Helsinki, 27 January 2023

Addressees
Registrants of 4,4'-isopropylidenedi-o-cresol listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: 4,4'-isopropylidenedi-o-cresol
EC number: 201-240-0
CAS number: 79-97-0

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. Combination of Fish Short Term Reproduction Assay (FSTRA - according to OECD TG 229) and Fish Sexual Development Test (FSDT – according to OECD TG 234) must be conducted on adult Zebrafish (*Danio rerio)*, Japanese medaka (*Oryzias latipes*) or Fathead minnow (*Pimephales promelas*) with the Substance, taking into account the following specifications:

    - At least four test concentrations must be used; the highest test concentration must cause clear systemic (i.e., non-endocrine-specific) toxicity or must be set at the maximum water solubility in case that the LOEC of non-endocrine-specific toxicity is above water solubility of the Substance.
    - The exposure must take place via testing water and the use of a solvent must be avoided.
    - The test must be started according to the protocol laid down in OECD TG 229 using adult fish and must cover all standard endpoints of the OECD TG 229 and of the OECD TG 234.
    - You must document the fertility (i.e., the number of fertilised and viable eggs) after 21 days (as per the OECD TG 229).
    - The OECD TG 229 must be conducted with four replicates to match the requirements of the OECD TG 234 protocol and to have four independent replicates throughout the whole test design. The same exposure concentrations must be used as those used in the OECD TG 229.
    - The OECD TG 234 protocol must be started with the eggs collected from the breeding pairs of the OECD TG 229 study, for each concentration and control, in the 4**-** or 8**-**cell stage.
    - Histopathology of gonads (evaluation and staging of oocytes and spermatogenic cells) must be conducted at the end of the OECD TG 234 study in all concentrations and the control(s) and at the end of OECD TG 229 only when there is an impact seen on fecundity and fertility in F0 fish, unless plasma vitellogenin (VTG) or secondary sex characteristics are clearly impacted; the number of fish to be examined for OECD TG 234 and 229 must be 16 per test and control replicates.
    - The histopathology of the liver must be included in both tests (at the end of OECD TG 229 and OECD TG 234) in all concentrations and the control(s). The number

of fish to be examined must be half the number of fish in the OECD TG 229 and 16 per test and control replicates in the OECD 234.
- If a test with Japanese medaka is conducted, the genetic sex and secondary sex characteristics must be examined.

Deadlines

The information must be submitted by ***4 May 2026***.

Conditions to comply with the information requested

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

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

You will find the justifications for the requests in this decision in the Appendix entitled ''Reasons to request information to clarify the potential risk'.

You will find the procedural steps followed to reach the adopted decision and some technical guidance detailed in further Appendices.

Appeal

This decision may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] by Mike Rasenberg, Director of Hazard Assessment.

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Basis for substance evaluation

The objective of substance evaluation under REACH is to allow for the generation of further information on substances suspected of posing a risk to human health and/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 and/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.

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

1. Potential risk

1.1 Potential hazard of the Substance: Potential endocrine disrupting properties

Following the assessment of the available relevant information on the Substance, the evaluating MSCA and ECHA have identified a potential hazard of endocrine disrupting (ED) properties for the environment, more specifically related to the oestrogenic, androgenic and and/or steroidogenic (EAS) mode of action (MoA) of the Substance, which must be clarified.

Publicly available scientific literature and registration data from *in vitro* and *in vivo* tests raise a concern for ED properties of the Substance in environmental species, which is suspected to be mediated via the hypothalamic–pituitary–gonadal (HPG) axis for EA MoA and hypothalamic–pituitary–thyroidal (HPT) axis for the thyroidal (T) MoA. Furthermore, *in silico* analyses yielded positive results for endocrine activity of the Substance, and its structural similarity to other bisphenols (Pelch et al., 2019; Kitamura et al., 2005b) with known ED properties, such as Bisphenol A (BPA; EC/List number 201-245-8) and Bisphenol B (EC/List number 201-025-1), also underpin the identified hazard concerns.

The current request aims only at clarifying the EAS concern (the strongest) and not the concern related to the T MoA, which may need to be further investigated at a later stage.

a) *In silico* and *in vitro* data

The evaluating MSCA analysed the data available for the Substance in the endocrine disruptor screening program EDSP21 (US EPA) based on ToxCast high-throughput screening (HTS) data evaluation. The Substance was active in 23 out of 55 *in vitro* high-throughput screening assays:

- 17 of the 23 assays were disregarded due to e.g., high deviation between two groups of replicates/runs, not reliable model fitting and/or derived AC50;
- 6 of the 23 assays were regarded as reliable: the Substance screened as a weak oestrogen receptor (ER) antagonist (1 study) and a moderate androgen receptor (AR) antagonist (3 studies). Furthermore, there were 2 assays indicating thyroid-stimulating hormone receptor (TSHR) agonistic and steroidogenic activity.

In line with the ToxCast *in vitro* high-throughput screening results, (anti)oestrogenic and antiandrogenic activity was also observed in several *in vitro* assays with the Substance identified in the scientific literature as discussed below. One study included in the registration dossier (Kitamura et al., 2005b) confirmed oestrogenic and anti-androgenic activity for the Substance.

Furthermore, effects of the Substance on thyroid receptor (TR), glucocorticoid receptor (GR), progesterone receptor (PR) and mineralocorticoid receptor (MR) were seen in *in vitro* studies (Chen et al., 2020; Grimaldi et al., 2019; Kitamura et al., 2005a; Zhang et al., 2017).

Oestrogenic and antioestrogenic activity of the Substance in vitro and in silico

- A mechanistic *in silico* modelling study suggested simultaneous ER agonistic and antagonistic activities of the Substance (Chen et al., 2020). Molecular modelling of the Substance predicted ERα binding, favouring the agonist conformation of the receptor protein, i.e., in the 17β-oestradiol (E2) and coactivator peptide src-1 bound state (Pelch

et al., 2019).
- Modelling of the Substance using the Danish QSAR database[2] yielded a positive outcome for ERα binding and activation.
- Oestrogenic effects of the Substance were noted in a yeast oestrogen screening assay (Okuda et al., 2011).
- The Substance showed clear agonistic activity on ERα and/or ERβ in several assays using human derived cell lines (Chen et al., 2020; Grimaldi et al., 2019; Kitamura et al., 2005b; Pelch et al., 2019). Gene expression analysis on two well-characterised ER target genes (GREB1 and PGR) indicate that the Substance can directly alter their transcriptional regulation (Pelch et al., 2019). However, in another study (Lin et al., 2021) the Substance did not alter gene expression of steroidogenic genes in H295R cells and showed no oestrogenic activity in MVLN cells (a bioluminescent MCF-7-derived cell line to study the modulation of oestrogenic activity).
- While the Substance did not inhibit aromatase (CYP19) activity in a human-derived cell line, it inhibited the oestrogenic activity of E2 on oestrogen receptor (ER)-mediated vitellogenin (VTG) production in hepatocytes from male carp (*Cyprinus carpio*) (Letcher et al., 2005). Similarly, several reporter gene assays using human ERα and/or ERβ reporter systems, found that the Substance elicits weak oestrogen antagonistic activity, particularly towards human ERα, while ERβ agonistic activity was slightly decreased by treatment with the Substance (Chen et al., 2020; Maruyama et al., 2013). Another reporter gene assay using human ERα and ERβ reporter systems in turn did not confirm an antioestrogenic mode of action of the Substance (Pelch et al., 2019).

Overall, several publicly available *in vitro* studies show that the Substance can elicit clear oestrogen agonistic activity. Its potency was roughly in the range of that of BPA (e.g., EC_{50} BPC 0.42 µM vs. BPA 0.63 µM, Kitamura et al. (2005b)). Furthermore, weak oestrogen antagonistic activity was reported for the Substance (Letcher et al., 2005).

These findings are in line with results from ToxCast HTS Data evaluation, which as well showed oestrogen antagonistic activity for the Substance in the one reliable assay available. Oestrogen agonistic activity was also reported in some of these ToxCast assays. However, due to large data variability or concomitant test-specific cytotoxicity, the evaluating MSCA considers the reliability of these tests as borderline or as insufficient for a firm assessment.

Antiandrogenic activity of the Substance in vitro and in silico

ToxCast HTS Data evaluation (accessed on 21 July 2021) yielded three reliable assays with regards to AR binding and AR-mediated activity, respectively. The human AR-binding assay was positive for the Substance and results of the other two reporter gene assays in human-derived cells are indicative of an antiandrogenic mode of action of the Substance.

In addition, there were three reporter gene assays identified in the scientific literature which demonstrated that the Substance is a potent antiandrogen in human-derived cell lines (Grimaldi et al., 2019; Kitamura et al., 2005b; Pelch et al., 2019). No androgen agonism was observed in these *in vitro* assays. Moreover, the Substance did not act as an AR agonist in a molecular modelling approach (Pelch et al., 2019). In a cell assay measuring the steroid hormone production (Lin et al., 2021), the Substance decreased the testosterone concentration and increased the E2/T (oestradiol to testosterone) ratio which is indicative of an antiandrogenic mode of action (MoA).

[2] https://qsardb.food.dtu.dk/db/index.html

b) *In vivo* tests

In vivo tests in mammals - HPG-axis

- Studies with the Substance

Mechanistic *in vivo* studies are not available.

Repeated dose toxicity tests contained in the registration dossier (OECD TG 407 and OECD TG 421) show effects of the Substance on EAS sensitive endpoints in mammals, i.e., effects on reproductive organ weights (ovaries, seminal vesicles, testes, including histopathological findings in single individuals), number of implantations, fertility index. However, diagnostic endpoints related to the mode of action (such as anogenital distance (AGD) and nipple development in offspring of treated rats) were not affected in the OECD TG 421 study.

The identification of a specific MoA of the Substance regarding the identified fertility effects (ovary, uterus, testes, fertility index) is currently not possible.

The Substance elicited reproductive toxicity in the reproduction/developmental toxicity screening test (OECD TG 421, gavage) available in the registration dossier. Reproductive toxicity comprised a significantly decreased number of implantation sites and significantly decreased ovary weights in female rats without corroborating decreases in bodyweight at doses ≥ 250 mg/kg bw/d. Histopathological examinations of the ovaries are not available. At the highest tested dose of 1000 mg/kg bw/d, weights of seminal vesicles, testes, ovaries and uteri were affected by treatment with the Substance. In single individuals, adverse histopathological findings were noted at the high dose, including cysts in the cervix of the uterus, minimal focal atrophy of seminiferous tubules, minimal lymphocyte infiltration into the interstitium of the epididymis and minimal sperm granuloma. Moreover, 3 out of 12 females did not become pregnant at 1000 mg/kg bw/d, which yielded a decreased fertility index of 75% (91.7% in controls; effect not statistically significant but outside historical controls).

In a supporting oral subacute OECD TG 407 study, treatment effects on absolute testes weight (-8%, only after 14 days of recovery) and absolute ovaries weight (-18%) were noted at a high dose of 1000 mg/kg bw/d as well. Sperm hypoplasia was confirmed in one high-dose male with macroscopically enlarged testes. However, only testes of this one male were assessed, while histopathological evaluation of reproductive organs was generally not performed in this study. Moreover, no information on additional sperm parameters, spermatogenesis and/or folliculogenesis stages was included in the study report.

Treatment-related effects on pups were not observed in the OECD TG 421 study. No specific developmental toxicity study (e.g., a PNDT study according to OECD TG 414) with the Substance is available, hampering a firm assessment of potential hazards of the Substance with respect to developmental toxicity.

- Supporting information from structurally similar substances

In studies with the structurally similar substances Bisphenol AF (BPAF; EC/List number 216-036-7) and Bisphenol S (BPS; EC/List number 201-250-5), similar adverse fertility effects were observed in OECD TG 421/422 studies when compared to the above-mentioned study results on the Substance, but effects were noted already at lower test doses.

Effects of BPAF and BPS on female rats included irregularities in the oestrus cycle, ovarian cysts, and decreased numbers of implantation sites. Effects in males included Leydig cell atrophy, reductions in sperm counts, effects on male mammary glands and changes in the weight of male reproductive organs. BPS and BPAF also decreased the number of litters/pair and further reduced the fertility index down to 60% and 0%, respectively, at 300 mg/kg bw/d. It is noted that effects on fertility (especially on fertility index) were not as marked for the Substance when compared to the other two bisphenols.

The use of sodium carboxy-methyl-cellulose solution as vehicle (used in the studies with the Substance and BPS, but not with BPAF) may have reduced the oral absorption of the test substance(s) resulting in observable differences in effective doses. The Substance might be less potent in eliciting adverse fertility effects *in vivo*, potentially because of the additional methyl substituents at the phenol rings of the Substance.

Differences in toxicity of the Substance and BPA due to the occurrence/lack of these methyl groups were, for instance, suggested in a recent *in vitro* study (Padberg et al., 2019). This study showed that the Substance with its additional methyl groups affects cellular and physiological responses differently compared to the 'parent compound' without these methyl groups (i.e., BPA). Differences were observed particularly with regards to effects on mitochondrial function, cell proliferation and regulation of pro-inflammatory cytokines in a human derived liver cell line (Padberg et al., 2019). Binding affinity to specific hormone receptors or endocrine-mediated activity was not assessed in this comparative study.

The similarity of the types of reproductive effects elicited by BPA, BPAF, BPS and BPC (the Substance) *in vivo* are overall indicative of a similar MoA of all these bisphenol substances, despite the observed differences in effective dose levels, and suggest ED-mediated modes of action of the Substance on the HPG-axis.

In vivo tests in fish/aquatic vertebrates

For the Substance, no studies are available in fish or aquatic vertebrates measuring endpoints which are sensitive to or diagnostic of endocrine modalities.

c) Summary and conclusion on ED hazards

There are indications from *in vitro* and *in vivo* studies that the Substance interacts with the endocrine system of mammals.

- The available data indicate that the Substance particularly interacts with the HPG axis in mammals affecting EAS-sensitive reproductive parameters and hence probably elicit ED-mediated adverse effects. *In vivo*, adverse effects on fertility parameters were observed in rat studies, for which specific modes of action of the Substance could not be identified.
- Respective *in vitro* data are indicative of (anti)oestrogenic and antiandrogenic properties of the Substance.

Since the HPG axis is well conserved within all vertebrates, available mammalian data also raise a significant concern for ED properties of the Substance in the environment. Particularly, the effects of the Substance on fertility and thyroid function suggest a biological relevance of these study results for environmental species, potentially leading to adverse population effects. However, the available mammalian data are not, on their own, sufficient to conclude on the environmental concern.

In addition to the hazard concern identified for the Substance itself, structurally related substances (bisphenols) have also exerted concerns regarding ED and reproductive toxicity:

- BPS, 2,2’,6,6’-tetrabromo-4,4’-isopropylidenediphenol (TBBPA; EC/List number 201-236-9) or 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol (EC/List number 204-279-1) are currently under substance evaluation due to their suspected ED properties in humans and/or the environment.
- The RAC concluded on the need for a harmonised classification of BPS, BPAF and BPA as Repr. 1B, H360FD (for BPS) and Repr. 1B, H360F (for BPA and BPAF), due to their adverse and (potentially) ED-mediated effects on reproduction.
- BPA and BPB were identified as SVHC substances due to their proven ED effects on human health and the environment.

Overall, ECHA concludes that the currently available information is not sufficient to draw a conclusion on the concern raised regarding the EAS MoA of the Substance, particularly in aquatic species. Therefore, further information on potentially adverse and population-relevant effects mediated via endocrine modes of action is required for the Substance.

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

1.2 Potential environmental exposure

Environmental exposure to the Substance is likely based on the following information:

- The Substance can be regarded as a likely substitute for specific environmentally relevant uses of BPA and further bisphenols, e.g., in thermal papers (which is a current registered use of the Substance). Thermal papers have been shown to contribute to the release of BPA and BPS to the aquatic environment via paper recycling processes and hence the same exposure potential is assumed for the Substance if used as a dye in thermal papers.
- Based on the identified uses, the Substance is included in polymers and articles with an open and wide dispersive use from which release into the environment during service life and end of life stages cannot be excluded. Additionally, if used as a substitute for environmentally relevant BPA and BPB (also added to the Candidate List as SVHC based on its endocrine disrupting properties) uses, the exposure potential will further increase.
- In two biomonitoring studies, the Substance was found in urine and/or blood samples of female patients (age 18-40) suffering from unspecified endocrine disorders (Milczarek-Banach et al., 2021; Owczarek et al., 2018). The studies include no statement on potential sources of the Substance. However, these studies demonstrate that the Substance is already released from articles within the EU and hence, also exposure of environmental compartments cannot be excluded.

Therefore, exposure to the environment cannot be excluded.

1.3 Identification of the potential risk to be clarified

Based on all information available in the registration dossiers and in the published literature, the Substance is suspected to be an endocrine disruptor in the environment according to the WHO/IPCS definition.

More specifically, as explained in Section 1.1 above, the available information is not sufficient to conclude on the hazard and in particular the ED properties in the environment. Consequently, further data is needed to clarify the potential risk related to endocrine properties, in particular the EAS modality. In addition, exposure to the environment cannot

be excluded. Therefore, based on the available hazard and exposure information, the Substance may pose a potential risk to the environment.

1.4 Further risk management measures

If the ED properties of the Substance are confirmed, the evaluating MSCA will analyse the options to manage the risks. New regulatory risk management measures could be an identification as a substance of very high concern (SVHC) according to Article 57(f) of REACH as an ED for the environment. The 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 or a restriction proposal to further limit the use(s) of the Substance. These measures would result in stricter risk management measures, such as improved measures at manufacturing sites, better waste management and revised instructions on safe use, if appropriate.

2. How to clarify the potential risk

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

a) Aim of the study

As detailed in section 1.1., information on endocrine activity and subsequent adverse effects in environmental species are required to conclude on the potential ED hazard. More specifically, the available *in vitro* and mammalian data point to potential adverse effects of the Substance on the sexual development and reproduction capacity of wildlife vertebrate species in relation to an EAS MoA. Therefore, a study that investigates potential ED properties in fish is required.

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

b) Specification of the requested study

Test material and concentration

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

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

In your comments to the draft decision, you disagree with the request of testing at least four test concentrations. You point out (1) that neither the OECD TG 229 nor OECD TG 234 require four or more concentrations, but at least three concentrations are proposed

in the respective guidelines, (2) you propose to base the number of test concentrations selected on the range-finding tests and discussions with laboratory experts and (3) you explain, that including an extra concentration would lead to an increase in the number of fish used in the study.

ECHA agrees to base the test concentrations selected on the range-finding tests and discussion with laboratory experts. However, ECHA maintains the requirement for at least four test concentrations. In addition to the reasons stated above, at least four testing concentrations are required:

1) To minimise the risk that this study yields inconclusive results from a regulatory perspective, and hence would potentially need to be followed up by another animal study to clarify the concern;
2) To ensure that the highest concentration tested elicits systemic toxic effects. This will allow for an adequate concentration spacing and will minimise the risk of not including the highest possible concentration at which ED specific effects are most prominent.
3) Furthermore, such a concentration setting will allow for a sound regulatory assessment of all effects evoked by the Substance.
4) The minimum of three test concentrations described in the OECD guidelines can be sufficient to identify a substance as an ED but might be insufficient to conclude that the Substance tested is not an ED.
5) The FSDT is an extension of the OECD TG 210 and as such, is principally able to also assess systemic toxicity and to derive a full dose-response curve.
6) Additionally, if e.g. in the highest test concentration at the end of the first part of the study (OECD TG 229) there are not enough eggs to start the second part of the study (OECD TG 234), this concentration can be left out and it still would be possible to proceed with the remaining three test concentrations required as a minimum in the respective OECD TG 234 and to yield conclusive results for the ED properties of the Substance.

The highest test concentration must cause clear systemic (i.e., non-endocrine-specific) toxicity or must be set at the maximum water solubility in case that the LOEC of non-endocrine-specific toxicity is above water solubility of the Substance.

In your comments to the draft decision, you raise concerns on the selection of the highest test concentration. You refer to the OECD TGs 229 and 234 requirements, which state that, the highest test concentration should be lower than 10% of the LC50 (acute adult), or 10 mg/L, or the maximum solubility in water, whichever is lowest. Therefore, you propose that "*the highest test concentration must follow the OECD TGs in that the solubility limit, MTC, acute toxicity data, range-finding data, etc. should all be considered whichever is lowest should be selected*."

ECHA disagrees with your proposal to refer to the OECD TGs 229 and 234 requirements for dose setting for the following reasons:

1. To yield regulatory conclusive results, the highest tested concentration should be set at the LOEC for non-specific systemic toxicity. This will ensure a full dose-response curve and will minimize the risk of missing the dose window where ED specific effects are most prominent.
2. Additionally, the risk of obtaining inconclusive results that might trigger further animal testing will be minimised. If the LOEC for non-specific effects is above the water solubility, the highest tested concentration must be the maximum water solubility of the Substance. The decision was amended to clarify this issue.

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

Route of exposure

The Substance is soluble in water (22 mg/L). Therefore, the exposure must take place via testing water, as described in OECD TG 229 and OECD TG 234. The use of a solvent must be avoided.

Specification of the assay protocol

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

ECHA agrees with your comment regarding the age of fish for zebrafish at the start of the test and amended the draft decision accordingly. Additionally, ECHA notes that, irrespective of the fish species chosen, according to the OECD TG 229 assay reproductively mature animals that are actively spawning must be used. This can be commonly assumed at average spawns of >10 eggs/female/day for each species. Thus, the age of the fish to start the requested test with can vary according to culture conditions (e.g., water temperature).

In your comments to the draft decision, you request ECHA to include the possibility to select an alternative fish species (i.e., Fathead minnow or Japanese medaka). You considered that "*Zebrafish have no quantifiable secondary sex characteristics (SSC). Fathead minnow (Pimephales promelas) and Japanese medaka (Oryzias latipes) possess SSC which can be assessed, Nuptial tubercles and anal fin papillae, respectively".*

ECHA agrees that it is also possible to perform the requested study with Japanese medaka and Fathead minnow and that secondary sex characteristics could be better monitored using medaka. Hence, the decision was amended accordingly.

If a test with Japanese medaka is conducted, the genetic sex and secondary sex characteristics must be examined. The presence of a genetic sex marker is a considerable advantage as it increases the power of the sex ratio statistics and enables the detection of individual phenotypic sex reversal.

The first part of the test (OECD TG 229) must be conducted with four replicates to match the requirements of the OECD TG 234 protocol and to have four independent replicates throughout the whole test design.

After 21 days, the eggs of all exposure replicates and the control are collected and used to start an assay protocol following the OECD TG 234 (63 days). The eggs of each OECD

TG 229 replicate are divided to set up four replicates in the OECD TG 234. If there are not enough eggs available at day 21, the start of the OECD TG 234 can be postponed for 1 or 2 days. Note that in this interim period, the fish are to be further exposed. It is also possible to start collecting eggs already at day 19 to avoid a shortage of eggs to start the OECD TG 234 part of the requested study.

In your comments to the draft decision, you requested clarification on how to proceed if the number of eggs is insufficient on day 21 regarding initiating the OECD TG 234 part of the study.

ECHA confirms that if the number of eggs produced in the OECD TG 229 part of the study is not sufficient to proceed with OECD TG 234 at day 21 the study should be extended for one or two days until day 23 and the OECD TG 234 part should be conducted with the number of eggs available from OECD TG 229 part at day 23. It is also noted that if the number of eggs from only specific replicates is not sufficient to continue with the OECD TG 234 part, the assay must be continued but without the specific replicates. ECHA does not consider the difficulties in comparing e.g., VTG from this prolonged exposed fish with historical control data as a problem, as the data from the controls within the test is available and should preferably be used for comparison and interpretation of the results.

The collected eggs for the use in the OECD TG 234 test must be stored immediately in petri dishes. The fertilised eggs in cell stage 4 or 8 must directly be selected via binocular and exposure must start immediately afterwards, using the same exposure concentrations, as in the OECD TG 229 test.

All standard endpoints of the OECD TG 234 must be covered (in addition to those described in the section below). This section of the assay will provide data on endpoints related to sexual development like sex ratio and gonad histology and also covers the early life stages of the fish.

Parameters to be measured in addition to those already included in OECD TG 234 and OECD TG 229

The additional endpoints allow to conclude on the underlying endocrine modes of action and consider effects owing to unspecific liver toxicity.

- For the OECD TG 229 study, the fertility (the number of fertilised and viable eggs) after 21 days must be documented.
- Histopathology of gonads (evaluation and staging of oocytes and spermatogenic cells) must be conducted at the end of the OECD TG 234 test in all concentrations and the control(s). The number of fish to be examined must be 16 per test and control replicates. In addition, histopathology of gonads must be conducted at the end of the OECD TG 229 when there is an impact seen on fecundity and fertility in F0 fish, unless VTG or secondary sex characteristics are clearly impacted. In your comments to the PfA made you ask for more clarification on the following issues: the merit of histopathology of the gonads in both the OECD TG 229 and 234 phases, the potential triggers (e.g., effects on survival, reproduction, and/or other apical endpoints) and the number of fish to be sampled. Furthermore, you state that in any case, if clear impacts on VTG and secondary sex characteristics (where appropriate) are observed, there should not be a requirement to assess the samples for gonadal histopathology. With respect to your last point ECHA agrees that histopathology of the gonads in the OECD TG 229 part of the assay only needs to be performed if there is an impact seen on fecundity and/or fertility and there are no significant effects on vitellogenin and/or secondary sex characteristics. Histopathology of the gonads under the OECD TG 229

protocol, triggered by effects observed on fertility and/or fecundity but in the absence of clear effects on VTG and secondary sex characteristics, will allow to conclude whether fecundity and/or fertility are affected via an endocrine mode of action or not. However, histopathology of the gonads at the end of the OECD TG 234 part must be performed in all cases since this enhances the conclusiveness of the assay. Effects on sex ratio are determined via gonadal histology making the conduct of histopathology of gonads mandatory in the OECD TG 234. As regards the number of fish to be histopathologically examined for the OECD TG 229, if triggered, there must be 16 per test concentration and control replicates.

- Histopathology of the liver must be included at the end of both tests (at the end of OECD TG 229 and OECD TG 234) in all concentrations and the control(s) to detect effects on hormone levels and synthesis caused by specific target organ toxicity of the Substance. This information is necessary to distinguish specific endocrine-mediated effects from effects owing to unspecific liver toxicity. The number of fish to be examined must be half the number of fish in the OECD TG 229 and 16 per test and control replicates in the OECD TG 234.

In your comments to the draft decision, you requested clarification on the number of fish per test concentration or control where histopathology is required. ECHA amended the decision to include this clarification.

The requested assay design (combination of OECD TG 229 and OECD TG 234) will provide relevant information both on reproductive effects, and effects on sexual development and their underlying modes of action which are required to conclude on the EAS-related endocrine disrupting properties of the Substance in the environment.

Request for the full study report

You must submit the full study report which includes:

- a complete rationale of the 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 of Endocrine disruption for the environment by the Substance.

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

- The request is appropriate because it will provide information which will clarify potential adverse effects of the Substance on fish sexual development and reproduction due to an (anti)oestrogenic, (anti)androgenic activity and interference with steroidogenesis. This will enable the evaluating MSCA to conclude on these potential ED properties, and to confirm whether the Substance is an endocrine disruptor according to the WHO/ IPCS criteria.
- The request is the least onerous measure because the combination of OECD TG 229 with OECD TG 234 allows to evaluate effects of the Substance on reproduction (OECD TG 229) as well as including sensitive juvenile life stages of fish and the evaluation of effects on sexual development (OECD TG 234). Beside the presumed effects on sexual development, the available mammalian data suggest that the Substance effects reproduction of fish. Additionally, effects on number of fertilised eggs, hatchability as

well as larval development were observed for the structural analogues BPA and BPB and therefore effects on reproduction must be evaluated for the Substance as well. Both would not be possible using only a standard FSDT study (OECD TG 234). Furthermore, in contrast to performing an OECD TG 229 and OECD TG 234 assay as sequential assays, the requested combination is expected to be much more sensitive. This is because the combination allows for starting the OECD TG 234 assay with eggs from fish already treated with the Substance. Hence, effects transferred from parental fish to their F1 can also be detected in this setting.

Possible alternatives covering the same endpoints would be fish full life cycle testing at Level 5 of the OECD Conceptual Framework (CF) (OECD, 2018) such as a Medaka or Zebrafish Extended One Generation Reproduction Test (MEOGRT/ZEOGRT, OECD TG 240). A fish full life cycle or multi-generation test would include all sensitive life stages and would be robust enough to conclude on the environmental ED concern relating to the EAS mode of action. However, the evaluating MSCA considers that there is scientific evidence that transgenerational effects (i.e., from F1 to F2) are of minor importance in this case. Hence, as the MEOGRT/ZEOGRT also includes part of the F2 generation, this would be disproportionately time and resource consuming. In comparison with the combination of OECD TG 229 and OECD TG 234 requested herein (duration 84 days), the MEOGRT has a considerably longer duration of 133 days. Furthermore, the number of fishes is lower in the requested combination of OECD TGs 229 and 234 (max. 720 fishes in F0 and F1 for four concentrations) than in the MEOGRT (924 fishes in F0 and F1).

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

d) Consideration of time needed to perform the requested studies

In your comments on the draft decision, you requested an extension of the timeline of 24 months to at least 39 months. You sought to justify this request as follows:

- 21 – 36 months would be needed for the performance of the study and receipt of the final study report due to laboratory capacities.

  ECHA has exceptionally granted an extension of 12 months from the standard deadline to take into account the currently longer lead times in contract research organisations.

- At least 3 months would be needed after the receipt of the final study report to make the necessary dossier update to include the required information and make any other changes to the dossier depending on the study results.

  The time required to update the IUCLID dossier is already considered within the standard deadline. Therefore, the deadline was not extended on this basis.

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

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 and for Reproductive toxicity, the Member State Committee agreed to include the Substance (EC number 201-240-0, CAS RN 79-97-0) in the Community rolling action plan (CoRAP) to be evaluated in 2021. 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 for the environment

Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 16 March 2022.

Decision-making

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

(i) Registrant(s)' commenting phase

ECHA received your comments and forwarded them to the evaluating MSCA.

The evaluating MSCA took your comments into account (see Appendix A). The request(s) and the deadline were amended.

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

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

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

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

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

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

(iii) MSC agreement seeking stage

The Member State Committee reached a unanimous agreement in its MSC-81 written procedure and ECHA took the decision according to Article 52(2) and Article 51(6) of REACH.

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

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

Test methods, GLP requirements and reporting

Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[3].

In your comments to the draft decision you requested that not every part of the requested study is to be completed to GLP, as this is a non-standard test and QA departments may potenially not be able to fully validate all test procedures.

ECHA agrees that for certain parts of the requested study could be conducted without GLP certification if it is transparently documented.

Test material

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

1. Selection of the Test material(s)

The Test Material used to generate the new data must be selected taking into account the following:

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

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

a) You must report the composition of the Test Material selected for each study, under the 'Test material information' section, for each respective endpoint study record in IUCLID.
b) The reported composition must include all constituents of each Test Material and their concentration values.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

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

Technical instructions on how to report the above is available in the manual "How to prepare registration and PPORD dossiers"[4].

[4] https://echa.europa.eu/manuals