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Helsinki, 8 March 2017

Substance name: propyl 4-hydroxybenzoate
EC number: 202-307-7
CAS number: 94-13-3
Date of Latest submission(s) considered[1]: 07.04.2014
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressees: Registrant(s)[2] of propyl 4-hydroxybenzoate (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the registered substance propyl 4-hydroxybenzoate:

1.1 Extended one-generation reproductive toxicity study (oral route, with rats, with the developmental neurotoxicity and immunotoxicity (DNT/DIT) cohorts and with the extension of Cohort 1B to mate the F1 animals to produce an F2 generation); test method: EU B.56/OECD 443, as specified in Appendix 1;

1.2 Daphnia magna Reproduction Test; test method: OECD 211, as specified in Appendix 1;

1.3 Fish sexual development test; test method: OECD 234; with Japanese medaka (*Oryzias latipes*) or zebrafish (*Danio rerio*), including gonadal histopathology. If the test species is Japanese medaka, genetic sex shall also be determined, as specified in Appendix 1.

You shall provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the Chemical Safety Report by **17 June 2019**. Within this time frame, you shall also provide the full study reports of above mentioned studies to the evaluating Member State Competent Authority (MSCA). The deadline takes into account the time that you, the Registrant(s), may need to agree on who is to perform any required tests.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not

[1] This decision is based on the registration dossier(s) at the end of the 12 month evaluation period / This decision is based on the registration dossier(s) on the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

[2] The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

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included in the public version of this decision.

2. Who performs the testing

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all Registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

3. Appeal

You can appeal this decision to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

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

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Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on propyl 4-hydroxybenzoate (propylparaben) and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (MSCA) to complete the evaluation of whether the substance constitutes a risk to human health and the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested in order to clarify the concern for endocrine disruption for human health, toxicity and endocrine disruption for the environment.

1.1 Extended one-generation reproductive toxicity study (oral route, with rats, with the developmental neurotoxicity and immunotoxicity (DNT/DIT) cohorts and with the extension of Cohort 1B to mate the F1 animals to produce an F2 generation

The Concern(s) Identified

In vitro tests and *in vivo* studies show **endocrine disrupting (ED) modes of action** for propyl 4-hydroxybenzoate. These are studies from level 2 and 3 of the "OECD conceptual framework and standardized test guidelines for evaluating chemicals for endocrine disruption" (OECD guidance document No. 150):

In *in vitro* assays, corresponding to the **OECD Conceptual Framework (CF) level 2**, estrogen agonistic activity has been demonstrated for propyl 4-hydroxybenzoate (ER binding affinity, ER transactivation, MCF7 proliferation) (Blair *et al.*, 2000; Kim *et al.*, 2011; Routledge *et al.*, 1998; Nishihara *et al.*, 2000; Okubo *et al.*, 2001; Byford *et al.*, 2002; Van Meeuwen *et al.*, 2008; Terasaki *et al.*, 2009; Vo *et al.*, 2010; Vo *et al.*, 2011; Wrobel *et al.*, 2013).

In Kim *et al.* (2010), the substance was shown to weakly bind to the androgen receptor. Berger *et al.* (2015) showed that propyl 4-hydroxybenzoate acts as an hAR antagonist in the Yeast Antiandrogen Screen (YAAS). Anti-androgenic activity is suggested by one study at 10µM propyl 4-hydroxybenzoate (Chen *et al.*, 2007). At the highest dose tested (10µM), propyl 4-hydroxybenzoate significantly inhibited the transcriptional activity of testosterone by 19%. Anti-androgenic activity was also shown by Kjærstad *et al.* (2010), but this can be a secondary effect since it was seen at cytotoxic concentration (>10µM) Another study shows glucocorticoid receptor agonistic activity and peroxisome proliferator-activated receptor (PPARγ) agonistic activity for propyl 4-hydroxybenzoate (Hu *et al.*, 2013). In addition, glucocorticoid-like activity has been shown in vitro for propyl 4-hydroxybenzoate at 1µM in human breast carcinoma cell line (Klopčič *et al.*, 2015).

No test is available regarding thyroid Mode of action.

Based on the above data, ECHA concludes that an estrogen agonistic activity has been demonstrated *in vitro*, while indications of other ED modes of action (anti-androgenic, glucocorticoid-like and PPARγ agonistic) are also suggested by some studies.
Estrogen agonistic activity was also observed in vivo. Three uterotrophic studies with

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propyl 4-hydroxybenzoate are available (with some deviations from the OECD TG 440[4]), which correspond to the **OECD CF level 3** (*in vivo* studies):

- **Hossaini et *al.* (2000)**: Based on TG 440, with a reliability of 3 as assessed by the evaluating MSCA (reliability 2 according to you). The authors concluded on no significant difference in uterine weight compared to the control at 100 mg/kg bw/d (subcutaneous) or at 1, 10, 100 mg/kg bw/d (oral route) in immature female rats and mice. However, the volume of vehicle exceeded the maximum allowed for the highest dose, information on animals body weight is missing and blotted uterine weights are not reported, making the assessment of results difficult, as this can influence uterus weight.

- **Lemini *et al.* (2003)**: Based on TG 440, with a reliability of 3, as assessed by the evaluating MSCA (reliability 2 for you). The authors concluded on a significant increase in relative uterine weight with immature and ovariectomized mice (at 20, 65 and 195 mg/kg bw/day) and with immature rats (at 65 and 195 mg/kg bw/day). TG 440 is not validated for immature mice. Information on rats body weight is missing and, in ovariectomized mice, a significant body weight reduction has been observed at highest dose (-14%), indicating that the maximum tolerated dose was possibly reached. Moreover, blotted uterine weights were not reported for ovariectomized mice and uterine wet and blotted weight were not reported for immature rats. This makes the assessment of results difficult.

- **Lemini *et al.* (2004)**: Based on TG 440, with a reliability of 2. In this study, with ovariectomized (Ovx) mice, significant increase in relative uterine weight was observed at 65 and 195 mg/kg bw/day of propyl 4-hydroxybenzoate (subcutaneous). This weak estrogenic effect (relative uterotrophic potency related to Estradiol (E2) resp. of 0.03 % and 0.005 %) was confirmed by histological observations on both endometrium and myometrium, similar to effects of E2. The assay shows minor deviations with some information missing or not reported (effect of the vehicule on uterus, bedding used...). Moreover, the experiment has been performed for 3 days only, which is according to TG 440 too short to detect weak estrogenic substances in Ovx mice. However, significant effects have been observed on the weight of the uterus (relative) and confirmed by histological observations. This study was assessed as the most reliable of the 3 available uterotrophic assays.

This last *in vivo* study confirmed the estrogen agonistic mode of action of propyl 4-hydroxybenzoate shown *in vitro*.

According to the WHO (2002) definition, "An ED is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations." This means that a chemical is identified as an ED if an adverse *in vivo* effect can be plausibly linked to an endocrine mode of action.

Tests from **OECD CF level 4** (or 5) can provide information about **adverse effects**. Very few *in vivo* tests following OECD guidances are available for the substance:

One reliable test (following OECD TG 422) with propyl 4-hydroxybenzoate is available in

[4] The OECD TG 440 was validated for immature and ovariectomized rats and for ovariectomized mice. Immature model for mice is not under the scope of the test guideline.

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the registration dossier. In this **Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test** (2012), rats were exposed via the diet to 0, 59-137, 178-432 or 605-1380 mg/kg bw/day of propyl 4-hydroxybenzoate. In this study, no adverse effects are reported, apart from changes in epididymides weight: statistically significant increase of absolute right epididymides weight is reported in males at mid and high dose group. A significant increase in relative weight of right epididymides is also reported (at high dose, when relative to body weight and at mid- and high dose group, when relative to brain weight). Absence of effects in organs of exposed adult rats doesn't preclude possible effects in organs of rats exposed during ED more sensitive life stages (e.g. when exposed in utero). Moreover, the test was performed according to the old study design and doesn't include the recently added parameters for ED. The OECD 422 has indeed been recently updated to include more parameters for ED. The OECD 422 is primarly a screening test and it is not conclusive due to lower statistical power.

Two studies with propyl 4-hydroxybenzoate (not following any OECD test guideline) are looking at **male reproductive function**:

- In **Oishi (2002)**, rats were exposed via food to 0, 12.4, 125 and 1290 mg/kg bw/d of propyl 4-hydroxybenzoate from post-natal day 19-21 to 46-48. The cauda epididymal sperm reserves and the sperm concentration decreased dose-dependently and the difference was significant at the mid and high dose. The daily sperm production in testis was significantly decreased in all treated groups. Testosterone concentration in serum decreased in a dose dependent manner and was significant at the highest dose. No differences in weight of the testes, epididymides, prostate, seminal vesicles and preputial glands were observed in any group. It is however not possible to conclude based on these results. It was not reported how animals were allocated. Histopathology of the testis and epididymis was not performed. Moreover, the number of animals was too small to conclude on testosterone effects.

- In **Gazin *et al.* (2013)**, rats were exposed via gavage to 0, 3, 10, 100 or 1000 mg/kg bw/d propyl 4-hydroxybenzoate from post-natal day 21 to 77. Some animals were allowed to recover during a period covering 3 spermatogenic cycles (26 weeks). Only significant higher weights of right testis in 100 and 1000 mg/kg bw/day groups at the end of the recovery period (non monotonic dose response) and significant higher weights of the left epididymis in all groups at the end of the recovery period were observed.

The differences in both studies could possibly be related to the different periods of exposure. Indeed, starting at PND 21 (juvenile), a 4 weeks treatment ends during the peri-pubertal stage (PND 49), while a 8 weeks treatment ends at the adult stage (PND 77).

Both studies indicate some concern for reproduction but are not sufficient to draw a definite conclusion.

Two studies (not following OECD test guidelines) are looking at **female reproductive function**:

- In **Vo *et al.* (2010)**, significant myometrial hypertrophy of the uterus was observed after oral exposure to 1000 mg/kg bw/day of propyl 4-hydroxybenzoate

in Sprague-Dawley rats (exposure from PND 21 to PND 40).

- In **Ahn *et al.* (2012)**, neonatal Sprague-Dawley female rats were subcutaneously exposed to 0, 62.5, 250 or 1000 mg/kg/d of propyl 4-hydroxybenzoate for 7 days. A significant increase in the number of primordial follicles at 1000 mg/kg bw/day and a decrease in the number of early primary follicles at 250 and 1000 mg/kg bw/day were observed. Also a significant increase in expression of the CaBP-9k gene, associated with follicle development, was observed at doses of 250 and 1000 mg/kg bw/day, indicating estrogenic activity of propyl-4-hydroxybenzoate.

Both studies indicate some concern for reproduction but are not sufficient to draw a definite conclusion.

In the paraben family, estrogenic activity tends to increase with increasing chain length (Darbe and Harvey, 2008)(methy<ethyl<propyl<butyl 4-hydroxybenzoate). The results of the Estrogen Receptor Binding Profiler (OECD Toolbox) are "weak binder" for methyl and ethyl 4-hydroxybenzoate and "moderate binder" for propyl and butyl 4-hydroxybenzoate.

Information from butyl 4-hydroxybenzoate, a structurally related substance gives alerts about fertility and developmental disorders, supporting the need for further information on propyl-4-hydroxybenzoate :

- In **Kang *et al.* (2002)**, pregnant rats were exposed to butyl 4-hydroxybenzoate (subcutaneous) from gestation day (GD) 6 to post-natal day (PND) 20. Statistically significant observed effects in F1 offspring were among others:
  - a decrease in the proportion of pups born alive (at 100 and 200 mg/kg bw/day) and
  - a decrease in the proportion of pups surviving to weaning (at 200 mg/kg bw/day) (the high standard deviations for these endpoints support the need for a higher tier study with more robust data).
  - a decrease of the sperm count and the sperm motile activity in the epididymis (at 100 and 200 mg/kg bw/day). In accordance with the sperm count in the epididymis, the number of spermatids of stage VII in the seminiferous tubule were significantly decreased.

These data on butyl 4-hydroxybenzoate are not suitable for the final determination of endocrine disruption for propyl 4-hydroxybenzoate, but they can be considered as supporting information.

No OECD CF level 5 test is available for propyl 4-hydroxybenzoate.

Taking into account the wide dispersive use, high tonnage (100-1000 T/year) and consumer uses, a risk for human health cannot be excluded. Possible adverse effect of propyl 4-hydroxybenzoate on fertility and development and possible related ED mode of action need to be clarified. In view of the results for butyl 4-hydroxybenzoate, in utero exposure or neonatal exposure are of particular concern.

Moreover, in vivo fish studies (level 3) also indicate an estrogenic mode of action of the substance (increase of Vitellogenin (VTG)) (see section 1.3).

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Why new information is needed

There is an estrogen agonistic mode of action. Effects on the sexual organs, following in utero exposure or neonatal exposure, could have an impact on the fertility of the F1 generation. Further studies are required as the substance is widely used and exposure occurs.

Currently the substance has no harmonised classification. The results of an Extended one-generation reproductive toxicity study (EOGRTS) can elucidate Human Health ED adverse effects. This could lead to a classification as reprotoxic and/or to an identification of the substance as Substance of very high concern (SVHC) (Reprotoxic and/or ED for Human Health) and possible inclusion in Annex XIV of the REACH Regulation (for Human Health).

Considerations on the test method and testing strategy

In the OECD Guidance Document No.150, two options are proposed when the OECD 422 does not show strong effects, but with positive ED mechanistic *in vitro* results and when *in vivo* data are equivocal (scenario L in Table Annex 2.8):

- repeating the *in vitro* test with metabolites, or
- performing a level 5 OECD CF test (e.g. EOGRTS).

Parabens are alkyl esters of para-hydroxybenzoic acid that differ at the para position in the benzene ring by the alkyl chain: methyl, ethyl, propyl, etc. A common metabolite in this family is the 4-hydroxybenzoïc acid (pHBA). This metabolite is not expected to exhibit endocrine disrupting activity based on the current data (Darbe and Harvey, 2008). The difference in *in vitro* potency of the different members of the paraben family is closely linked to the side chain. The concern is likely related to the substance in its free form. The level of free parabens in the body is determined by the efficiency of the metabolic pathway, which is less efficient at early life stages (SCCS/1446/11). The most sensitive life stages are expected to be covered by the EOGRTS, which is thus considered to be the most appropriate method to assess ED adverse effects of propyl 4-hydroxybenzoate.

Besides endpoints of relevance for endocrine disruption, the EOGRTS will address reproductive toxicity as well as developmental neurotoxicity and immunotoxicity.

The extended one-generation reproductive toxicity study (EOGRTS) is requested with the following study design:

i. Inclusion of the extension of Cohort 1B

The triggers for extension of Cohort 1B to produce the F2 generation are listed in REACH annex IX and are further explained in the ECHA guidance (Chapter R.7a). Significant exposure (to consumers and professionals) and indications of relevant mode(s) of action related to ED (from *in vivo* studies or non-animal approaches) trigger the inclusion of the extension of Cohort 1B to produce the F2 generation.

Hence, extension of cohort 1B to produce F2 generation is especially warranted in this case due to consumer exposure and estrogenic mode of action of propyl 4-hydroxybenzoate. The substance is used in cosmetics and pharmaceuticals, but the Scientific Committee on Consumer Safety (SCCS) and the European Medicines Agency

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(EMA) in their evaluation of the substance did not assess the ED properties of the substance. Moreover, In SCCS/1514/13, the SCCS indicated a lack of data on human exposures to propyl 4-hydroxybenzoate and about toxicokinetics in humans. Adequate evidence was not available for the safe use of the substance in cosmetics. In its reflection paper (2013), EMA indicated uncertainties for children below the age of two.

Available data on the substance indicate some effects of propyl 4-hydroxybenzoate on spermatogenesis in males (Oishi, 2002) and on folliculogenesis in females (Ahn *et al.*, 2012). The extension of Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) will provide information on the fertility of the offspring, i.e. the F1 generation, which has been exposed already during germ cell formation, preimplantation, *in utero* and postnatal periods. Due to the ED mode of action, possible impaired fertility in F1 generation could occur at lower doses than in the parental generation.

Moreover, Kang *et al.* (2002) demonstrated that maternal exposure to butyl 4-hydroxybenzoate, a structurally related member of the paraben family, has adverse effects on the F1 male offsprings which could affect their fertility (e.g. effect on spermatogenesis).

ii. Inclusion of Cohort 2A/2B (developmental neurotoxicity)

Inclusion of the DNT cohort may be important in relation to certain types of adverse effects caused by endocrine disrupters, i.e. effects on the sexual dimorphic development of the brain.

In the ECHA guidance (Chapter R.7a: Endpoint specific guidance Version 4.1 – October 2015)(Appendix R.7.6–2, EOGRTS Study Design), information on specific hormonal mechanisms/modes of action with clear association with the developing nervous system, such as estrogenicity (Fryer *et al.*, 2012) and antiandrogenicity (Pallarés *et al.*, 2014) is a trigger for inclusion of cohorts 2A/2B.

Hence, inclusion of cohort 2A/2B is especially warranted in this case due to the estrogenic mode of action of propyl 4-hydroxybenzoate.

In a study of Koeppe *et al.* (2013), based on data of the National Health and Nutrition Examination Survey (NHANES) from the general population of the U.S., urinary paraben levels were associated with thyroid hormone levels. Negative associations were observed between urinary propyl 4-hydroxybenzoate and thyroid levels. These were borderline significant ($p=0.04$) in the total group of adults (age: 20+), and stronger if women were considered separately ($p=0.01$ for fT4 and $p=0.02$ for fT3).

Another epidemiological study (Meeker *et al.*, 2011) could not give indication of possible association between propyl 4-hydroxybenzoate exposure and serum thyroid hormone levels, but only 167 adult men were subject to this study (while in the Koeppe et al., 2013, measures were obtained from 1479 adults and 352 adolescents).

Knowing that even moderate and transient reductions in human maternal T4 levels during pregnancy may affect the child's neurological development, resulting in impaired motor- and neurological function in childhood (Draft Review Feasibility study for minor enhancements of TG 421/422, 2014), the available data on possible alteration of thyroid hormone levels trigger the need for a more in-depth investigation.

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Moreover, the Guidance on Information Requirements and Chemical Safety Assessment (Version 4.1; Chapter R.7a: Endpoint specific guidance) indicates that: "Existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action" can be used to justify requests for DNT and DIT cohorts.

In the study by Ali and Elgoly (2013), butyl 4-hydroxybenzoate was administered to pregnant rats from gestation day 1 to lactation day 21 (200 mg/kg bw/d, orally or subcutaneously). Offspring male rats were subjected at the last 3 days of lactation to Morris water maze and three chamber sociability test.

The results showed social and learning and memory behavioral deficits in the offspring. Alterations in brain neurotransmitters and brain derived neurotrophic factor BDNF were also observed, following excision and dissection of the brain.

The same research group (Hegazy *et al.*, 2015) investigated mechanistic similarities between pups of pregnant rats exposed to butyl 4-hydroxybenzoate (200 mg/kg bw/d, orally or subcutaneously) and to valproic acid, which is commonly used in autistic models. The results suggest that mitochondrial dysfunction (disruption of ATP/AMP production) and oxidative stress (changed redox potential) may play key roles in autism. The brain injury symptoms observed after butyl 4-hydroxybenzoate exposure show similarities to those observed with valproic acid.

This corresponds to the findings of Gargus and Imtiaz (2008), who have reviewed the mitochondrial energy-deficient endophenotype in autism, a neurologic disorder which is influenced by a combination of various genetic, environmental and immunological factors. Evidence has suggested that increased vulnerability to oxidative stress may be involved in the etiology of this multifactorial disorder.

In Nakagawa and Moore (1999), propyl 4-hydroxybenzoate has been shown to cause dose-dependent cytotoxicity through mitochondrial dysfunction, involving induction of membrane permeability transition (MPT). Reduction of mitochondrial membrane potential was accompanied by abrupt loss of intracellular ATP.

Moreover, propyl 4-hydroxybenzoate has the ability to cause oxidative stress (e.g in Martín *et al.* (2010), a significant increase of the 8-OHdG index, widely recognized as a sensitive marker of oxidative stress, was observed in Vero cells following 24h-exposure of ≥50 µM PPB).

Taking into account the mechanistic properties of propyl 4-hydroxybenzoate (related to oxidative stress and mitochondrial dysfunction) and given the neurodevelopmental effects of butyl 4-hydroxybenzoate (a structurally related substance), there is a need to further investigate developmental neurotoxicity of propyl 4-hydroxybenzoate.

Therefore, taking into account the available data, inclusion of the DNT cohort is warranted.

iii. <u>Inclusion of Cohort 3 (developmental immunotoxicity)</u>

In the ECHA guidance (Chapter R.7a: Endpoint specific guidance Version 4.1 – October 2015)(Appendix R.7.6-2, EOGRTS Study Design), information on hormonal

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mechanisms/modes of action with clear association with the immune system, such as estrogenicity (Adori *et al.*, 2010), is a trigger for inclusion of Cohort 3.

Hence, inclusion of the DIT cohort is warranted here due to the estrogenic mode of action of propyl 4-hydroxybenzoate.

Moreover, the Guidance on Information Requirements and Chemical Safety Assessment (Version 4.1; Chapter R.7a: Endpoint specific guidance) indicates that: "Existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action" can be used to justify requests for DNT and DIT cohorts.

In the study by Hegazy *et al.* (2015), brain neuroinflammation and a significant increase in pro-inflammatory cytokine levels (IL-1β, TNF-α and IL-6) were measured in male pups of dams exposed to butyl 4-hydroxybenzoate (200 mg/kg bw/day, orally or subcutaneously, from gestation day 1 to lactation day 21). In this study, the level of those immune proteins was investigated because of their known effect on neural-development and activity.

This indication about the effect on immune proteins by a structurally related substance supports the need to investigate developmental immunotoxicity of propyl 4-hydroxybenzoate.

iv. Premating exposure duration

According to the ECHA guidance (Chapter R.7a), the pre-mating exposure duration shall be 10 weeks in order to cover the full period of spermatogenesis and folliculogenesis. However, due to the request of the extension of cohort 1B, ECHA is of the opinion that the pre-mating exposure period could be reduced to 2 weeks, as these development periods will be covered in the F1 generation.

v. Dose level setting

The test guidance (OECD TG 443) recommends to include at least 3 dose levels and a concurrent control. Two- to four-fold intervals are optimal. The dose levels shall be based on toxic effects. You should ensure that appropriate doses are selected by taking into account the available data and, if needed, perform a range finding study.

As the propyl 4-hydroxybenzoate metabolism in rats seems to be faster than in humans, as indicated below, special attention should be paid to a proper selection of the upper dose:

In SCCS/1514/13, A comparison of the human dermal toxicokinetic study (Janjua *et al.*, 2007, 2008) with the toxicokinetic data of the study in juvenile male rats (Gazin *et al.*, 2013) reveals that the systemic exposure to free paraben in human males (dermal application of 10 mg butyl 4-hydroxybenzoate/kg bw/day) is similar to that in juvenile male rats exposed to a 100-fold higher oral dose (1000 mg propyl 4-hydroxybenzoate/kg bw/day).

The SCCS wondered if the differences of the study conditions could explain this similar internal dose, at 100-fold different external dose (oral versus dermal, butyl- versus

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propyl 4-hydroxybenzoate, concomitant dermal application of 2 phthalates esters). However, none of these arguments (single or in combination) were considered as convincing.

For the SCCS, despite the uncertainties, it seems more likely that rats metabolise propyl (and butyl) 4-hydroxybenzoate in a much more rapid and effective way than humans.

For this reason, ECHA recommends a sufficiently high upper dose (possibly 1000 mg/kg bw/day which was the highest dose tested in the OECD 422 test).The internal dose of the free form of propyl 4-hydroxybenzoate shall be monitored during the study, to allow comparison with human exposure and possible extrapolation of the animal effects.

vi. Route of exposure

The substance shall be administered via the oral route.

You shall submit the full study report of the required information. Indeed a complete rational and access to all available information (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.) are needed to fully assess the provided information and to efficiently clarify the concerns.

Alternative approaches and proportionality of the request

The request for the EOGRTS is suitable and necessary to obtain information that will allow to clarify whether there is a risk for human health. More explicitly, there is no equally suitable alternative way available of obtaining this information. Where the data, once obtained, confirm that there is risk for endocrine disrupting effects and/or neuro/immune developmental effects, it will allow authorities to consider further regulatory risk management such as SVHC identification (authorization procedure). ECHA notes that there is no experimental study available at this stage that will generate the necessary information without testing on vertebrate animals.

Consideration of Registrant(s)' comments on the original draft decision

In your comments you recommended to develop a common assessment strategy for endocrine activity for propyl and methyl 4-hydroxybenzoate and indicated that the estrogen receptor binding activity of parabens may increase with increasing alkyl chain length (Dabre and Harvey, 2014) and thus there might be a higher potential for estrogenic activity for long-chain length molecules.

In this regard, you agreed to perform an OECD TG 443 study on propyl 4-hydroxybenzoate with the inclusion of Cohort 1B only, to clarify the potential effect of the substance on the reproductive performance.

You did not agree with the extension of Cohort 1B to mate the F1 animals to produce an F2 generation. In your opinion, there are no data (neither in vitro, nor in vivo) which clearly provide evidence for an estrogen agonistic mode of action or even give hint for it. You also rejected the argument on wide dispersive and consumer uses. For you, consumer uses have already been assessed by SCCS for cosmetics and EMA for pharmaceuticals.

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You questioned the suggested upper dose (1000 mg/kg bw/day) and considered utilization of lower doses more reasonable, since endocrine acting agents/hormones show their mode of action at very low concentrations.

You did not agree to include the DNT/DIT cohorts. For you, the available data on the substance (e.g. OECD TG 422) did not provide any hint to trigger the performance of such investigations. Furthermore, you do not consider the data on butyl 4-hydroxybenzoate as an indication of a propyl 4-hydroxybenzoate related concern for affecting the neurological development.

The evaluating MSCA took your comments into account and agreed that testing is to be performed on propyl 4-hydroxybenzoate to clarify the concern. Moreover, additional explanation is provided in this statement of reasons above to clarify and strengthen the reasons for requesting the extension and inclusion of cohorts and to justify the requested dose level setting.

Consideration of proposals for amendment and Registrant(s) comments

One MSCA indicated in its proposal for amendment (PfA) that the DNT and DIT cohorts should be triggered based on existence of a substance specific particular concern and this concern should be a serious and severe effect. The same MSCA indicated that propyl 4-hydroxybenzoate is not a potent estrogen, which is supported by the negative uterotrophic assay conducted by Hossaini *et al.* (2000) and the available *in vitro* studies. In their opinion they state that weak estrogens do not meet the requirement for a severe and serious effect or raise a particular concern and should not be used to support the inclusion of the DNT or DIT cohort.

In your comments to the PfAs, you agreed with this PfA. You state that there are no data (neither *in vitro*, nor *in vivo*) which clearly provide evidence for an estrogen mode of action or even give hint to it. Moreover, you mentioned that binding to the receptor at cytotoxic and overloading ranges might be rather an artificial effect than a real substance-receptor binding.

ECHA disagrees with this analysis. ECHA acknowledges that propyl 4-hydroxybenzoate shows weak estrogenic activity. However other substances showing weak estrogenic activity (*in vitro* or *in vivo*) are known to have effects on neurodevelopment and immunodevelopment (for example Bisphenol A), or are suspected to have such effects and are under investigation (for example Bisphenol S). Therefore, weak estrogenicity is no reason not to include the DIT or DNT cohorts.

Binding to the estrogen receptor and estrogenic activity have been demonstrated for propyl 4-hydroxybenzoate with various models *in vitro*:

- ER binding assay, rat uterine cytosol (Blair *et al.*, 2000),
- hERα transactivation assay (Kim *et al.*, 2011),
- Recombinant hEr yeast assay (Routledge *et al.*, 1998),
- Yeast two-hybrid assay with ERα (Nishihara *et al.*, 2000),
- Competitive binding for ERα and ERβ and MCF-7 proliferation (E-screen) (Okubo
- *et al.*, 2001),
- Competitive binding assay using cytosol of MCF-7 breast cancer cells, ERE-CAT transfected human breast cancer cells, RNA analysis of pS2 gene in MCF-7 cells (northern blot) and Cell proliferation in MCF-7 (Byford *et al.*, 2002)

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- Proliferation assay with MCF-7 cells (E-screen) (Van Meeuwen *et al.*, 2008).

ECHA acknowledges that a conclusion on anti-androgenic activity cannot be drawn based on the results from Kjærstad *et al.* (2010). In this study, an effect was indeed observed at cytotoxic concentration. Otherwise, from a general point of view, concentrations used in different *in vitro* models for determination of modes of action cannot be used to (un)predict adverse effects *in vivo*. For instance, *in vitro*, in Routledge *et al.*, 1998, the effect of propyl and butyl 4-hydroxybenzoate (tested at 4.10^{-5}M and 1.10^{-5}M respectively) was inhibited by addition of the antiestrogen 4-hydroxy tamoxifen, demonstrating that the estrogenic activity shown in this study was a consequence of an estrogen receptor interaction. In this study, butyl 4-hydroxybenzoate was able to compete with 3H-oestradiol to the rat estrogen receptor with an affinity approximatively 5 orders of magnitude lower than diethylbestrol (DES) and between 1 or 2 orders of magnitude less than 4-nonylphenol.

The estrogenic mode of action of propyl 4-hydroxybenzoate has also been demonstrated *in vivo*, with significant increase in relative uterus weight and histological observations (Lemini *et al.*, 2004). In this study, the relative uterotrophic potency to E2 (RUPE2) compares the weight of uterine exposed to propyl-4-hydroxybenzoate with the weight of uterine exposed to E2. This value only considers the uterine weight and does not integrate the histopathological findings. Furthermore, the RUPE2 value of propyl-4-hydroxybenzoate (0.030 at 65 mg/kg bw/day and 0.005 at 195 mg/kg bw/d) in this study is comparable to a RUPE2 value of 0.016 for bisphenol A (at 100mg/kg bw/d), another well-known weak estrogenic substance, calculated from Papaconstantinou *et al.* (2000).

The uterotrophic assays investigated by Hossaini *et al.* (2000) and by Lemini *et al.* (2003) show several deviations (as mentioned above in this Appendix). These deviations make the assessment of results in both studies difficult. Therefore, the study from Lemini *et al.* (2004) was assessed as the most reliable of the 3 available uterotrophic assays.

You stated in your comments that in the available higher tier studies (according to OECD TG 422, 2012; Oishi, 2002; Gazin *et al.*, 2013) no adverse effects were reported in thyroid, ovaries, testes, epididymis, adrenal glands, prostate, uterus, seminal vesicles, oestrus cycle and sperm analysis. In addition, you stated that effects of propyl 4-hydroxybenzoate on female reproductive functions are not clear and no robust results were obtained leading to any conclusions on the endocrine potential (e.g. Vo *et al.*, 2010; Ahn *et al.*, 2012).

ECHA emphasises that, as indicated in the test guidance OECD 422, this test offers only limited means of detecting postnatal manifestations of prenatal exposure, or effects that may be induced during postnatal exposure. Moreover, the test was performed according to the old study design and doesn't include the recently added parameters for ED. From the studies looking specifically at male sexual organs (Oishi, 2002 and Gazin *et al.*, 2013) or female reproductive function (Vo *et al.*, 2010; Ahn *et al.*, 2012), no definite conclusion can be drawn with regard to adverse effects, but some concerns forreproduction were observed.

You further stated that various parameters and the functional/motor activity battery that are commonly accepted as indicative for immunological or neurological impairment were

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examined in the course of an OECD TG 422 study with propyl 4-hydroxybenzoate and no effects recorded (differential leucocyte count, thymus, spleen, lymph nodes).

For ECHA, the concern for immunological or neurological impairment is coming from effects on the brain development of male pups which were exposed to butyl 4-hydroxybenzoate (Ali and Elgoly, 2013; Hegazy *et al.*, 2015). The available studies on propyl 4-hydroxybenzoate do not cover this endpoint.

The MSCA also indicated in its PfA that no justification has been provided to enable an independent assessment of the read-across hypothesis to other benzoate esters.

In your comments you agreed on the PfAs since you do not consider data derived with structural analogues with longer chained substitutes suitable for the final determination of endocrine potential of propyl 4-hydroxybenzoate.

ECHA notes that there was no intention for a read-across analysis with other benzoate esters. However, the Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7a : endpoint specific guidance. Version 4.1. October 2015) mentions that " *existing information on effects caused by substances* ***structurally analogous*** *to the substance being studied, suggesting such effects or mechanisms/modes of action*" can be used to justify requests for DNT and DIT cohorts. In this way, two studies performed with butyl 4-hydroxybenzoate, a structurally analogous substance, showing neurological effects are considered relevant: The Ali and Elgoly study (2013) showing social, learning and memory behavioral deficits in rat offspring and the Hegazy *et al.* study (2015) showing similar effects to those observed with valproic acid commonly used in autistic models.

In addition, further mechanistic properties (related to oxidative stress and mitochondrial dysfunction) (Martin *et al.* (2010) et Nakagawa and Moore (1999)) of propyl-4-hydroxybenzoate were provided, which could be involved in potential similar neurodevelopmental and immunological effects.

Both the PfA submitting MSCA and you argued that the provided justification for the DIT and DNT examinations was not sufficient. ECHA disagrees with this analysis since for the DNT and DIT cohorts, weighing all the information, there is sufficient evidence to raise a **reasonable expectation** that the substance could be a developmental neuro/immunotoxicant.

Therefore, based on the overall evidence on propyl 4-hydroxybenzoate and the effects seen with a structurally analogous substance, ECHA considers it appropriate to include the DNT/DIT cohorts.

Note for your consideration

It was noted during Member State Committee meeting that Morris Water Maze testing can be included in the DNT cohort as specified in paragraph 50 of the OECD test guideline 443. This kind of testing would cover the concern seen with butyl 4-hydroxybenzoate in Ali and Elgoly (2013), as described above.

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Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the substance subject to this decision: Extended one-generation reproductive toxicity study (oral route, with rats, with the developmental neurotoxicity and immunotoxicity (DNT/DIT) cohorts and with the extension of Cohort 1B to mate the F1 animals to produce an F2 generation); test method: EU B.56/OECD 443, as specified above.

1.2 Daphnia magna Reproduction Test (OECD 211)

The Concern(s) Identified

In the registration dossier, the acute data indicate that fish is the most sensitive species.

For chronic toxicity, only data for algae (NOEC > 1 mg/l) are reported in the registration dossier.

However, in a literature study (Dobbins et al, 2009) information is given about acute and subchronic toxicity for invertebrates (Daphnia magna) and fish (Pimephales promelas): While, for acute toxicity, LC50s reported in this study are in the same order of magnitude as those reported in the registration dossier, subchronic data show that invertebrates are more sensitive than fish.

In this study, fish were exposed for 7 days to propyl 4-hydroxybenzoate, while D. magna was exposed for 10 days. It was concluded that for the endpoint growth, D.magna is more sensitive than fish (LOECgrowth of 2.5 mg/L for fish and 0.4 mg/L for Daphnia). In case only a LOEC is determined, it can be used to derive a NOEC with the following calculations (see Guidance on information requirements R10, Table R.10-1):

- If the LOEC value is between 10 and 20% effect, the NOEC can be calculated as LOEC/2.
- If the effect percentage of the LOEC is unknown, no NOEC can be derived.

The authors of Dobbins et al. (2009) confirmed that the effect at the lowest level in the 10d NOEC growth Daphnia was 16%. Therefore, the evaluating MSCA used an approximate value of 0.2 mg/L to reassess the RCR values using the EUSES tool. Taking into account that the test duration of the Daphna study was only 10 days instead of 21 days, this is probably still an underestimation of the risk. When applying an assessment factor of 100 and using the EUSES default exposure values, RCR values slightly above 1 were calculated.

Why new information is needed

The exposure duration of 10 days is not long enough to conclude on reproduction toxicity in Daphnia magna as sexually mature D. magna start to release neonates from their brood chamber at day 8-10, followed by a release of a second brood at day 10-12, a third by day 12-14 and a 4th by day 14-17. Above data show a concern for subchronic toxicity in invertebrates, however a definite 21-day Daphnia NOEC is not available. At

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present no classification for the environment is reported in the registration dossier. Furthermore a risk for the environment cannot be excluded.
The 21 day No observed effect concentration (NOEC) derived from the Daphnia reproduction toxicity test will be used for classification and labelling purposes and for a more accurate assessment of the risk for the environment.

Considerations on the test method and testing strategy

A Daphnia magna reproduction test according to OECD 211 shall be performed.

You shall submit the full study report for the information required. Indeed a complete rational and access to all available information (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.) are needed to fully assess the provided information and to efficiently clarify the concern.

Alternative approaches and proportionality of the request

The request for the Daphnia magna reproduction test is suitable and necessary to obtain information that will allow to classify and label for the environment, as well as to refine the environmental risk assessment. More explicitly, there is no equally suitable alternative way available of obtaining this information.

Consideration of Registrant(s)' comments

In your comments you agreed that there are differences in sensitivity between fish and Daphia. For the registrant(s) however, no risk is expected for the environment based on the calculations presented in Dobbins et al., 2009 (hazard quotient (HQ)<1 and Chemical toxicity distribution). Based on this publication and existing data for acute toxicity in Daphnia, you considered that the request for an OECD TG 211 study was highly unjustified. The evaluating MSCA disagreed with this analysis since the CTD (Chemical toxicity distribution) method presented in Dobbins et al., 2009 does not include exposure values and is therefore not considered relevant for the risk assessment.

Moreover, the hazard quotient (HQ) was calculated by dividing the measured or predicted environmental concentration by the most sensitive no-observed-effects concentration (NOEC) for each paraben (if HQ >1, there is potential for toxic effects in the environment). This method doesn't make use of an assessment factor and to determine the measured environmental concentration for propyl 4-hydroxybenzoate, exposure values from the literature (from the 3 articles as referred below) were averaged.

> *- Lee HB, Peart TE, SvobodaML. 2005. Determination of endocrinedisrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatographymass spectrometry. J Chromatogr A 1094:122–129.*

(Concentration in influent and effluent samples from 8 WWTPs in Southern Ontario, Canada)

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- *Benijts T, Lambert W, De Leenheer A. 2004. Analysis of multiple endocrine disruptors in environmental waters via wide-spectrum solid-phase extraction and dual-polarity ionization LC-ion trap MS/MS. Anal Chem 76:704–711.*

(concentration in river water, WWTP effluent and industrial effluent, probably from Flanders (Belgium) as the Flemish Environment Agency supplied the samples)

- *Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res 42:3498–3518.*

(concentration in surface waters in South Wales, United Kingdom)

The evaluating MSCA asked the authors of the Dobbins *et al.* (2009) study about the NOEC they obtained for propyl 4-hydroxybenzoate. Following values were provided:
- 7d NOEC growth fish (fathead minnow, *Pimephalse promelas*): 1.25 mg/L;
- 10d NOEC growth Daphnia: <0.375 mg/L (this was the lowest treatment tested, all treatment groups were significantly different from the control). The effect at the lowest level (0.375 mg/L) was 16%;
- 10d NOEC reproduction Daphnia: 3 mg/L.

From the " guidance on information requirement R10" (Table R.10-1):
"a LOEC (lowest observed effect concentration) stands for the lowest concentration where an effect has been observed. It may therefore not be used as a NOEC. In case only a LOEC is given in the report, it can be used to derive a NOEC with the following procedures:
- LOEC > 10 and < 20% effect: NOEC can be calculated as LOEC/2.
If the effect percentage of the LOEC is unknown no NOEC can be derived."

The evaluating MSCA reassessed the RCR values with the results obtained by Dobbins *et al.*, 2009, using the EUSES tool:
Using a value of 0.2 mg/L as a proxy for a NOEC (Daphnia, growth)(after 10day instead of 21day, therefore still an underestimation), an assessment factor of 100 and the EUSES default exposure value, following local RCR were calculated using EUSES 2.1.1:
RCR (formulation) for freshwater = 3.87
RCR (formulation) for soil = 4.05
RCR (private use) for freshwater = 1.85

Based on the LOEC growth for Daphnia from Dobbins *et al.* (2009), a risk for the environment cannot be excluded. Therefore, ECHA is convinced that a Daphnia magna Reproduction Test (OECD TG 211) is needed.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the substance subject to this decision: Daphnia magna Reproduction Test; test method : EU C.20/OECD 211, as specified above.

1.3 Fish sexual development test

The Concern(s) Identified

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In vitro and *in vivo* studies show endocrine disrupting (ED) modes of action for propyl 4-hydroxybenzoate. These are studies from level 2 and 3 of the "OECD conceptual framework and standardized test guidelines for evaluating chemicals for endocrine disruption" (OECD guidance document No. 150):

In vitro assays (guideline and non-guideline tests), corresponding to the **OECD Conceptual Framework (CF) level 2,** showed estrogenic agonist activity of propyl 4-hydroxybenzoate (ER binding affinity, ER transactivation, MCF7 proliferation) (see the above section 1.1).

Three fish studies with propyl 4-hydroxybenzoate, which correspond to the **OECD CF level 3** (*in vivo* studies), are available (non-guideline tests). Vitellogenin (VTG) increase is observed in two out of these studies (Bjerregaard *et al.*, 2003; Inui *et al.*, 2003), while a decrease is observed in the third one (Mikula *et al.*, 2006).

In Bjerregaard *et al.* (2003) a clear and dose dependent induction of vitellogenin was observed after oral exposure via food (7, 33, 36, and 39 mg/kg every second day) of juvenile rainbow trout and no mortality was observed due to exposure. This confirms the estrogenic agonist effect of propyl 4-hydroxybenzoate. Increase in plasma VTG concentration was also observed following exposure via water, but the dose-dependency was more pronounced in the fish exposed via food.

In the Inui *et al.* (2003) study an induction of vitellogenin was found and the highest plasma vitellogenin concentration (around 100 µg/ml) in the exposed fish according to figure 1 of the article (please note that the authors sent in a corrigendum stating that the units to figure 1 are µg/ml and not ng/ml as indicated in the paper). Inui *et al.* (2003) confirms the induction of vitellogenin by measuring the gene expression of VTG 1 and 2 in the liver, so the estrogenic agonist results are clearly confirmed in this experiment performed with adult male Medaka exposed via water.

In Mikula *et al.* (2006) juvenile zebrafish were exposed for 20 days to 3 different concentrations of propyl 4-hydroxybenzoate via water (0.1, 0.4 and 0.9 mg/L). The exposed fish showed statistically significant lower VTG concentrations in whole body homogenates compared to the control (no dose dependency). However, differences in VTG concentration compared to control were minor. The authors concluded on antiestrogenic effects.

Tests from **OECD CF level 4** provide some information about adverse effects.

In the Mikula *et al.* (2009), feeding study with juvenile zebrafish, the anti-estrogenic effect observed in 2006 could not be seen. No difference in VTG concentrations in whole body homogenates was observed in treated fish compared to the control, while a statistically significant skewed sex ratio towards females was observed at the lower dose of 500 mg/kg (not at 1000 or 2000 mg/kg).

The study of Gonzalez-Doncel *et al.* (2014) concludes on no shift in sex ratio at 28 and 43 dpf in Medaka exposed to 4000 µg/L propyl 4-hydroxybenzoate via water during the 10 days of embryonic development (hatching began on day 11). Taking into account the short exposure duration, the limited number of fish (7 fish at 28 dpf and 19 fish at 43 dpf), the histological analysis for only one exposure concentration and the fact that gonadal differentiation is only fully completed after 60 dpf, this experimental approach

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does no allow to conclude on sex ratio effects. However severe adverse effects (macroscopical and histopathological) were observed in some indivuduals at 13 dpf. As no signs of histological damage were found in surviving 28 and 43 dpf larvae it can be concluded that those severe adverse effects lead to subsequent mortality. VTG was not measured in this study.

Some effects on fish population relevant endpoint are suspected (sex ratio), but the data do not allow to draw a definite conclusion. Indeed, available studies show weaknesses which do not allow to conclude on ED adverse effects.

No OECD CF level 5 test has been performed for propyl 4-hydroxybenzoate.

Taking into account the wide dispersive use, high tonnage ([redacted] T/year) and consumer uses, a risk for the environment cannot be excluded.

During the manufacture and formulation stages, it can be expected that the substance will mainly be discharged via waste water during cleaning processes. Based on the Level III Fugacity model, it is estimated that the majority of the substance (98.8%) will partition to that compartment.

It can be expected that in view of the use of the substance as an additive in consumer pharmaceutical and cosmetic products, such as ointments, shampoos and conditioners, the majority of the substance will be emitted to waste water.

In view of this, it can be expected that the water compartment will be the most affected. Therefore, the possible ED effects on fish need to be clarified.

Why new information is needed

The above data show a concern for fish reproduction but are not sufficient to draw a definite conclusion. Three studies show clear estrogenic agonist mode of action, both at gene expression and protein level. Moreover, Mikula *et al.* (2009) observed skewed sex ratio towards female zebrafish.

No OECD CF level 5 test has been performed for propyl 4-hydroxybenzoate.

Performing a standard level 4 study could elucidate the ED mode of action and allow to conclude on effects on sex ratio.

Currently the substance has no harmonised classification. Results of a level 4 fish study will elucidate ENV ED adverse effects, which could lead to an identification of the substance as SVHC (ED for ENV) according to Art.57(f) and possible inclusion in Annex XIV of the REACH Regulation (for ENV).

If the ED concern is not confirmed, the results of this long term fish study will be used to derive a No-observed effect concentration (NOEC) for a more accurate assessment of the risk for the ENV, as it would take into account possible long term effects. This could also possibly lead to a classification for the ENV.

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Considerations on the test method and testing strategy

A Fish Sexual Development test, OECD TG 234, is requested to be performed. An OECD TG 234 study is particulary relevant when the test chemical is suspected to act primarly on the sexual development phase of the fish lifecycle (as opposed to the reproductive phase). The test will provide apical information on phenotypic sex ratio which is fixed during fry or juvenile stages of the species used in this test.

The study shall be performed with Japanese medaka (*Oryzias latipes*) or zebrafish (*Danio rerio*).

The most environmentally relevant exposure route is water. The test shall be conducted under flow through conditions since a concern is mostly expected in case of continuous exposure to propyl 4-hydroxybenzoate, as biodegradability has been demonstrated. Gonadal histopathology shall be included and data on male / female / intersex / undifferentiated shall be provided. To increase the statistical power of the sex ratio, genetic sex shall also be determined if the test species is Japanese medaka.

You shall submit the full study report for the information required. Indeed a complete rational and access to all available information (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.) are needed to fully assess the provided information and to efficiently clarify the concerns.

Alternative approaches and proportionality of the request

The OECD TG 229 is a screening assay which informs about in vivo ED Mode of Action (MoA) but not on adversity. The OECD TG 234 informs on in vivo MoA AND adverse effects. As estrogenic mode of action has already been shown in vivo, the OECD TG 234 needs to be performed.
The request for Fish Sexual Development Test (FSDT)(OECD TG 234) is suitable and necessary to obtain information that will allow to clarify whether there is a risk for environment. More explicitly, there is no equally suitable alternative way available of obtaining this information, since the study provides information on both an in vivo mode of action and adverse effects. Where the data, once obtained, confirm that there is a risk for endocrine disruption for the environment, it will allow authorities to consider further regulatory risk management in the form of SVHC identification. ECHA notes that there is no experimental study available at this stage that will generate the necessary information and does not need to test on vertebrate animals.

ECHA note that a FSDT has been requested for a structurally analogue substance, methyl 4-hydroxybenzoate (ECHA decision dated 6 September 2016). ECHA highlights that you should consider whether read-across is possible, if appropriate, as a way to avoid unnecessary animal testing. ECHA notes that methyl 4-hydroxybenzoate has a weaker estrogen receptor binding activity and therefore, a negative result of the FSDT for methyl 4-hydroxybenzoate cannot be predictive for a negative result of the FSDT for propyl 4-hydroxybenzoate. A positive result in the FSDT for methyl 4-hydroxybenzoate however could be considered for read-across to propyl 4-hydroxybenzoate.
The result of the FSDT is to be considered positive when an effect is seen both on the VTG level and on the sex ratio (see OECD 150, C3.4).

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Consideration of Registrant(s)' comments

You considered the level 3 cited studies of low relevance for the assessment of endocrine properties of propyl 4-hydroxybenzoate due to mortality and questions about VTG concentration.
You also considered that the available evidence is not sufficient to directly request a Fish Sexual Development Test (OECD TG 234). You suggested to run instead a Fish Short Term Reproduction Assay (OECD TG 229).

The evaluating MSCA disagreed since the OECD TG 229 is only a screening assay which informs about *in vivo* ED Mode of Action (MoA), but not on adversity. The OECD TG 234 informs on *in vivo* MoA and adverse effects. Moreover, an estrogenic MoA has already been shown *in vivo*, therefore the OECD TG 234 is considered the most appropriate test to perform.

Consideration of proposals for amendment and Registrant's comments

One MSCA in its PfA requested to allow the Registrant(s) to read-across from methyl paraben if considered relevant.

The evaluating MSCA agreed with this PfA and the possibility for read-across was taken up as a consideration for you.

In your comments to the PfAs you agreed with the PfA proposing the possibility for read-across and in reference to this PfA, you stated that further discussion would be needed on the test concentrations used for the requested fish sexual development test.

ECHA notes that the OECD TG 234 specifies that the highest test concentration should be either 10% of the juvenile LC 50 value, 10% of the adult LC50 value or 10 mg/L (whichever is the lowest). In this specific case, there is an LC 50 value of 6.4 mg/L (IUCLID : short term toxicity to fish, 2012; test species *Danio rerio*).

The fish sexual development test should be performed according to test method OECD 234. Therefore, it is recommended to follow the test guideline and use 10% of this lowest LC50 value as the highest test concentration.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the substance subject to this decision: Fish sexual development test; test method: OECD 234; with Japanese medaka (Oryzias latipes) or zebrafish (Danio rerio), including gonadal histopathology. If the test species is Japanese medaka, genetic sex shall also be determined, as specified above.

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Draft Review Feasibility study for minor enhancements of TG 421/422

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(Reproduction/Developmental Toxici-ty Screening Test) /(Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) with ED-relevant endpoints from DTU FOOD, draft 28 August 2014 http://www.oecd.org/chemicalsafety/testing/Review-of-TGs-421-422-28-August-2014-and-Appendix-1-2a-2b-3.pdf

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected reproductive toxicity, potential endocrine disruptor, wide dispersive use, consumer use, exposure of sensitive populations and exposure of environment, propyl 4-hydroxybenzoate, CAS No 94-13-3 (EC No 202-307-7) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015. The Competent Authority of Belgium (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

Pursuant to Article 45(4) of the REACH Regulation the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 15 March 2016.

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

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took into account you comments, which were sent within the commenting period, and they are reflected in the Reasons (Appendix 1).

Commenting by other MSCAs and ECHA

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. They are reflected in the Reasons (Appendix 1).

Referral to Member State Committee

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

ECHA invited you to comment on the proposed amendment(s). Any comments on the proposal(s) for amendment were taken into account by the Member State Committee and are reflected in the Reasons (Appendix 1). The Member State Committee did not take into account any comments on the draft decision as they were not related to the proposal(s) for amendment made and are therefore considered outside the scope of Article 52(2) and Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision during its meeting and ECHA took the decision according to Article 52(2) and 51(6) of

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the REACH Regulation.

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Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

   Further advice can be found at
http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.

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Appendix 4: List of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

EC number: 202-307-7
CAS number: 94-13-3
Public name: propyl 4-hydroxybenzoate

This decision is addressed to the Registrant(s) of the above substance with active registration pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided below.