design of the study based on this proposal for amendment, for the reasons outlined in the paragraphs below.

In your comments to the proposals for amendment, you disagreed with this additional requirement, arguing that it is not appropriate to overlay a metabolic profile study from mammals over *Xenopus* considering their drastic differences. However, ECHA and the evaluating MSCA consider the additional measurement as feasible, relevant and as an added value since, as pointed out below in more detail, the mechanisms of metabolism and detoxification in amphibians may not be different from those of other vertebrates (EFSA, 2018). Thus, it is expected that both MPHP and OH-MPHP will be formed *in vivo* in amphibians. Furthermore, it is not the intention of these measurements to conclude in detail on the metabolism of the Substance in amphibians or on detailed absorption, distribution, metabolism, and excretion (ADME) parameters, but measuring these metabolites aims to provide a more detailed picture on the exposure situation of the animals at the end of the study. This will reduce the risk of inconclusive results of the requested study and hence make the proposed test design more robust.

ECHA considers that observed effects can be comprehensively related to the bioavailable fraction of the Substance. Recent evidence from the literature showed that the Substance quickly hydrolyses and metabolises into two monoesters, mono-(2-propylheptyl) phthalate (MPHP) and mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP) in both rats and humans. In rats, MPHP is further metabolised via omega-1 oxidation, yielding mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP) (Klein et al., 2016). In human volunteers orally exposed to the Substance, the maximum concentration of the monoester MPHP in blood occurs earlier than that of the Substance, which is consistent with the interpretation that systemic MPHP is initially governed by its hydrolysis in the stomach and the gastrointestinal tract. In humans, OH-MPHP is also a major metabolite excreted in urine

(Klein et al., 2018; Kessler et al., 2012; German Commission HBM, 2015). ECHA notes that the analytical methods published in Klein et al. (2016, 2018) studies for the Substance, MPHP and OH-MPHP have been developed in collaboration with a registrant. Therefore, ECHA considers the measurement of these two metabolites technically feasible.

ECHA agrees with your comment to the draft decision that, with the dietary protocol requested in this draft decision, exposure may occur through dermal uptake or filtering of the water. However, these routes of exposure are considered to be environmentally relevant and may contribute to the total systemic exposure and therefore any effects seen will be related to the Substance introduced into the test chambers. Furthermore, the uptake through these routes is expected to be low, due to low solubility of the Substance.

Furthermore, in your comments to the proposal for amendment you reiterated your concern that homogenisation of the whole larvae will not distinguish the Substance that has adsorbed to the skin or is inside the gastrointestinal tract but not systemically available to the organism from the bioavailable fraction of the Substance. ECHA agrees that measurement of the Substance alone may not sufficiently reflect systemic exposure to the Substance. However, the concentration of the Substance in the homogenates will likely be small and variable and may be representative of recent dietary exposure rather than dermal accumulation, given the very low water soluble fraction of the Substance that might adsorb to the surface of the larvae. Hence, ECHA considers that the analytical measurement of MPHP and OH-MPHP metabolites in homogenates will allow to demonstrate systemic exposure to the Substance and limit the possibility to yield inconclusive results. As you pointed out in your comment on the proposals for amendment, metabolisation of the Substance might also occur due to the release of enzymes during the homogenisation process rather than during *in vivo* metabolisation. However, ECHA considers this transformation as negligible as homogenisation and sample storage should be performed at or below 4 °C. Furthermore, if the Substance is transformed after homogenisation this reinforces the need to also measure metabolites of the Substance in the homogenates to avoid detecting only part of the Substance taken up.

Another proposal for amendment requested, in order to enhance the conclusiveness of the requested AMA study with dietary exposure, to add besides the thyroid histopathology (already required as a standard investigation in OECD TG 231) also liver histopathology and assessment of hepato-somatic index. As raised in the proposals for amendment by a Member State, conducting liver histopathology and determining the liver weight in the requested study will aid in determining whether the thyroid-mediated effects might be associated with hepatotoxicity or not.

In your comments to the draft decision you argue that liver hypertrophy was observed in the RDT study together with increased liver weights. In your comments to the proposals for amendment, you reiterate this argumentation and state that if the Substance would be systemically available to the tadpoles and would cause effects, it can be expected that the liver will be affected as well and probably cause an indirect effect on the HPT axis. Furthermore, in your comments to the draft decision you expressed doubt on the added value of conducting an AMA as probable adverse effects would be caused due to hepatotoxicity and not due to a direct effect on the endocrine system.

However, ECHA considers that effects on the liver in rats were seen at higher doses than the observed thyroid hypertrophy/hyperplasia. Thus, it needs to be clarified via which type of mechanism possible effects on the HPT axis in amphibians are mediated. Therefore, the decision was amended so that histopathology of the liver and assessment of the hepatosomatic index shall be included in the test protocol in order to enhance the conclusiveness of the requested study.

The AMA is further seen to provide more conclusive results than a LAGDA assay since the exposure of the animals via the diet would be more complicated in the LAGDA setting. This is due to the fact that the LAGDA is not only based on dry food, as the AMA is, but also includes e.g. shrimps and different food for different life stages of the animals. Hence, it is considered that it would be much more complex to achieve a uniform dotation of the various feed in a LAGDA setting compared to the AMA. Establishing a LAGDA protocol only based on dry food would need extensive pre-testing and is considered disproportionate here. Furthermore, the AMA mostly covers life stages where the animals are filter feeders and thus passively take up finely dispersed dry food particles via the water leading to a more reproducible exposure of the animals than in the LAGDA setting, which also covers developmental stages during which the tadpoles actively start to hunt for food. Due to the poor water solubility of the Substance, dietary exposure is in this case the only way to achieve significant uptake into the test animals.

In case the effects on metamorphosis remain inconclusive with regard to population relevance, but the mechanistic data obtained from the requested AMA strengthen the environmental ED concern by showing interference of the Substance with the HPT axis, the environmental ED concern should be followed up by further testing. The need for further information to clarify the remaining concern will be considered during the evaluating MSCA's follow-up evaluation of the information requested in the present Decision. Any subsequent requests for information to clarify the concern will be made in a new draft Decision after the follow-up evaluation is completed.

b) Specification of the requested study

Test method and test material

The test material must be representative for the Substance as put on the market, in particular with respect to the concentrations of isomers, constituents and impurities.

With respect to the low water solubility (2 ng/L) of the Substance, the AMA must be conducted according to OECD TG 231 with dietary exposure and the non-standard adaptations of the test guideline as requested and justified below.

Study design

The study design also includes the additional investigations resulting from the proposals for amendment:

- The Substance must be dissolved in acetone, afterwards mixed with dry Sera Micron® and evaporated with filtered air to dryness again. Caution should be taken to ensure that the Substance does not crystallise as the solvent is removed.
- A negative control with Sera Micron® similarly treated with acetone without the Substance must be prepared. Recommendations regarding a potential inclusion of a second control group using non-pre-treated Sera Micron® are included below in section "Recommendations for considerations of Registrants".
- The test must be conducted under flow-through conditions to ensure an acceptable water quality.
- The spiked Sera Micron® must be fed as a suspension prepared with dilution water. This solution must be prepared shortly before the beginning of the test and divided into individual aliquots, e.g. in scintillation vials, so each aliquot holds enough food for an entire treatment for a single day.
- Before the beginning of the test the intended concentration of the Substance in the spiked food must be verified by analytical measurements. To check the concentration of the treated diet, triplicate samples of the dosed food must be extracted with a

suitable extraction method and the Substance concentration in the extracts must be measured by an appropriate analytical method.

- A dose range-finding test must be performed in order to reduce technical challenges and increase the robustness and quality of the data obtained in the main study. The dose range-finding will clarify at which dose level systemic (toxic) effects may occur to decide on the top dose for the main study.
- At least five concentration levels with four replicates must be tested in the main study to obtain a full dose-response relationship that also allows for a sound LOEC/NOEC derivation for further regulatory measures if needed. Since the variability in bioavailability and toxicokinetics of the Substance are unknown in amphibians, the dose level differential between the highest and lowest dose levels should be increased at about two orders of magnitude. The highest dose level must give a clear systemic (i.e. non endocrine-specific) toxicity.
- The concentration of the Substance and the two metabolites mono-(2-propylheptyl) phthalate (MPHP) and mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP) in the animals, must be analytically measured. These data are essential to adequately interpret the assay results, especially if no hints for an endocrine activity of the Substance can be found.
  A conclusion that the Substance is not an ED for the environment can only be drawn when you can demonstrate that the animals were adequately exposed to the different test concentrations of the Substance. Analytical measurements of the internal body burden of the test Substance, MPHP and OH-MPHP must be performed in full body homogenate at the end of the test.
  From each treatment group at least three animals must be pooled and analysed using an adequate sample preparation and analytical set up.

- In addition to thyroid histopathology (already required as a standard investigation in OECD TG 231), the liver histology at day 21 (study termination) must be investigated in the randomly chosen tadpoles for thyroid histopathology (5 tadpoles per replicate tank).
  Tissue collection, fixation and analysis must be performed as explained in OECD series on testing and assessment No. 228[3]. It should be noted that after stage 60, measurement of wet weight cannot appropriately be used in statistical analyses (replicate means or medians) for differences in growth because tadpoles show a reduction in size and weight due to tissue resorption and reduction of absolute water content. In such case you can consider only tadpoles ≤ stage 60 if it concerns only a small portion of the test animals that is removed from the statistical analysis or if an increased number of tadpoles shows development beyond stage 60 (>20%) in one or more nominal concentration(s), then a two-factor ANOVA with a nested variance structure should be undertaken on all tadpoles. Guidance is provided in Annex 3 of the OECD TG 231 (AMA).

In light of the proposals for amendment, leading to the expansion of the study design, you have also provided comments reiterating your concern with the feasibility of the overall, currently expanded, study design. For the sake of completeness, they are addressed in separate paragraphs.

In particular, you claimed that ECHA and the commenting member state "*obviously propose to the registrants to undertake an Amphibian Metamorphosis Assay (AMA) using unvalidated deviations for regulatory purposes*".

[3] Guidance Document on Histopathology Techniques and Evaluation (OECD, 2015) for the Larval Amphibian Growth and Development Assay.

ECHA considers that although the dietary route is not commonly used, OECD TG 231 considers this route as an option in case it is not possible to administer a chemical via the water, e.g due to physchem properties. The Substance is poorly soluble in water and can easily adsorb to particulate matter based on its physical and chemical properties. Furthermore, several long-chain phthalates have been shown to adsorb to particulate matter and accumulate in the sediment, where tadpoles tend to feed. Also, phthalate-laden food minimally leaches into the water from the food; and thus, the primary route of phthalate exposure to tadpoles is expected to be dietary (Larsson and Thuren, 1987). Therefore, the dietary exposure is in this case the only way to achieve significant uptake into the test animals.

You also stated that:

- exposure concentration and dose cannot be inferred directly from body burden and thus, effects directly attributable to exposure to the test substance cannot be accurately determined.
  To conclude on the ED properties of the Substance ECHA considers the correlation of the body burden to adverse effects as sufficient. There is, with respect to this, no need to infer from the body burden to the exposure concentration.

- the amount of tissue required to acquire the analytical sensitivity needed was grossly underestimated and will impact the acquisition of other data requiring an excessive number of test organisms.
  ECHA considers the full-body homogenates of the pooled tadpoles as sufficient to yield aliquotes that fulfill the needs for adequate analytical sensitivity. Hence, ECHA cannot understand why this should impact the acquisition of other data or will lead to an excessive number of test animals.

As raised in the PfAs by a Member State, conducting thyroid histopathology, which is a standard data requirement in the requested AMA study, is a crucial parameter to assess the thyroid activity of the Substance: As per the ECHA/EFSA Guidance on Endocrine Disruptors (EFSA/ECHA, 2018) in the case of amphibians, changes in thyroid histopathology should be considered adverse at the population level only when observed together with effects on development (i.e. delay or acceleration). This is due to the fact that histopathological findings in the thyroid often represent compensation to thyroid insufficiency (Marty et al., 2017). Nevertheless, changes in development in amphibians even if observed in the absence of investigation of thyroid histopathology are considered population relevant effects. However, the degree of delay or acceleration in the development that can be considered adverse at population level is uncertain (Marty et al., 2017). Therefore, such effects should be considered relevant at the population level unless available information demonstrates the contrary. Furthermore, the ED guidance stipulates: "*Accelerated and asynchronous development (characterised by disruption of the relative timing of the morphogenesis or development of different tissues and the inability to clearly establish the developmental stage of an animal by morphological landmarks) are thyroid-mediated effects. Delayed development is not by itself an indicator of anti-thyroidal activity and needs to be confirmed by histopathological analysis of the thyroid.*"

In your comments to the proposals for amendment, you also proposed for the first time an alternative tiered approach to the test design, covering the following steps (the following is quoted verbatim from your comments):

a) *Perform an initial 21 day feasibility pilot study focusing on acetone addition to Sera Micron® powder which will include a standard Sera Micron® control (no acetone) and Sera Micron® with added acetone subsequently allowed to evacuate to assess the impact of the solvent ob quality and performance of the feed.*

b) *If this feasibility study indicates dietary administration is feasible and will not compromise the study, a second pilot evaluation step will be initiated in which DPHP will be added to the diet as described. Verification of homogeneity of DPHP in the fully solubilized diet will then be performed. If the DPHP in the diet is determined to be homogeneous, the AMA will be performed as proposed by ECHA with the following modification: only DPHP will be measured in the diet (initially only), test solution after application at set interval, and tissue residue (at conclusion of exposure only). No measurements of metabolites will be performed.*
c) *If at any point, steps a or b suggest lack of feasibility; the ECHA-proposed design will be supplanted by the design described in step d.*
d) *A standard AMA will be performed following an adequate range-finding study. A solid-phase saturator column series (4 columns in series) will be used to produce a stock solution at maximum soluble levels. Practical water solubility will be assessed as described in OECD 231. The practical water-soluble concentration will represent the high concentration unless a lower MTC is identified in a range-finding study. If the registrant determines that it is unlikely adverse effects will be observed below the practical limit of water solubility, a limit test may be performed. All other criteria that apply to an OECD 231 AMA study will apply.*

ECHA reflected on this tiered approach and considers the following:
- information on the effect of the solvent on the quality and performance of the food is beneficial to ensure that the physical nature or the nutritional quality of the food is not altered. Therefore, a non-solvent control could be included as a separate control group to the main AMA study or the dose-range finding study (see Recommendations for considerations of Registrants below);
- in terms of animal welfare, it is not necessary to carry out a separate pilot study in addition to the dose range-finding study;
- a liquid-liquid saturator method cannot yield concentrations of the Substance that are higher than water solubility. As pointed out above, there is a concern that the tadpoles accumulate the Substance more via their food than via the water soluble fraction.

Thus, to reflect this real-case scenario, a feed-based exposure is requested to lead to possibly higher internal concentrations than can be expected from solely water-borne exposure. Additionally, the above described and requested verification steps may allow to demonstrate homogeneity of the Substance in the diet and no further feasibility study is needed. Thus, ECHA disagrees with the proposed tiered approach and maintains the requested study including exposure of the animals via the food as the most realistic pathway in this specific case taking into account the physical and chemical properties of the Substance.

Recommendation for considerations of Registrants

In your comments to the PfAs, you raised arguments regarding suspected issues with the quality and consistency of the food due to the use of acetone. To alleviate your concerns, as explained above, addition of a non-solvent control as a separate control group to the main AMA study or the dose-range finding study could be considered. Therefore, you are recommended to consider inclusion of a second control group with pure Sera Micron® untreated with acetone, in addition to the already requested control using acetone-treated Sera Micron®. Inclusion of a second control group would allow to discern whether treatment of the Sera Micron® with acetone alone would in fact impact the quality and consistency of the food.

Request for the full study report

You must submit the full study report which includes:

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

This will enable the 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 is:

- appropriate, because it will provide information to further clarify whether the Substance shows endocrine activity and related adverse effects in the environment via an interaction with the thyroid system. This will enable the evaluating MSCA to either conclude on potential ED properties of the Substance or to decide whether and which further testing may be necessary to come to a conclusion regarding environmental ED effects;
- the least onerous measure because there is no equally suitable alternative method available to obtain the information that would clarify the potential hazard.
  If inconclusive, possible alternatives would be a level 4 test of the OECD Conceptual Framework (CF) (OECD, 2018) such as a LAGDA (OECD TG 241). This assay could clarify the proposed thyroidal activity as well as EAS modalities of the Substance. However, due to the extremely poor water solubility of the Substance and the difficulties to establish a feeding protocol, it is very likely that a LAGDA will yield inconclusive results.
  Level 3 testing with fish, e.g. according to Fish Short-Term Reproduction Assay (OECD TG 229) or the 21-day fish assay (OECD TG 230) and higher tier fish testing like a Fish Sexual Development Test (OECD TG 234) or the Medaka Extended One Generation Reproduction Test (OECD TG 240) are concluded to be less appropriate at this stage compared to an amphibian study since the available mammalian data clearly point to thyroidal activity of the Substance. As explained above, validated thyroidal endpoints are not yet covered by the available OECD fish test guidelines.

  One of the Member States Competent Authorities suggested in a proposal for a amendment that, while it agrees with the requested AMA, a Xenopus Eleutheroembryonic Thyroid Assay (XETA, OECD TG 248) assay should be reflected in the decision. XETA is an aquatic screening test and may provide some mechanistic information. However, the available data clearly point to an interaction of the Substance with the HPT axis although the underlying mode of action is currently unclear. Therefore and in accordance with the ECHA/EFSA guidance, an AMA (OECD TG 231) is more appropriate as it covers a broader range of pathways and endpoints. Additionally, the AMA can provide more sound information on adverse effects on metamorphosis and hence reduce the likeliness to the need of follow-up testing to conclude on the ED properties, which must be done in case of a positive XETA. Additionally, as the available mammalian data (presented above) raise a substantial concern as to whether the Substance shows T-mediated activity, it is very unlikely that the XETA would be completely negative. Finally, the XETA assay cannot be perfomed via a feeding protocol and hence is not able to address the very low water solubility of the Substance. Therefore the XETA is not considered as an appropriate alternative to the requested AMA.

In your comments on the draft decision, you suggested an alternative test in rodents instead of the required AMA, to obtain information on the mechanism of action of the observed thyroid effects in mammals. As explained in section 2.1.a, ECHA does not consider the proposed test to be appropriate to further investigate and clarify the identified endocrine disruption concern for the environment. The endpoints in the requested study design comprise effects on metamorphic development, comparable to those included in the LAGDA protocol, and hence can be conclusive with regard to population relevant adverse effects. This is also stated in the OECD guidance document 150.
The available data already provide evidence that the Substance acts via the HPT axis in vertebrates. The mechanistic information provided by the requested AMA will serve to increase the clarity on whether the observed adverse effects could also be mediated via a non-ED mechanism which you suggested in your comments to the draft decision. Hence, ECHA considers that there is a high likelihood that (in case of a positive outcome) the requested AMA can be used to conclude on adversity and hence can be used for ED identification taking all available evidence into account.

Since there are to-date no environmental studies investigating adversity, available for the Substance, a positive outcome of the AMA will add further and relevant information to the existing database. Based on this information, a conclusion with respect to the environmental ED properties of the Substance can most likely be drawn. Hence, the requested study is the least onerous measure compared to e.g. higher tier testing with a LAGDA study at this stage of the assessment.

Additional investigations may be required in a follow-up investigation to clarify the concern for human health which arises from the available mammalian data set if deemed necessary with regard to potential risk management measures. ECHA considers that the request of a single study which would sufficiently investigate the concern for endocrine disruption both with respect to the environment and human health is not possible. Therefore, ECHA considers that the concern for the environment should first be clarified with the AMA requested in this decision.
Furthermore, with respect to the concern raised, there is no other experimental study available at this stage that will generate the necessary information and does not require the testing of vertebrate animals.

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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 258-469-4, CAS RN 53306-54-0) in the Community rolling action plan (CoRAP) to be evaluated in 2020. Germany is the competent authority ('the evaluating MSCA') appointed to carry out the evaluation.

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

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

Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 11 March 2021.

Decision-making

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

For the purpose of this decision-making, dossier updates made after the date the draft of this decision was notified to you (Article 50(1) of REACH) will not be taken into account.

(i) Registrant(s)' commenting phase

ECHA received your comments and forwarded them to the evaluating MSCA. The evaluating MSCA took your comments into account (See Appendix A).

(ii) Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment. Subsequently, the evaluating MSCA received proposals for amendment to the draft decision and modified it accordingly (see Appendix A and B).

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

ECHA invited you to comment on the proposed amendments. You provided comments. The comments extending to the proposals for amendment were taken into account and the draft decision was amended accordingly (see Appendix A and B).

The following aspects of the comments received were not taken into account at this stage as they were considered to be outside of the scope of Article 52(2) and Article 51(5): The reiteration of the explanation of the observed effects in rats with a secondary mode of action (hepatotoxicity) and the argumentation against the justification of the AMA request in Section 1.3 of the decision based on the hazard and exposure information.

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement during its MSC-78 meeting and ECHA took the decision according to Article 52(2) and 51(6) of REACH.

The deadline of the decision was exceptionally extended by 12 months from the standard deadline granted by ECHA to consider currently longer lead times in contract research organisations.

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[4].

Test material

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

1. *Selection of the Test material(s)*

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

2. *Information on the Test Material needed in the updated dossier*

   a) You must report the composition of the Test Material selected for each study, under the 'Test material information' section, for each respective endpoint study record in IUCLID.
   b) The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested, in this case information on the exact stereoisomers of the Test Material that were used.

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"[5].

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