Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

propyl 4-hydroxybenzoate

EC Number: 202-307-7
CAS Number: 94-13-3

CLH-O-0000007263-77-01/F

Adopted

16 March 2023

]

16 March 2023

CLH-O-0000007263-77-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **propyl 4-hydroxybenzoate**

EC Number: **202-307-7**

CAS Number: **94-13-3**

The proposal was submitted by **Belgium** and received by RAC on **29 March 2022.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Belgium has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **11 April 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 June 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Gerlienke Schuur**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 March 2023** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-VW-Y	propyl 4-hydroxybenzoate	202-307-7	94-13-3	Repr. 2, H361fd	H361fd	GHS08 Wng	H361fd			
RAC opinion	607-RST-VW-Y	propyl 4-hydroxybenzoate	202-307-7	94-13-3	No classification						
Resulting Annex VI entry if agreed by COM	607-RST-VW-Y	propyl 4-hydroxybenzoate	202-307-7	94-13-3	No classification						

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Propyl 4-hydroxybenzoate, referred to as propyl paraben in this RAC opinion, is an antifungal and antimicrobial agent. The substance is used as a preservative in personal care products and pharmaceuticals and as food-additive E216.

The scope of the CLH report by the Dossier Submitter (DS) and the RAC opinion is only on harmonised classification (CLH) for reproductive toxicity (adverse effects on sexual function and fertility, adverse effects on development, and effects on or via lactation).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS described the results of 8 different studies relevant for reproductive toxicity.
Studies with reliability score 1 (reliable without restriction) or 2 (reliable with restrictions) assigned by the DS:

- Extended One-Generation Reproductive Toxicity Study (EOGRTS) in rats according to OECD TG 443 (2021), with developmental neurotoxicity, developmental immunotoxicity and additional learning and memory testing cohorts, and extension of cohort 1B to produce the second generation. Reduced sperm motility and morphology, increased post-implantation loss (F0), and changed anogenital distance (AGD) and nipple retention (F1 and F2 pups) were reported.
- Dose range finding study comparable to reproductive/developmental toxicity screening test in rats (DRF to EOGRTS, 2018). Increased percentage of pre- and post-implantation loss were reported.
- Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in rats according to OECD TG 422 (2012). Increased post-implantation loss and decreased birth index were reported.
- Prenatal Developmental Toxicity study (PNDT) in rats according to OECD TG 414 (2019). No effects reported.

Further studies with reliability score 3 (not reliable) assigned by the Registrant or the DS:

- A non-guideline 1-generation study with male and female rats exposed from post-natal day (PND) 4 to PND90 mated with untreated animals (Sivaraman *et al*., 2018). No effects were reported on mating and fertility index.
- Study with male rats dosed for four weeks. Effects on sperm count and morphology were reported (Oishi, 2002)[1].
- Study with juvenile male rats dosed for 8 weeks. No effects on male sperm parameters were reported (Gazin *et al*., 2013).
- A mice study with subcutaneous injections from gestational day (GD)1 to GD4. The mean number of implantation sites reported as unaffected on GD6 (Shaw & deCatanzaro, 2009).

[1] *In the CLH report, 2012 is given as year of publication. This probably should be 2002.*

The DS noted that no human data are available.

Classification

The DS concluded Repr. Category 2 for fertility based on severe effects in sperm in absence of clear general toxicity as demonstrated in the EOGRTS (2021) and study by Oishi (2002).

The DS concluded Repr. Category 2 for developmental toxicity based on the effects on AGD in EOGRTS (2021), and post-implantation loss modifications in the EOGRTS (2021) and the PNDT (2019).

The DS noted that no data are available for adverse effects on or via lactation.

Comments received during consultation

Two Member State (MS) comments were received:

- One MS wondered if the DS envisaged to propose Repr. Category 1B instead of Category 2. Coherent effects are seen on sperm (count and morphology) among the studies, including EOGRTS. Fertility index was not affected, however the premating time in the study was 2 instead of 10 weeks. Furthermore, the effects may be common to the family of substances (at least methyl-, ethyl and butylparaben). DS noted that family members are currently not harmonised classified.
- Another MS noted that based on the contradicting data and overall weight of evidence for sperm effects, the case is borderline between Repr. Category 2 and no classification for fertility. Furthermore, the MS questioned if AGD is to be used rather for classification for fertility than for developmental toxicity. In females, it is unclear what type of adversity is associated with a decreased AGD or AGD relative to body weight (Anogenital Index, AGI), and why this parameter should be used for classification. The DS agreed with the borderline case, and that decreased male AGD or AGI can be used as supportive information for fertility classification.

One European national authority referred to historical control data (HCD) noted in the registration dossier on post-implantation loss in the Combined repeated dose toxicity study with the reproduction/developmental toxicity screening (2012) and on AGD in the EOGRTS (2021). The DS provided the HCD for post-implantation loss but for AGD HCD were not available.

Three Industry or Trade Associations comments were received:

- One Trade Association commented that the classification proposal was based on effects in the EOGRTS (2021) on sperm parameters, decreased absolute AGD in male pups and apparent increases in post-implantation loss. However, no toxicologically relevant effects on sperm (noting HCD on motile counts of the conducting laboratory, ranging between 65.25% to 98.17% (mean -/+ 2SD)), AGD (concurrent HCD at the conducting laboratory = mean of 2.6 mm from 2073 male pups) or post-implantation loss were reported. For the purpose of weight of evidence, CLH proposal should also have referred to the study by Sivaraman *et al*. (2018).
- Another Trade Association noted that the classification was not based on a total weight of evidence. Negative data were not given equal weight compared to seemingly positive outcomes. For example, reduced AGD values were considered only from the F1 pups, despite not occurring in F2 pups, being not significantly significant after normalisation to cube root or body weight, not dose-dependent and well within the range of historical control data. Further, it was not discussed that the post-implantation loss observed in the EOGRTS (2021)

was statistically not significant, not confirmed in cohort 1B, and well within the range of HCD. Comments noted that the study by Oishi (2002) was not conducted in accordance with OECD guidelines, and had shortcomings. Additional data (Hoberman *et al*. (2008), Sivaraman *et al*. (2018) and Gazin *et al*., 2013) not showing similar effects was not taken into account by the DS. The DS responded that all data on F1 pups and F2 pups are available in Tables 42 and 45 of the CLH report. Data regarding the post-implantation loss in the cohort 1B is available in Table 18 and noted in the CLH report that it is not confirmed in the cohort 1B, and not dose-related. The DS noted that the study by Oishi (2002) was available in the registration dossier and qualified as "acceptable, well documented publication which meets basic scientific principles" despite the study was assigned a reliability score 3 (not reliable) in the CLH report and in the registration dossier[2].

- The third Trade Association also noted the isolated evaluation of single biological parameters, statistical significance, consideration of dose-dependency, and use of HCD. It also noted that there is an ongoing ECHA project with regard to the evaluation of OECD TG 443 EOGRTS studies. DS replied that this project is not linked to the CLH process.

One Academic Institution noted that the classification proposal was lacking scientific justification and did not take into account the scientific principles on toxicological evaluation (e.g., historical control data, biological variability, adversity of effects and dose dependency).

One company did not agree with the proposed classification, considering that effects were judged in isolation, endpoints were lacking statistical significance as well as dose-dependency and were well within the range of historical control data.

In addition to the studies reported by the DS, the European Commission's Scientific Committee on Consumer Safety (SCCS) described several other studies with propyl paraben (SCCS opinion on propyl paraben, 2021); these are described further in the background document.

Assessment and comparison with the classification criteria

EOGRTS according to OECD TG 443 (2021) with Wistar rats (N=30/sex in control and high dose, 25/sex in low and mid dose) was available, with cohorts

- 1A and 1B (N=20/sex/dose) for reproductive and developmental toxicity testing,
- 2A for neurobehavior testing and neurohistopathology assessment,
- 2B for neurohistopathology assessment at post-natal day (PND)21 or 22,
- 3 for developmental immunotoxicity testing on PND56, and
- an additional cohort (cohort 4) for learning and memory testing (N=10/sex/dose).

Wistar rats were dosed orally by gavage with dose levels of 0, 100, 300 and 1000 mg/kg bw/day.

EOGRTS parental animal results

With regard to clinical signs, increased salivation and moving bedding at mid dose in females and in both sexes at the highest dose were noted. No effects were found on parental body weights. TSH was severely increased in females (1634.46, 2015.93, 2037.14, and 3801.42* pg/ml, resp. at 0, 100, 300 and 1000 mg/kg bw/day), while no effects were seen on T4 levels. Absolute and relative prostate weight, and relative liver weight was statistically significant decreased in male

[2] *The reliability score by the registrant was explained further in the registration dossier with control values being outside normal range, and not consistent with literature data and other Oishi studies, absence of dose-response for daily sperm production (DSP), small group size, and because full study protocol and raw data were not available.*

rats at the highest dose, absolute and relative thymus weight was decreased in female rats at the highest dose.
Male reproduction parameters: At 1000 mg/kg bw/day, not statistically significant effects were found on sperm motility (72.7% vs 77.1% in control) and sperm morphology (tail only, 8.2% vs 3% in control).
Female reproduction parameters: At 1000 mg/kg bw/day, the percentage of post-implantation loss was increased, not statistically significant (9% vs 6% in control). The pre-coital interval was slightly increased in all tested doses.
Histopathological examination did not reveal treatment-related effects.

EOGRTS F1 generation results (offspring)

Concerning pups, the viability index was not changed, mean pup body weight was significantly lower only at PND14 in the highest dose group. The anogenital distance (AGD) was somewhat (but statistically significant) decreased in male F1 pups in the highest dose (2.84, 2.78, 2.73, and 2.71*, and relative[3] AGD 1.51, 1.48, 1.46, and 1.46, respectively at 0, 100, 300 and 1000 mg/kg bw/day). More effects on AGD are seen in female F1 pups (1.26, 1.15***, 1.13***, and 1.12*** in mm, and relative AGD 0.68, 0.62***, 0.61***, and 0.61***, at 0, 100, 300 and 1000 mg/kg bw/day). Nipple retention in male pups was decreased at the highest dose (0.23, 0.35, 0.21, and 0.04*, at 0, 100, 300 and 1000 mg/kg bw/day).

EOGRTS F1 generation results

Cohort 1A:

Male reproduction: In the male pups, absolute testis weight was reduced (not stat. sign.; 1.817, 1.782, 1.839 and 1.677 g, at 0, 100, 300 and 1000 mg/kg bw/day). The percentage of motile sperm count (72.4% vs 79.1% in control) was reduced, percentage of static sperm count was higher (27.58% at 1000 mg/kg bw/day vs 20.90% in control group) and percentage of rapid sperm was also reduced (58.11% at 1000 mg/kg bw/day vs 64.83% in control group). Furthermore, total number of abnormal sperm was increased at the highest dose (19.06 at 1000 mg/kg bw/day vs 10.35 in control group) but not statistically significant.
Female reproduction: mean estrous cycle duration was not changed.
Immunological parameters were reported to be affected, however without a clear dose-response and statistical significance.

EOGRTS Cohort 1B and F2 results

The pre-coital interval increased in a dose-related manner (1.94, 2.20, 2.74 and 2.83 days at 0, 100, 300 and 1000 mg/kg bw/day). Mean pup weight was not different amongst the different groups at PND0, 4, 7, 14 and 21. AGD was statistically significantly decreased in male F2 pups (2.98, 2.89, 2.87, and 2.77*** in mm, as well as relative AGD 1.61, 1.55, 1.55, and 1.52**, at 0, 100, 300 and 1000 mg/kg bw/day). No effects on AGD were found in female F2 pups. The nipple retention was increased in male F2 pups (0.33, 0.20, 0.42, and 0.68** at 0, 100, 300 and 1000 mg/kg bw/day). No other effects on reproductive parameters were found.
No relevant effects were found in *Cohort 2A, 2B, 3 and 4*.

[3] *Assumed to be relative to pup weight.*

The study reported a NOAEL for general toxicity of >1000 mg/kg bw/day, and a NOAEL for fertility of 1000 mg/kg bw/day, regarding male fertility. However, the DS was in favour of a NOAEL of 300 mg/kg bw/day based on the sperm effects.

Dose range finding study for the EOGRTS comparable to OECD TG 421 (Anonymous, 2018), dosing Wistar rats (N=5 for control, other groups N=10/sex/dose) orally by gavage with 0, 500, and 1000 mg/kg bw/day. No general toxicity was observed. The precoital interval was decreased in tested groups (7.20, 3.00 and 2.30, resp. at 0, 500 and 1000 mg/kg bw/day). The percentage of pre- and post-implantation loss was increased (0.00, 0.79 and 1.74% and 6.47, 6.74 and 8.72%, resp. at 0, 500 and 1000 mg/kg bw/day). No effects were found on number of live pups.

Other studies

A combined repeated dose toxicity study with the reproduction/developmental toxicity screening test according to OECD TG 422 (Anonymous, 2012) was presented, dosing Wistar rats (N=11/sex/group) orally by feed (corresponding to 59.3–98.0, 178.3–305.1, and 605.0–980.9 mg/kg bw/day for the males and 116.0–137.3, 341.9–431.8, and 1 076.4–1 380.0 mg/kg bw/day for females), from 28 days for males and 14 days for females prior to pairing, and through pairing and gestation until PND4. No general toxicity was seen. Male and female reproduction parameters were not affected. Only the percentage of post-implantation loss was higher at the highest dose (12.4% vs 5.9%). Mean number of live pups was lower at the low and high dose group (11.2, 9.8, 11.6 and 9.9 respectively). The birth index was decreased at the highest dose (87.6% vs 94.1% in control).

Sivaraman *et al*. (2018) performed a study with propyl paraben to assess potential estrogen-mimetic effects. Male and female SD rats were dosed orally by gavage with 0, 10, 100 and 1000 mg/kg bw/day from PND4 to PND90 (n=25/sex/dose). To assess reproductive function, they were mated with untreated partners. Mating and fertility index were unaffected. Preputial separation was not affected, other male parameters were not examined. Mean age of vaginal patency was significantly lower at the highest dose (31.2 vs 33.9 in control), however within HCD (29.0 to 33.9 days). There were no effects on estrous cyclicity. Mean number of implantation sites was significantly higher in the low dose group (14.3, 17.4**, 16.1 and 15.6, resp. at 0, 10, 100 and 1000 mg/kg bw/day). No effects on litter weight and viability index, and no treatment-related effects were reported in the pups.

Gazin et al. (2018) performed a study with juvenile male Wistar rats orally dosed by gavage to 0, 3, 10, 100, and 1000 mg/kg bw/day (N=20/group). Exposure was a single dose at PND31 in the preliminary study and for 8 weeks starting at PND21 in the main study. No marked general toxicity was observed. No effects on balano-preputial separation, on mean epididymal, testis sperm count and on testis weight and microscopy were found. DS noted slight variations in sperm motility parameters, however they were not statistically significant and without any dose-response relationship.

Oishi (2002[4]) performed a study with male Wistar rats (N=8/group), dosed orally by feed in resulting doses of 0, 12.4, 125 and 1290 mg/kg bw/day for four weeks. No effects were reported on the male reproductive organ weights. Sperm counts in the cauda epididymis was severely affected. The sperm reserves were statistically significant decreased (43.6, 31.1, 25.7*, and

[4] *In the CLH report, 2012 is given erroneously as the year of publication whereas the publication year should be 2002.*

22.5* x10^7/cauda) and the sperm concentration was statistically significant decreased (108, 70.8, 63.1*, and 48.8* x10^7/g), respectively for 0, 12.4, 125 and 1290 mg/kg bw/day dose groups. Daily sperm production (DSP) in testis and its efficiency was severely reduced (DSP 37.5, 26.2*, 27.0*, and 25.9* x10^6; Efficiency 30.0, 20.6*, 22.4*, and 21.4* x10^7, respectively for 0, 12.4, 125 and 1290 mg/kg bw/day dose groups. Mean testosterone concentration in serum decreased in a dose-dependent way and was significant at the highest dose (9.08, 8.20, 7.17 and 5.86* ng/ml, respectively for 0, 12.4, 125 and 1290 mg/kg bw/day dose groups). Author reported a LOAEL for fertility of 12.4 mg/kg bw/day.

Shaw & deCatanzaro (2009) performed a study in mice using subcutaneous injections of 0, 35 or 40 mg propyl paraben (per animal) from GD1 to GD4. The mean number of implantation sites on GD6 was unaffected.

Prenatal developmental toxicity study according to OECD TG 414 (Anonymous, 2019) was performed in Wistar rats with 0, 100, 300 and 1000 mg propyl paraben/kg bw/day orally by gavage from GD5-19. No effects were reported on body weight, pre- and post-implantation loss and percentage resorptions. No treatment-related histopathological changes were observed. The number of live pups was similar in all groups, and no effect of treatment on the litter and fetus weight was found. External, and visceral, craniofacial and skeletal examinations did not find treatment-related effects.

Comparison to the classification criteria

Fertility

RAC concludes that since there is no evidence for effects of propyl paraben on fertility in humans, Reproductive Toxicity classification in Category 1A is not appropriate.

Furthermore, RAC considers Reproductive Toxicity classification in Category 1B for fertility not appropriate because there is no clear evidence of effects on fertility from animal studies.

Reproductive Toxicity classification in Category 2 is possible based on evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility (and where the information is not sufficiently convincing to place the substance in Category 1B).

The available studies provide some evidence that propyl paraben may affect sexual function and fertility. Overall, RAC considers this evidence too inconsistent and uncertain to classify propyl paraben for Reproductive Toxicity in Category 2. More specifically (see also Table on sperm parameters below):

- In males, some sperm parameters were affected, without marked general toxicity.
- Sperm motility was slightly affected (not statistically significant) in F0 in the EOGRTS (2021), however this was not found in the OECD TG 422 screening study (2012) and Gazin *et al*. (2013).
- Sperm counts in the testis were reported to be statistically significantly decreased at all dose levels (about similar levels), without a dose-response by Oishi (2002). However, sperm counts were not decreased in other studies (EOGRTS, 2021; OECD TG 422 screening study, 2012; Gazin *et al*., 2013).
- No treatment-related effects were reported on testis weight.
- In several studies the fertility index was not affected (EOGRTS, 2021; Sivaraman *et al*., 2018; OECD TG 422 screening study, 2012).
- The effect on AGD found in the EOGRTS (2021) could be seen as a signal for perturbed masculinisation in the developing pups, and could be discussed under fertility endpoint.

However, the effect on relative AGD is only statistically significant in F2 pups, it is only slightly changed compared to controls, and it is not accompanied by an effect on nipple retention expected for a substance with an anti-androgenic mode of action (Schwartz *et al*., 2021). Further, no clear effects are found on sperm parameters in the EOGRTS (2021).

Due to the lack of overall homogeneity of the data on sperm parameters and no clear effect on sperm parameters in the EOGRTS (2021), together with no effect on functional parameters, RAC considers the evidence not sufficient for Reproductive Toxicity classification.

RAC concludes based on the available data that there is insufficient evidence for effects on sexual function and fertility in experimental animals, and that no classification for effects on fertility is warranted.

Developmental toxicity

RAC concludes that since there is no evidence for effects of propyl paraben on development in humans, Reproductive Toxicity classification in Category 1A is not appropriate.

RAC considers classification of propyl paraben in Category 1B not appropriate because the evidence on developmental toxicity is considered too weak in the animal studies. According to the CLP criteria, the data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on reproduction should be considered not to be a secondary non-specific consequence of other toxic effects.

RAC also considers Reproductive Toxicity classification in Category 2 not appropriate because the evidence from animal experiments for an adverse developmental effects is too inconsistent and uncertain. More specifically, no classification of propyl paraben is justified for the following reasons (see also the overview Tables on post-implantation loss and AGD/nipple retention below):

- No visceral, craniofacial or skeletal malformations were reported.
- No effects on pup weight and pup viability were reported.
- The effects on post-implantation loss were not statistically significant, did not show a dose-response relationship, and was not found consistently in all rat studies (PNDT, 2019; DRF for EOGRTS, 2018; EOGRTS, 2021; OECD TG 422 screening study, 2012; Sivarman *et al*., 2018).
- Relative AGD decrease and nipple retention increase in male pups are seen as sensitive anti-androgenic endpoints. The reported decrease in relative AGD in male pups was slight, without a clear dose-dependency, and found in the F1 and F2 pups (only statistically significant in F2 at the highest dose group of EOGRTS, 2021). Nipple retention was significantly increased only in F2 male pups, however decreased in F1 male pups at the highest dose. The decrease in AGD in female F1 pups seems to be caused by a higher control value, and it was not reported for female F2 pups.

Based on the above, RAC concludes that propyl paraben warrants no classification for Reproductive Toxicity for developmental effects.

Lactation

No data for effects on or via lactation were described in the CLH report. In the description of the EOGRTS (2021) in the registration dossier it is noted that "Exposure at PND4 demonstrated transfer of test item via milk" (Cohort 4). RAC agrees with the DS proposal that no classification for effects on or via lactation is warranted.

In summary, RAC concludes on **no classification for fertility and sexual function, for developmental toxicity, and for effects on or via lactation.**

Additional references

Danish EPA (2022). Analyses and risk assessment of endocrine disruptors in product for pregnant women and children. Survey of chemical substances in consumer products No. 189. Rapport (mst.dk)

EMA (2015). Reflection paper on the use of methyl- and propylparaben as excipients in human medicinal products for oral use. EMA/CHMP/SWP/272921/2012. Reflection paper on the use of methyl- and propylparaben as excipeints in human medicinal products for oral use (europa.eu)

Oishi (2001). Effects of butylparaben on the male reproductive system in rats. Toxicology and Industrial Health, 17, 31-39.

Oishi (2004). Lack of spermatotoxic effects of methyl and ethyl esters of *p*-hydroxybenzoic acid in rats. Food and Chemical Toxicology, 42, 1845-1849.

SCCS (2021). Opinion on Propylparaben (PP). sccs_o_243.pdf (europa.eu)

Schwartz et al. (2021). On the use and interpretation of areola/nipple retention as a biomarker for anti-androgenic effects in rat toxicity studies. Frontiers in Toxicology, 3, 1-22

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).