Committee for Risk Assessment

RAC

Annex 1
Background document
to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 March 2023

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Chemical name:

propyl 4-hydroxybenzoate

EC Number: 202-307-7

CAS Number: 94-13-3

Index Number: 607-RST-VW-Y

Contact details for dossier submitter:

FPS Public Health, Food Chain Safety and Environment
DGEM/ Department of Product Policy and chemical Substances / Management of Chemical Substances
Avenue Galilée, 5/2
1020 Brussels
Belgium

Version number: 2 **Date: March 2022**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PROPYL 4-HYDROXYBENZOATE

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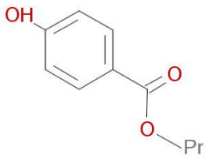
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PROPYL 4-HYDROXYBENZOATE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	propyl 4-hydroxybenzoate
Other names (usual name, trade name, abbreviation)	Propylparaben Propyl paraben 4-hydroxybenzoic acid propyl ester Benzoic acid, 4-hydroxy-, propyl ester p-Hydroxybenzoic acid propyl ester Propyl p-Hydroxybenzoate n-Propylparaben Other names (Trade names): Faracide P Microcare OHB Paratexin P Solbrol P
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	202-307-7
EC name (if available and appropriate)	propyl 4-hydroxybenzoate
CAS number (if available)	94-13-3
Other identity code (if available)	/
Molecular formula	C10H12O3
Structural formula	
SMILES notation (if available)	/
Molecular weight or molecular weight range	180.21 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	/
Description of the manufacturing process and identity of the source (for UVCB substances only)	/
Degree of purity (%) (if relevant for the entry in Annex VI)	> 98.0 % (w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
propyl 4-hydroxybenzoate EC n° 202-307-7	**> 98.0 % (w/w)**	**/**	**Aquatic chronic 3, H412**

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
See confidential annex				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
/					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 submitter's proposal	607-RST-VW-Y	propyl 4-hydroxybenzoate	202-307-7	94-13-3	Repr. 2	H361fd	GHS08 Wng	H361fd	/	/	/

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	Repr. 2, H361fd	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Propylparaben is a chemical substance which is registered under REACH (1907/2006/EC). The substance is not listed in annex VI of CLP and classification and labelling was not previously discussed by the TC C&L.

The substance is self-classified in the public registration dossier as :

Aquatic Chronic 3, H412

The substance is also under substance evaluation (REACH).

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).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

Formulation : Manufacturing of cosmetic products and pharmaceutical preparations (ointments)

Consumer uses : Consumer End Use of cosmetic products or pharmaceuticals

6 DATA SOURCES

- Registration dossier
- Literature search
- Full study report

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101.3 kPa	White crystalline solid	Anonymous 1, 2012 (registration dossier)	Rel. 1

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Melting/freezing point	97 °C (mean value of 2 peer reviewed article reports and 1 peer reviewed handbook report. No experimental details are given)	Publications 1990, 2006 and 2001 (Registration dossier)	Rel. 4
Boiling point	301 °C ± 16 °C (The sample shows a broad endothermic boiling peak in the 290 - 350 °C region)	Anonymous 2, 2012 (registration dossier)	Rel. 2 OECD TG 103 Non-GLP
Relative density	1.287 g/cm^3	Publication 1999 and publication 2006 (registration dossier)	Rel. 2
Vapour pressure	0.00034 Pa at 20 °C (OECD TG 104) by vapour pressure balance (effusion method)	Anonymous 3, 2011 (registration dossier)	Rel. 2 Non-GLP
Surface tension	No surface activity expected	Registration dossier	Based on structure
Water solubility	500 mg/L at 25 °C 579.6 mg/L at 25 °C (EPI Suite v4.0)	Publication 2003 EPI suite v4.0 (registration dossier)	Rel. 4 Rel. 2
Partition coefficient n-octanol/water	**2.8 (mean of results of the most reliable studies)** 2.94 at 37 °C and pH3 (Shake flask method) 3.04 (HPLC method) 2.876 at room T° (HPLC method) 2.34 at 20 °C (HPLC method) 3.00 at pH7.5 (shake flask method) 3.04 (HPLC method) 2.71 (no method stated)	 Publication 2008 Publication 1995 Publication 2003 Publication 2009 Publication 1981 Publication 1990 Publication 2005 (registration dossier)	Rel. 2 Rel. 4 Rel. 4 Rel.1 Rel.2 Rel.2 Rel.4
Flash point	NA for solids		

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Flammability	No flammable solid (EU Method A.10) No pyrophoricity No flammability on contact with water	Anonymous 4, 2011 (registration dossier)	Rel. 2 Non-GLP
Explosive properties	No	Registration dossier	The substance does not contain reactive groups associated with exploviness
Self-ignition temperature	NA		
Oxidising properties	No	Registration dossier	The substance does not contain any oxidizing groups and all oxygen atoms present in the molecular structure are bonded directely to carbon.
Granulometry	Median particle diameter (d50): 16.2 ± 0.7 µm Fraction less than 1 µm diameter: 5.00 ± 0.1 vol % Fraction less than 4 µm diameter: 16.4 ± 0.15 vol % Fraction less than 10 µm diameter: 37.8 ± 1.0 vol % Fraction less than 100 µm diameter: 88.4 ± 0.6 vol %	Anonymous 5, 2011 (registration dossier)	Rel. 2 ISO 13320-1 EN 481
Stability in organic solvents and identity of relevant degradation products	The functial groups of the substance indicate no instabilities in common organic solvents		
Dissociation constant	pKa = 8.87 (HPLC) pKa = 8.16 at 25 °C (potentiometric titration) pKa = 8.35	Publication 2008 Publication 2009 Publications 1979 and 2006	Rel. 2 Rel. 2 Rel. 2
Viscosity	NA		Substance is a solid

8 EVALUATION OF PHYSICAL HAZARDS

Hazard class not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this CLH dossier

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier

10.7 Skin sensitisation

Hazard class not assessed in this dossier

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier

10.9 Carcinogenicity

Hazard class not assessed in this dossier

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
EOGRTS with DNT and DIT Oral, gavage Rat (Wistar) F0: 30/sex in control and high dose and 25/sex in low and mid doses Cohorts 1A and 1B: 20/sex/dose Cohorts 2A, 2B, 3 and 4: 10/sex/dose OECD TG 443 GLP Reliability 1	Propylparaben Purity: 99.7 % Vehicle: 1 % of hydroxyethyl-cellulose Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: F0: min. 10w in males and 14d of pre-mating, max 14d of mating, gestation and through weaning in females F1: from weaning (PND 22) to terminal sacrifice and the respective cohorts	<u>F0 parental:</u> Clinical signs: increased salivation and moving bedding at mid dose in females and in both sexes at the highest dose Bw: no significant change Male reproduction parameters: reduced sperm motility (72.67 % vs 77.05 % in control), sperm morphology affected (tail only) (8.17 vs 2.96 % in control) at 1000 mg/kg bw/d Female reproduction parameters: precoital interval slightly increased in all tested doses, increase percentage of post-implantation loss at 1000 mg/kg bw/d (8.98 % vs 5.99 % in control) Necropsy: few organ weight changes (liver and prostate in males and only thymus in females). No microscopic changes observed <u>F1 pups:</u> Viability index not modified <u>Cohort 1A:</u> Clinical signs: moving bedding observed at the highest dose Bw: changed at the highest dose in males at D64 Balano-preputial separation slightly reduced Thyroid hormone: significantly higher in females of the mid and high doses Male reproduction parameters: reduced testes weight, percentage of motile sperm count and higher percentage of abnormal sperms	Registration dossier (study report, 2021)

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Immunological parameters affected (see table 15) Necropsy: few organ weight changed (see table 16) <u>Cohort 1B and F2 pups:</u> Moving bedding in female of the mid dose and in both sexes of the highest dose Bw: sign. higher during gestation and lactation Female reproduction parameters: precoital interval increased (dose-related) Pups: AGD and nipple retention sign. affected <u>Cohort 2A:</u> Moving bedding at the highest dose Neurotoxicity parameters: few modifications (see table 19) <u>Cohort 2B:</u> No abnormalities observed <u>Cohort 3:</u> Immunological parameters affected (see table 20 and 21) <u>Cohort 4:</u> Mean escape latency sign reduced in female during memory phase NOAEL (general toxicity) : > 1000 mg/kg bw/d NOAEL (fertility) : 1000 mg/kg bw/d for M and F according to registration dossier however, regarding male fertility, DS is in favour of a NOAEL of 300 mg/kg bw/d based on the sperm effects	
Dose range finding study for reproduction/developmental toxicity screening test Oral, gavage Rat (Wistar) 10/sex/dose (except for control group : 5/sex) Equivalent or similar to OECD TG 421 Non-GLP Reliability 1 (according to registration dossier. But not GLP)	Propylparaben Purity: 99.7 % Vehicle: 1 % hydroxyethyl-cellulose Doses: 0, 500 and 1000 mg/kg bw/d Duration of exposure: min 35d for males and during 21d for pre-mating, max 14d for mating, through gestation and up to PND 21 (except one dam of each treated	<u>Parental :</u> Bw: unaffected Female reproductive parameters: precoital interval decreased in tested groups, percentage of pre- and post-implantation loss increased. Other parameters unaffected Necropsy: no treatment-related change <u>Pups:</u> Mean nb of pups at birth, mean nb of live pups and viability index unaffected NOAEL (general toxicity) : > 1000 mg/kg bw/d	Registration dossier (study report, 2018)

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reliability 2 (according to DS)	group which was dosed up to GD 20). Surviving pups of one litter from each group were treated from PND 13 to PND 21	NOAEL (fertility) : 1000 mg/kg bw/d	
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Oral, feed Rat (Wistar) 11/sex/group OECD TG 422 GLP Reliability 1	Propylparaben Purity: 99.7 % Vehicle: not specified Doses: 0, 1500, 4500 and 15000 ppm (see table 25 for the mean achieve dose levels) Duration of exposure: min 4w for males and approx. 7w for females (14d of premating, max 14d of mating, gestation and through lactation day 3)	<u>Parental:</u> Bw: unaffected Male reproduction parameters: not sign. affected Female reproduction parameters: percentage of post-implantation loss severely higher at the highest dose (12.4 % vs 5.9 %) Necropsy: kidney and epididymide (right) weights sign. modified. No microscopic treatment-related changes observed <u>Pups:</u> mean nb of living pups lower at the low and high dose groups, birth index decreased at the highest dose. Pup bw at PND 1 and 4 unaffected No treatment-related abnormalities observed NOAEL (parental toxicity) : 15000 ppm NOAEL (reproduction/developmental toxicity : 15000 ppm mentioned in the registration dossier. However, based on the higher percentage of post-implantation loss observed at the highest dose, a NOAEL of 4500 ppm (mid dose) is proposed by DS.	Registration dossier (study report, 2012)
Assessment of propylparaben in juvenile rats 2 separate studies were conducted to assess the potential estrogen-mimetic effects on 1) reproductive developmental and function examination when animals exposed from PND 4 to 90, and 2) examination of uterus weight in immature females when exposed from PND 4 to PND 7 or 21 Oral, gavage Rat (SD) 25/sex/dose in phase 1 and	Propylparaben Purity: 99.7 % Vehicle: 1 % hydroxyethylcellulose Doses: 0, 10, 100 and 1000 mg/kg bw/d Duration of exposure: from PND 4 to PND 90 in phase 1 and from PND 4 to PND 7 or 21 in phase 2	Mean age of vaginal patency sign. lower at the highest dose (within HCD) Preputial separation similar in all groups Female reproductive parameters: mean nb of implantation sites sign. higher in the low dose group Male reproductive parameters: mating and fertility index unaffected (other parameters not examined) Necropsy: no treatment-related change observed <u>Pups:</u> Litter weight and viability index not modified No malformed pups observed NOAEL (parental toxicity) : 1000 mg/kg bw/d	Sivaraman *et al.*, 2018

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
15 or 30/sex/dose in phase 2 Reliability 2 (according to registration dossier, but only summary available, only few parameters examined, no individual data, no info about GLP conform) Reliability 3 (according to the DS)		NOAEL (fertility) : 1000 mg/kg bw/d	
Assessment of propylparaben on juvenile male rats Oral, gavage Rat (Wistar) Preliminary study: 3 males in control group and 18 males in treated groups Main study: 20 males per group GLP Reliability 4 (according to registration dossier (short abstract available)) Reliability 3 (according to DS : article available, GLP compliance)	Propylparaben Purity: 99.7 % Vehicle: 1 % hydroxyethylcellulose Doses: 0, 3, 10, 100 and 1000 mg/kg bw/d Duration of exposure: single exposure at PND 31 for preliminary study, 8 weeks for main study (divided into 2 subgroups: one euthaninzed at the end of exposure period and a second after a 26 weeks of recovery period)	Main study: Clinical signs: hypersalivation at the highest dose Bw: slightly increased (approx. + 7 %) Mean day of balano-preputial separation unaffected Plasma hormone levels (Testosterone, LH and FSH): only slight variations observed Mean epididymal and testis sperm count: unaffected Sperm motility: slight variations observed Necropsy: mean testis weight did not show modifications, and no microscopic changes observed NOAEL (general toxicity) : 1000 mg/kg bw/d NOAEL (male fertility) : 1000 mg/kg bw/d	Gazin *et al.*, 2013
Effects of propylparaben on the male reproductive system Oral, feed Rat (Wistar) 8 males per group No information about GLP compliance Reliability 3 (according to registrant)	Propylparaben Purity: 99.7 % Vehicle: corn oil Doses: 0, 0.01, 0.10 and 1.0 % (corresponding to 0, 12.4, 125 and 1290 mg/kg bw/d) Duration of exposure: 4 weeks	Bw: no information available Male reproductive parameters: organ weight unaffected Sperm counts in the cauda epididymis and sperm production in testis severely affected Daily sperm production and its efficiency was severely reduced Mean testosterone concentration in the serum decreased in a dose dependent manner and sign. at the highest dose LOAEL (fertility) : 12.4 mg/kg bw/d No NOAEL	Oishi, 2002
Assessment on the impact of parabens on early pregnancy Subcutaneous injection	Propylparaben and butylparaben Purity: unknown Vehicle: depending of	Mean nb of implantation sites unaffected NOAEL (parental toxicity) : 45 mg of propylparaben	Shaw and deCantazaro, 2009

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Mouse No information about GLP compliance Reliability 2 (according to registration dossier, but only summary available, only few parameters examined, no individual data, no info about GLP conform) Reliability 3 (according to the DS)	the experiment Doses: 35 or 45 mg of propylparaben Duration of exposure: GD 1 to GD 4 (euthanised at GD 6)	NOAEL (developmental toxicity) : 45 mg of propylparaben	

No human data or other studies available

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In an extended-one generation reproductive toxicity study (Registration dossier (study report, 2021)), performed following the OECD TG 443, groups of male and female Wistar rats were given propylparaben (purity of 99.7 %) by gavage at a concentration of either 0, 100, 300 or 1000 mg/kg bw/d.

For the F0 parental generation, 30 males and 30 females were exposed to either 0 or 1000 mg/kg bw/d and 25 males and 25 females were exposed to either 100 or 300 mg/kg bw/d. Animals were given propylparaben daily during a minimum of 10 weeks for males (14 days of pre-mating, maximum 14 days of mating and until terminal sacrifice) whereas females were exposed during 14 days of premating period, maximum 14 days of mating, during gestation and until weaning (at PND 21). Before weaning of the F1 pups on PND 21, animals were randomly selected and placed into cohorts.

- Cohort 1A was composed of 20 males and 20 females per dose group and animals were sacrificed at 13 weeks of age (approx. 10 weeks of treatment).
- Cohort 1B was composed of 20 males and 20 females per dose group and was selected to produce F2 pups. As for the F0 parental generation, males and females were mated. F1 animals were sacrificed shortly after weaning of F2 pups (approx. at 20 – 25 weeks old).
- Cohort 2A (neurotoxicity) was composed of 10 males and 10 females per dose group and animals were sacrificed at 12 weeks of age (approx. 9 weeks of treatment).
- Cohort 2B (neurotoxicity) was composed of 10 males and 10 females per dose group and animals were sacrificed at weaning.
- Cohort 3 (immunotoxicity) was composed of 10 males and 10 females per dose group and animals were sacrificed at 8 weeks of age (approx. 5 weeks of treatment).
- Cohort 4 (for learning and memory testing) was composed of 10 males and 10 females per dose group and animals were sacrificed at PND 35 – 42.

F0 parental and F1 pups (before weaning) :

Regarding the F0 parental generation, 3 females of the low dose group were sacrificed at PMD 6, GD 21 and PND 4 and 1 female of the mid dose was euthanized at PND 18. All these animals were sacrificed for animal welfare reasons. At the highest dose, 22 males exhibited increased salivation and all males exhibited moving bedding. While in females, clinical signs were already observed at the mid dose group. 4 females at the mid dose and 24 females at the highest dose exhibited excessive salivation, and moving bedding was observed in 5 females at the mid dose and 30 females at the highest dose. No significant and treatment-related bw change was observed (see Table 9). Thyroid hormones examination exhibited a severe increase in TSH levels in females exposed to 1000 mg/kg bw/d (1634.46, 2015.93, 2037.14 and 3801.42* pg/ml, resp. at 0, 100, 300 and 1000 mg/kg bw/d), while T4 level was not affected.

Table 9 : body weight data (in g)

Dose level (in mg/kg bw/d)		0	100	300	1000
Males					
Nb examined		30	25	25	30
D1		370.30	376.56	370.20	371.13
D21		394.87	398.56	390.64	392.73
D49		429.70	426.68	419.00	422.87
D70		449.63	443.08	438.16	440.70
Females					
Premating period	D1	227.30 (n=30)	229.72 (n=25)	229.28 (n=25)	224.07 (n=30)
	D14	232.97 (n=30)	235.71 (n=24)	235.92 (n=25)	230.37 (n=30)
Gestation period	D0	232.5 (n=26)	236.95 (n=21)	237.35 (n=23)	230.54 (n=26)
	D20	337.54 (n=24)	349.76 (n=21)	338.43 (n=23)	341.93 (n=27)
Lactation period	D0	263.92 (n=26)	268.27 (n=22)	267.23 (n=22)	266.68 (n=28)
	D7	286.81 (n=26)	288.33 (n=21)	281.59 (n=22)	280.75 (n=28)
	D21	286.72 (n=25)	289.05 (n=21)	288.90 (n=21)	287.36 (n=28)

Male reproduction parameters were examined and revealed a reduction of sperm motility at the highest dose. Furthermore, sperm morphology examination exhibited also changes. Percentage of tail only sperm was severely increased (approx. of 276 %) (see Table 10).

Table 10 : male reproduction parameters

Dose level (in mg/kg bw/d)		0	100	300	1000
Motility	Motile count (%)	77.05 (n=30)	77.60 (n=30)	77.98 (n=25)	72.67 (n=30)
	Static count (%)	22.97 (n=30)	22.40 (n=25)	22.02 (n=25)	24.00 (n=30)
	Rapid (%)	60.85 (n=30)	57.34 (n=25)	60.68 (n=25)	55.75 (n=30)
Testicular sperm count	Million sperms/g	113.5 (n=30)	115.5 (n=25)	124.0 (n=25)	114.9 (n=30)
Nb examined		24	0	0	24
Sperm morphology (in %)	Amorphous head	0.0	/	/	0.0
	Head only	2.46	/	/	2.63
	Bent tail	2.17	/	/	2.38

	Broken tail	0.42	/	/	0.08
	Coiled tail	0.25	/	/	0.08
	Tail only	2.96	/	/	8.17
Number of sperms evaluated		200.00	/	/	200.00
Total number of abnormal sperms		8.25	/	/	13.33
Total number of normal sperms		191.75	/	/	186.67
% of abnormal		4.13	/	/	6.67

Regarding female reproduction parameters, no significant or dose-related change was observed. However, precoital interval was slightly higher in all tested doses in comparison with the controls. Furthermore, percentage of post-implantation loss was increased at the highest dose (approx. of 149 % compared to the control group) (see Table 11). At the end of gestation, the mean number of live births was of 10.50, 11.18, 10.70 and 10.89, resp. at 0, 100, 300 and 1000 mg/kg bw/d.

Table 11 : fertility data

Dose level (in mg/kg bw/d)	0	100	300	1000
Mean oestrous cycle duration (in day)	4.07	4.05	4.02	4.01
Precoital interval (in day)	1.90	2.30	2.33	2.31
Duration of gestation (in day)	22.32	22.29	22.18	22.22
Mean nb of corpora lutea	11.62	12.59	12.35	12.04
Mean nb of implantation sites	11.12	12.14	11.70	11.67
Mean pre-implantation loss (in %)	4.88	3.30	5.42	3.20
Mean post-implantation loss (in %)	5.99	7.79	4.76	8.98

At necropsy, only spontaneous gross pathological findings were observed and final body weight was not significantly changed. Relative liver weight was significantly lower in males exposed to 1000 mg/kg bw/d (2.841, 2.751, 2.716 and 2.644**, resp. at 0, 100, 300 and 1000 mg/kg bw/d). Furthermore, absolute and relative prostate (with seminal vesicles and with coagulating glands) weights were significantly reduced at the highest dose (abs : 3.281, 3.226, 3.008 and 2.856** g and rela : 0.727, 0.716, 0.678 and 0.643**, resp. at 0, 100, 300 and 1000 mg/kg bw/d). While in females, only relative thymus weight was significantly changed (abs : 0.242, 0.188, 0.201 and 0.186 g and rela : 0.088, 0.067, 0.074 and 0.067*, resp. at 0, 100, 300 and 1000 mg/kg bw/d). Histopathological examination did not reveal treatment-related effects.

Concerning pups examination, viability index was not modified and mean pup body weight was only significantly lower at PND 14 in the highest dose group (29.68** g vs 32.81 g in control group).

Cohort 1A :

Three females, exposed to 100 mg/kg bw/d, were sacrificed at PMD 6, GD 21 and PND 4, and one female, exposed to 300 mg/kg bw/d was euthanized at PND 18. All these animals were sacrificed for animal welfare reasons. Clinical signs, such as moving the bedding, was observed in 17 males and 7 females of the highest dose group. Furthermore, at this highest dose, body weight was sign. lower in males at day 64 (see Table 12). Mean balano-preputial separation was slightly reduced (32.32, 31.75, 31.75 and 31.45 days, resp. at 0, 100, 300 and 1000 mg/kg bw/d) while mean vaginal opening was of 30.20, 30.55, 30.75 and 30.50 days, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Blood examination revealed also few haematological changes in both sexes (see Table 13), alkaline phosphatase was significantly reduced in females exposed to 300 and 1000 mg/kg

bw/d (138.394, 113.083, 78.795** and 80.986** U/L, respectively at 0, 100, 300 and 1000 mg/kg bw/d). Thyroid hormones analysis showed also variations and significant changes (see Table 14).

Table 12 : Body weight data (in g)

	Males				Females			
Dose level (in mg/kg b/d)	0	100	300	1000	0	100	300	1000
D1	55.4	52.9	52.8	49.8**	54.6	51.4	50.7	49.2**
D29	220.7	224.7	218.0	212.2	156.4	163.2	158.6	160.8
D64	339.4	342.8	331.8	312.3**	210.6	218.8	213.7	214.4

** : $p < 0.01$

Table 13 : haematological data (at week 11)

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
Nb examined	10	10	10	10	10	9	10	10
Ht (%)	47.63	48.16	49.28	48.97	43.89	44.51	46.84**	45.92*
Hg (g/dL)	16.02	16.33	16.45	16.76**	14.71	15.16	15.87***	15.55*
RBC (1^{12}/L)	8.845	9.036	9.017	9.285	7.960	8.038	8.515*	8.425
MCV (fL)	53.92	53.29	54.68	52.79	55.20	55.42	55.07	54.58
MCH (pg)	18.14	18.06	18.26	18.07	18.49	18.88	18.66	18.48
Plt (1^9/L)	701.4	681.3	659.9	613.9**	669.2	794.2	678.6	679.5
WBC (1^9/L)	5.376	5.580	6.660*	6.998**	2.907	4.734**	5.544***	4.479*
PT (sec)	21.49	21.58	21.94	21.74	22.33 (n=9)	21.84 (n=8)	22.95	24.19**
aPTT (sec)	9.88	9.44	9.49	9.14	9.94 (n=9)	10.34 (n=9)	10.11	9.51

* : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$

Table 14 : thyroid hormone data

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
N examined	10	10	10	10	10	10	10	10
T4 (nmol/L)	82.15	85.10	81.21 (n=9)	79.32	53.20	58.60	67.08*	68.85**
TSH (pg/ml)	1912.58	2387.28	2307.35	4001.28	1730.85	3304.06	1352.69 (n=9)	1369.46 (n=9)

* : $p < 0.05$; ** : $p < 0.01$

Regarding male reproduction paremeters, no significant change was observed, however some parameters were greatly modified. At the highest dose, absolute testes weight was decreased (1.817, 1.782, 1.839 and 1.677 g,

resp. at 0, 100, 300 and 1000 mg/kg bw/d). Sperm motility examination revealed also modification at the highest dose group, as well as sperm morphology. In this highest dose group, percentage of motile sperm count was reduced (72.42 % at 1000 mg/kg bw/d vs 79.10 % in control group), percentage of static sperm count was higher (27.58 % at 1000 mg/kg bw/d vs 20.90 % in control group) and percentage of rapid sperm was also reduced (58.11 % at 1000 mg/kg bw/d vs 64.83 % in control group). Furthermore, total number of abnormal sperms was greatly increased at the highest dose (19.06 at 1000 mg/kg bw/d vs 10.35 in control group).

In females, mean oestrous cycle duration was not changed (3.97, 4.00, 3.98 and 4.09 days, resp. at 0, 100, 300 and 1000 mg/kg bw/d).

In this cohort, immunological parameters were examined and revealed severe modifications (see Table 15).

Table 15 : immunological data

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
Mean lymphocyte count in spleen (lymphocytes x10^6/organ (g))	331	363	360	415	333	422	359	406
T cell count in spleen (T cells x10^6/organ (g))	121	138	143	175	127	166	149	169
CD4 T cell count in spleen (CD4 T cells x10^6/organ (g))	83	94	93	121	85	113	96	114
CD8 T cell count in spleen (CD8 T cells x10^6/organ (g))	35	41	47	51	38	49	49	52
NK cell count in spleen (NK cells x10^6/organ (g))	12	13	14	16	12	15	14	16
B cell count in spleen (B cells x10^6/organ (g))	133	145	130	140	132	157	126	146

No statistical analysis performed

At the end of the exposure period, at approx. 13 weeks of age, animals were sacrificed and necropsied. No dose-related or significant necropsy findings were observed. Final body weight was significantly lower in males exposed to the highest dose group. Furthermore, in this group, absolute adrenals weight, absolute heart weight and absolute liver weight were significantly decreased. In males exposed to 300 mg/kg bw/d, absolute and relative liver weights were also significantly reduced (see Table 16).

Table 16 : organ weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	100	300	1000	0	100	300	1000
N examined		20	20	19	18				
FBW		346.7	351.35	338.11	322.33*	215.15	223.30	215.80	217.78
Adrenals	Abs	0.0641	0.0633	0.0656	0.0574*	0.0768	0.0838	0.0803	0.0768
	Rela	0.0185	0.0181	0.0194	0.0179	0.0357	0.0376	0.0372	0.0352
Heart	Abs	1.060	1.036	1.031	0.955**	0.740	0.763	0.739	0.749
	Rela	0.306	0.295	0.305	0.297	0.344	0.342	0.432	0.344
Liver	Abs	11.805	11.499	10.775*	10.692*	7.468	7.400	7.081	7.204
	Rela	3.404	3.270	3.182**	3.311	3.465	3.317	3.278	3.311
Thyroid/parathyroid	Abs	0.0299	0.0329	0.0344	0.0335	0.0246	0.0233	0.0233	0.0267

	Rela	0.0087	0.0090	0.0069	0.0096	0.0114	0.0104	0.0108	0.0122
Epididymides L.	Abs	0.649	0.609	0.646	0.590	-	-	-	-
	Rela	0.187	0.173	0.193	0.183	-	-	-	-
Epididymides R.	Abs	0.651	0.603	0.646	0.595	-	-	-	-
	Rela	0.188	0.172	0.192	0.184	-	-	-	-
Prostate	Abs	1.843	1.745	1.896	1.729	-	-	-	-
	Rela	0.531	0.498	0.562	0.538	-	-	-	-
Testis L.	Abs	1.737	1.761	1.751	1.652	-	-	-	-
	Rela	0.5020	0.5025	0.5215	0.5112	-	-	-	-
Testis R.	Abs	1.702	1.699	1.719	1.604	-	-	-	-
	Rela	0.491	0.485	0.512	0.497	-	-	-	-
Ovaries	Abs	-	-	-	-	0.130	0.123	0.115	0.112
	Rela	-	-	-	-	0.0602	0.0554	0.0536	0.0517
Uterus with cervix	Abs	-	-	-	-	0.7670	0.8786	0.7195	0.8626
	Rela	-	-	-	-	0.3582	0.3970	0.3334	0.3992

* : $p < 0.05$; $p < 0.01$

Cohort 1B and F2 pups :

During the study period, 1 male and 1 female of the control group were found dead and 1 male of the mid dose group. The clinical sign "moving the bedding" was observed in all animals exposed to 1000 mg/kg bw/d and also in 1 female of the low dose and 6 females of the mid dose. Furthermore, significant body weight changes were observed in females during gestation and lactation periods (see Table 17).

Table 17 : body weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	100	300	1000	0	100	300	1000
In-life period for males and premating period for females	D1	56.2	53.3	53.6	53.8	52.6	52.3	49.7	52.6
	D29	221.2	222.8	222.9	222.9	160.2	160.2	161.1	163.0
	D57	315.9	312.2	324.6	321.1	203.6	204.1	207.2	214.2
	D71	344.1	339.6	351.4	343.8	213.6	218.2	223.4	226.0
	D92	376.3	368.2	374.8	372.4	-	-	-	-
	D120	406.3	404.6	415.3	405.8	-	-	-	-
Gestation	D0	-	-	-	-	217.41	220.37	226.88	227.18
	D7	-	-	-	-	233.65	238.10	242.24	247.94
	D14	-	-	-	-	254.00	258.70	266.12	271.81*
	D20	-	-	-	-	312.71	321.95	330.00	342.38**
Lactation	D0	-	-	-	-	241.33	242.80	253.00	262.53**
	D7	-	-	-	-	265.72	272.30	276.39	286.53**

	D14	-	-	-	-	280.83	287.35	294.28	303.16**
	D21	-	-	-	-	269.33	269.85	278.61	286.37**

* : p < 0.05 ; ** : p < 0.01

Regarding female reproduction parameters, precoital interval examination exhibited a dose-response increase as the parameter was of 1.94, 2.20, 2.74 and 2.83 days, resp. at 0, 100, 300 and 1000 mg/kg bw/d. Other reproductive parameters, such as mean number of corpora lutea, mean number of implantation sites, mean percentage of pre-implantation loss, mean percentage of post-implantation loss and mean number of duration of gestation did not exhibit this same trend.

Table 18 : fertility data

Dose level (in mg/kg bw/d)	0	100	300	1000
Mean nb of corpora lutea	10.37	11.55	12.17	12.42
Mean nb of implantation sites	10.32	11.05	10.94	12.11
Mean % of pre-implantation loss	0.38	3.45	8.90	2.50
Mean % of post-implantation loss	9.05	4.11	4.90	7.61
Mean duration of gestation (in d)	22.29	22.40	22.35	22.24

Shortly before weaning, parental animals were sacrificed. Necropsy did not reveal significant organ weight change or treatment-related histopathological effects

Regarding offspring examination, the mean number of pups (dead and alive) did not show variation (9.32, 10.75, 10.61 and 11.37, resp. at 0, 100, 300 and 1000 mg/kg bw/d).

Cohort 2A :

Only one female exposed to 300 mg/kg bw/d was euthanized during the study period. Clinical observation showed that moving the bedding was observed in all females of the highest dose and increased salivation was noted in 4 males and 3 females of this dose group. Furthermore, body weight was unaffected. In this cohort, neurotoxicity was examined and revealed some modifications as mentioned in Table 19. At necropsy, only one female of the mid dose group showed an uterus dilatation, and the final body weight and the brain weight were unaffected.

Table 19 : motor activity (sum interval 1, 2 and 3) and auditory startle response (at PND 24)

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
SM	33.50	27.50	29.70	29.20	30.20	30.10	30.33	27.70
FM	2789.10	3308.30	3865.10	3236.00	3312.70	3973.90	4487.22	4435.00
Sum SM and FM	2822.60	3335.80	3894.80	3265.20	3342.90	4004.00	4517.56	4462.70
SR	27.80	25.00	27.00	31.30	22.20	22.40	28.44	28.50
FR	145.40	139.25	148.10	140.40	128.40	113.20	144.56	122.60
Sum SR and FR	173.20	164.25	175.10	171.70	150.60	135.60	173.00	151.10

Mean max auditory startle response	0.664	0.573	0.613	0.690	0.683	0.582	0.622	0.666

Cohort 2B :

No abnormalities were observed during the necropsy.

Cohort 3 :

During the study period, three animals were found dead (one female of the low dose, one male of the mid dose and one male of the highest dose). Clinical observation revealed signs such as moving the bedding in all males and in 7 females of the highest dose and excessive salivation in 2 males and 1 female of this highest dose. No significant body weight change was noted. In this cohort, mean IgG and IgM serum levels were examined. Few modifications were observed (see Table 20 and Table 21).

Table 20 : IgM serum levels (in ng/ml)

		Males					Female				
Dose level (in mg/kg bw/d)		0	PC	100	300	1000	0	PC	100	300	1000
Anti-KLH IgM	Baseline	38306	37146	40129	33478	36858	43182	56579	59730	55998	53539
	D6	59517	45725	48187	47404	47050	52136	46821	52025	50412	51085
Total IgM	Baseline	50457	46246	53960	43916	44836	51163	44183	57456	46696	44951
	D6	85678	32436	65536	58650	61502	67790	37980	84141	80677	68915

Table 21 : IgG serum level (in ng/ml)

		Males					Female				
Dose level (in mg/kg bw/d)		0	PC	100	300	1000	0	PC	100	300	1000
Total IgG	Baseline	528458	475848	558915	472083	470908	744238	693940	859372	685535	587781
	D6	866785	569692	898070	713330	924306	1173465	714770	1610619	1209063	1183008

Anti-KLH IgG was below level of quantification

Cohort 4 :

All animals survived during the study period. As in the other cohort, clinical signs “moving the bedding” was observed in all males and in 2 females of the highest dose group. Body weight and gross pathology examination were unaffected by treatment. In this cohort, mean escape latency during learning and memory phases was examined and revealed a reduction during the memory phase which was significant in females.

Table 22 : mean escape latency during learning and memory phases (in sec)

	Male		Female	
Dose level (in mg/kg bw/d)	0	1000	0	1000
During learning phase (PND 28/29)	7.50 ± 4.89	9.21 ± 2.31	10.76 ± 3.80	8.65 ± 4.81
During memory phase (PND 35/36)	8.02 ± 3.41	6.95 ± 0.91	8.63 ± 0.86	6.07 ± 1.37*

* : $p < 0.05$

In a reproduction/developmental toxicity screening test (Registration dossier (study report, 2018)), performed as a range finding study preceding the EOGRTS, groups of male and female Wistar rats were given by gavage propylparaben (purity 99.7 %) at a concentration of either 0, 500 or 1000 mg/kg bw/d. Groups were composed of 5 males and 5 females in the control groups and 10 males and 10 females in the 500 and 1000 mg/kg bw/d groups. Males were exposed during minimum 35 days (21 days of premating period and maximum 14 days of mating period). Females were exposed during 21 days of premating period and up to 14 days. Thereafter, one dam of each group was dosed up to GD 20, the other dams received test substance during gestation and up to PND 21. The surviving pups of one litter from each group were treated from PND 13 to PND 21.

During the study period, 2 animals were found dead (one male of the low dose on PMD 8 and one female of the highest dose on PND 5). Body weight was unaffected by the treatment, as observed in Table 23.

Table 23 : body weight data (in g)

		Males			Females		
Dose level (in mg/kg bw/d)		0	500	1000	0	500	1000
Nb examined		5	10	10	5	10	10
Premating period	D1	272.40	271.40	269.20	182.80	173.60	174.80
	D14	362.20	323.00[A]	321.00	206.20	195.80	196.00
	D21	340.40	343.67 [A]	337.20	212.00	205.00	205.20
Mating and post mating period	D7	343.80	354.22 [A]	350.20	-	-	-
	D14	364.20	373.33 [A]	371.20	-	-	-
Gestation period	D0	-	-	-	219.80	210.22 [A]	210.40
	D14	-	-	-	267.60	266.20	263.56 [A]
	D20	-	-	-	331.20	337.90	333.20
Lactation period	D0	-	-	-	241.75[B]	252.33[A]	251.00[A]
	D9	-	-	-	280.25 [B]	271.67[A]	276.50[C]
	D21	-	-	-	273.00 [B]	284.00[A]	274.75[C]

[A] : Nb examined = 9 ; [B] : nb examined = 4 ; [C] : nb examined = 8

Regarding reproductive parameters, precoital interval was decreased in all treated groups (7.20, 3.00 and 2.30, resp. at 0, 500 and 1000 mg/kg bw/d). Furthermore, percentage of pre- and post-implantation loss were increased. Other parameters such as duration of gestation, mean number of corpora lutea and mean number of implantation sites were unaffected (See Table 24).

Table 24 : reproductive parameters

Dose level (in mg/kg bw/d)		0	500	1000
Duration of gestation	Nb examined	4	9	9
	Mean days	22.25	22.33	22.11
Corpora lutea	Nb examined	1	1	1
	Mean nb	14.0	13.0	14.0

	Nb examined	4	9	8
	Mean nb	13.75	13.22	13.63
Implantation sites	Nb examined	1	1	1
	Mean nb	12.0	11.0	14.0
	N examined	4	9	8
	Mean nb	13.75	13.11	13.38
Pre- and post-implantation loss	Nb examined	4	9	8
	% pre-	0.00	0.79	1.74
	% post-	6.47	6.74	8.72

At necropsy, one female exposed to 500 mg/kg bw/d had fluid filled uterus and an uterus horn dilatation and 1 female exposed to 1000 mg/kg bw/d exhibited dark lung accompanied by congestion and atelectasis. No other modifications were observed.

Pups were recorded and examined. The mean number of pups at birth was not modified (12.75, 12.44 and 12.56, resp. at 0, 500 and 1000 mg/kg bw/d).

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Registration dossier (study report, 2012)), groups of 11 male and 11 female Wistar rats received, in the feed, propylparaben (99.7 %) at a concentration of 0, 1500, 4500 and 15000 ppm (See Table 25 for the mean achieved dose levels in mg/kg bw/d). Animals were exposed during a minimum of 4 weeks in males and of approx. 7 weeks in females.

Table 25 : mean achieved dose levels (in mg/kg bw/d)

	Males		Females		
	Pre-pairing period	After pairing period	Pre-pairing period	Gestation period	Lactation period
0 ppm	0	0	0	0	0
1500 ppm	98.0	59.3	16.0	121.6	137.3
4500 ppm	305.1	178.3	341.9	349.2	431.8
15000 ppm	980.9	605.0	1076.4	1124.6	1380.0

All animals survived during the study period. At the highest dose, only one female exhibited malpositioned hind leg during the gestation period. No other abnormalities were recorded and the body weight examination did not reveal significant change (see Table 26 and Table 27).

Table 26 : mean male body weight (in g)

Dose level (in ppm)		0	1500	4500	15000
Pre-pairing period	D1	356	355	358	355
	D7	378	379	381	372
	D14	386	386	393	380
Pairing period	D1	389	394	398	386

	D8	407	408	411	397
After-pairing period	D1	410	411	415	401
	D6	424	425	430	415
	D11	439	440	446	430

Stat : Dunnett-test

Table 27 : mean female body weight (in g)

Dose level (in ppm)		0	1500	4500	15000
Pre-pairing period	D1	196	193	193	195
	D7	199	199	201	197
	D14	201	202	202	197
Gestation period	D1	208	209	211	203
	D7	226	231	232	224
	D14	255	258	260	248
	D21	319	317	323	304
Lactation period	D1	236	237	234	230
	D4	249	248	244	234

Stat : Dunnett-test

Regarding male reproductive parameters, sperm analysis did not reveal modifications. The mean testis sperm count was of 130.0 mio/g at the highest dose compared to 123.7 mio/g in control. Furthermore, sperm examination showed similar effects in all groups. Indeed, the percentage of progressive sperm was of 84.2, 85.5, 83.6 and 86.9 %, resp. at 0, 1500, 4500 and 15000 ppm. The percentage of stationary sperm was of 2.4, 2.3, 2.5 and 3.0 %, resp. at 0, 1500, 4500 and 15000 ppm. Furthermore, the percentage of not motile sperm was of 13.4, 12.2, 13.9 and 10.1 %, resp. at 0, 1500, 4500 and 15000 ppm.

Concerning female reproductive parameters, oestrous cycle, mean number of corpora lutea and mean number of implantation sites were unaffected. Furthermore, fertility index did not change (90.9, 90.9, 100.0 and 90.9 %, resp. at 0, 1500, 4500 and 15000 ppm). However, the percentage of post-implantation loss was severely higher at the highest dose group (5.9, 6.7, 5.2 and 12.4 %, resp. at 0, 1500, 4500 and 15000 ppm). And the mean living pups at the first litter check was lower at the low and the highest dose (11.2, 9.8, 11.6 and 9.9, resp. at 0, 1500, 4500 and 15000 ppm).

At necropsy, macroscopic findings were observed, however only enlarged liver was observed dose dependently (1, 1, 2 and 4 males, resp. at 0, 1500, 4500 and 15000 ppm). Final body weight was not significantly affected in both sexes. In males, relative and aboslute kidneys weights showed significant changes. Furthermore, absolute epididymide right weight was significantly higher at the mid and high dose group and relative weight was only significantly increased at the highest dose. Microscopic examination was also performed and did not reveal treatment-related changes (see Table 28).

Table 28 : microscopic findings

		Males				Females			
Dose level (in ppm)		0	1500	4500	15000	0	1500	4500	15000
Kidneys	Tubular basophilia	2/5	NT	0/1	0/5	1/5	0/1	NT	0/5

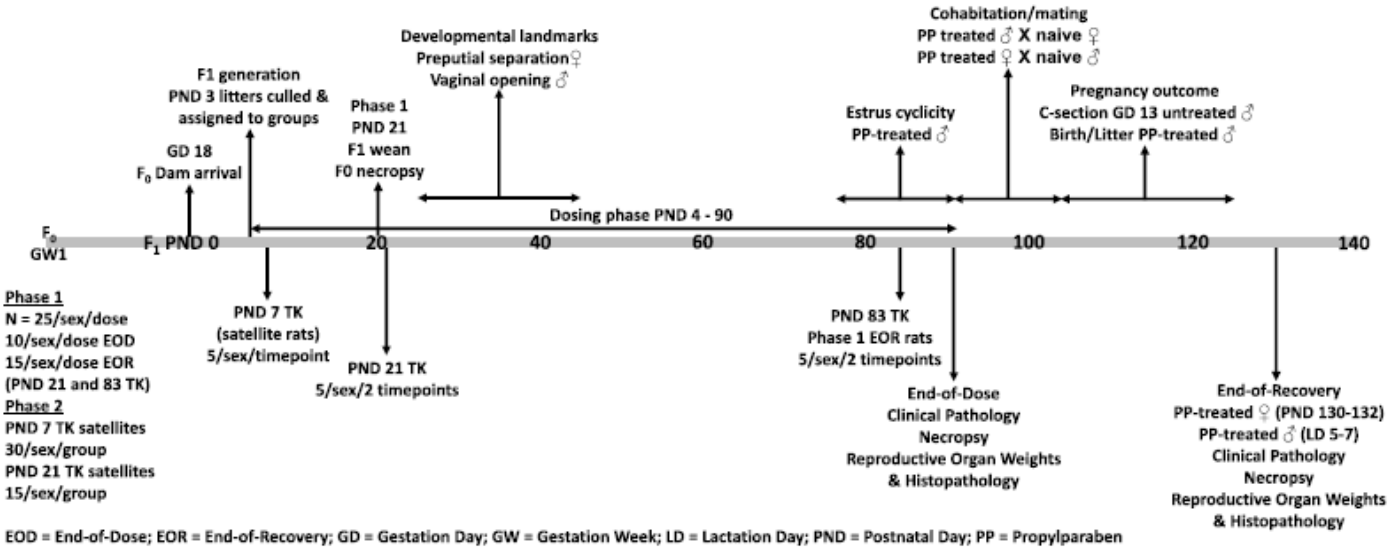
	Hyaline droplets	3/5	NT	1/1	3/5	0/5	0/1	NT	0/5
	Tubular cystic dilatation	0/5	NT	0/1	0/5	0/5	0/1	NT	1/5
	Pelvic dilatation	0/5	NT	1/	0/5	0/5	1/1	NT	0/5
Liver	Inflammatory foci	1/5	1/1	2/2	1/5	1/5	NT	NT	1/5
Thymus	Haemorrhage	0/5	NT	NT	1/5	0/5	2/2	NT	0/5
Testes	Tubular degeneration/atrophy	3/11	0/1	NT	2/11	-	-	-	-
	Sertoli cell vacuolation	5/11	0/1	NT	5/11	-	-	-	-
Epididymides	Cellular debris	1/11	0/1	NT	0/11	-	-	-	-
	Mononuclear foci	7/11	0/1	NT	8/11	-	-	-	-
Prostate	Inflammation	1/11	1/1	NT	2/11	-	-	-	-
Ovaries	Congestion	-	-	-	-	0/11	0/1	1/1	0/11

NT : not tested

At birth, mean number of living pups was lower at the low and high dose group (11.2, 9.8, 11.6 and 9.9, respectively at 0, 1500, 4500 and 15000 ppm). The birth index was of 94.1, 93.3, 94.8 and 87.6 %, resp. at 0, 1500, 4500 and 15000 ppm.

An article "Safety assessment of propylparaben in juvenile rats" (Sivaraman *et al.*, 2018) described a study which exposed male and female rats (F1 generation) to propylparaben on PND 4 through PND 90. Groups of male and female SD rats were exposed by gavage to propylparaben at a oncentration of either 0, 10, 100 or 1000 mg/kg bw/d. The study design is explained in Figure 1 in section 10.10.2.

Figure 1 : study design (Sivaranam *et al.*, 2018)



The F1 generation was observed. At the highest dose group, an increased incidence of abdominal distention during the pre-weaning period was noted as well as an increased incidence of excessive salivation immediately after dosing. Regarding body weight examination, males exposed to 1000 mg/kg bwd exhibited a slightly increased bw which was correlated to a higher food consumption. Developmental landmarks were examined. In females, mean age of vaginal patency was significantly lower at the highest dose (33.9, 32.4, 32.7 and 31.2** PND, resp. at 0, 10, 100 and 1000 mg/kg bw/d). The article's authors explained that this modification was within the range of the HCD (29.0 to 33.9 days) and that, in their study, 7 control females out of 25 had

late development (35 to 43 days) resulting in a high control value. In males, preputial separation was similar in all groups (42.1, 42.3, 42.3 and 43.2 PND, resp. at 0, 10, 100 and 1000 mg/kg bw/d).

Regarding female reproductive performance (treated female mated with non-treated male), mean number of implantation sites was significantly increased at the low dose (14.3, 17.4**, 16.1 and 15.6, resp. at 0, 10, 100 and 1000 mg/kg bw/d). Other parameters such as, mean duration of oestrous cycle, mating index, fertility index, duration of gestation, were unaffected (see Table 29)

Table 29 : reproductive data in females

Dose level (in mg/kg bw/d)	0	10	100	1000
Mean duration of oestrous cycle (in d)	4.19	4.43	4.29	4.29
Mating index (in %)	93.3	86.7	93.3	93.3
Fertility index (in %)	86.7	80.0	93.3	93.3
Mean duration of gestation (in d)	21.8	21.8	22.2	21.9
Live birth index (in %)	88.96	88.20	89.72	92.36

Concerning male reproductive performance, propylparaben-treated males did not exhibit treatment-related effects as mating index and fertility index were unaffected. Mating index was of 100 % in all groups and fertility index was of 92.9, 93.3, 80.0 and 86.7 %, resp. at 0, 10, 100 and 1000 mg/kg bw/d. However, other parameters such as sperm parameters were not examined.

Additional groups were used to examine reproductive performance. Untreated females were mated with treated males and females were examined at GD 13 after caesarean. Slight increase of the percentage of pre-implantation loss was observed. Other parameters did not show difference (see Table 30).

Table 30 : reproductive data

Dose level (in mg/kg bw/d)	0	10	100	1000
Mean nb of corpora lutea	17.8	17.9	18.9	17.0
Mean nb of implantation sites	16.8	17.0	17.4	15.8
Mean nb of live embryos	15.8	15.6	16.5	15.3
Mean % of pre-implantation loss	5.74	5.06	7.76	7.88
Mean % of post-implantation loss	5.15	8.15	5.10	3.68
Mean nb of early resorption + dead embryos	0.9	1.4	0.9	0.6

At necropsy, no treatment-related macroscopic and microscopic findings were noted. Higher absolute and relative uterus weight was observed (+ 36 % and + 43 % compared to control, resp.).

Concerning the second generation, percentage of male decreased slightly at the highest dose level (49.33, 48.22, 48.06 and 43.69 %, resp. at 0, 100, 100 and 1000 mg/kg bw/d).

The article “Oral propylparaben administration to juvenile male Wistar rats did not induce toxicity in reproductive organs” (Gazin *et al.*, 2013) describes a preliminary study which assessed pharmacokinetic parameters and a main study which assessed the effects of parabens on the male reproductive system. Groups of male Wistar rats were exposed by gavage to propylparaben at a concentration of either 0, 3, 10, 100 or 1000 mg/kg bw/d. In the preliminary study, 3 male rats in control group and 18 male rats in each treated groups received a single dose of propylparaben on PND 31 and animals were sacrificed after 24h. Whereas, in the main study, 20 males per group were used and divided into 2 subgroups : the first one was sacrificed and

necropsied at the end of the 8-week treatment period while the second one after a 26-week washout period (to cover 3 spermatogenic cycles). Additionally, satellite groups (17 males per group for treated groups and 9 males in control group) were used to assess juvenile toxicity study.

In the main study, hypersalivation was observed in animals of the highest dose and body weight was slightly increased in this group (approx. + 7 % compared to control group). Sexual maturation was examined and revealed that mean day of balano-preputial separation was unaffected as the mean day was of 44, 44, 44, 43 and 43 PND, respectively at 0, 3, 10, 100 and 1000 mg/kg bw/d. Hormone levels were tested and did not show significant modifications (see Table 31). Furthermore, mean epididymal and testis sperm count were not affected. As observed in Table 32, epididymal sperm motility parameters showed variations.

Table 31 : plasma hormone levels (at the end of treatment period)

Dose level (in mg/kg bw/d)	0	3	10	100	1000
Testosterone (nmol/l)	16.9	17.6	21.2	22.9	18.9
LH (ng/ml)	0.64	0.66	0.71	0.51	0.62
FSH (ng/ml)	13.6	12.7	12.4	13.4	12.5

Table 32 : epididymal sperm motility

Dose level (in mg/kg bw/d)	0	3	10	100	1000
Motile sperm ratio (%)	81.1	88.2	71.4	85.5	85.8
VAP (µm/s)	162.5	162.2	143.8	152.1	153.6
VSL (µm/s)	111	111.4	97.2	102.5	103.1
VCL (µm/s)	348.2	332.5	305.3	322.3	316.2
ALH (µm)	14.5	14.5	13.4	14	14.3
STR (%)	67	68	60	67	66
LIN (%)	33	35	30	33	34

At necropsy, mean testis weight was unaffected in animals examined at the end of the treatment period and in animals examined at the end of the recovery period. Furthermore, microscopic examination did not reveal treatment-related effects in these 2 groups.

An article “Effects of propylparaben on the male reproductive system” (Oishi, 2002) described a study, which exposed Wistar rats to propylparaben to examine the effects on the male reproductive system. Groups of 8 males were given by feed test substance, during 4 weeks, at a concentration of either 0, 0.01, 0.10 or 1.0 % which correspond approximately to 0, 12.4, 125 and 1290 mg/kg bw/d. At the end of the exposure period, animals were killed and examined.

During the study, no animals died. Information on daily clinical signs and body weight examination were not available.

Regarding male reproductive parameters, organ weights were not significantly modified (microscopic examination was not performed). Whereas, sperm counts in the cauda epididymis and sperm production in the testis were severely affected (see Table 33). The cauda epididymal sperm reserve and sperm concentration decreased in a dose-dependent manner and= these reductions were significantly at the mid and high dose groups. Daily sperm production and its efficiency showed also severe reduction which were significant in all tested dose groups. Furthermore, mean testosterone concentration in the serum exhibited also a severe and dose-dependent decreased, as it was of 9.08, 8.20, 7.17 and 5.86* ng/ml, resp. at 0, 0.01, 0.10 and 1.0 %.

Table 33 : male reproduction parameters

Dose level (in %)	0	0.01	0.10	1.0
Sperm counts in the cauda epididymis				
Reserves ($x10^7$/cauda)	43.6	31.1	25.7*	22.5*
Concentration ($x10^7$/g)	108	70.8	63.1*	48.8*
Sperm production in the testis				
DSP ($x10^6$)	37.5	26.2*	27.0*	25.9*
Efficiency ($x10^6$)	30.0	20.6*	22.4*	21.4*

* : $p<0,05$

An article, "Estrogenicity of parabens revisited : Impact of parabens on early pregnancy and an uterotrophic assay in mice" (Shaw and deCantazaro, 2009), describes two different studies. The first experiment tested butylparaben and the second experiment examined propylparaben. After these 2 parts, an uterotrophic assay was additionally performed with butylparaben.

In the second experiment which tested propylparaben, mouse were exposed by a subcutaneous injection during a period of 4 days (GD 1 to GD 4). Animals received 35 or 45 mg of the test substance.

On gestation day 6, animals were euthanized and examined. The mean number of implantation sites was unaffected by treatment.

10.10.3 Comparison with the CLP criteria

Criteria for Category 1	Criteria for category 2
"Known or presumed human reproductive toxicant Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human (category 1A) or from animal data (category 1B). Category 1A : known human reproductive toxicant. The classification is largely based on evidence from humans Category 1B : presumed human reproductive toxicant. The classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic	"Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."	

Since no human studies are available for effects on fertility, classification in Repr. 1A for fertility is not appropriate.

Available studies examined male and female fertility parameters. These parameters were disrupted by exposure to the propylparaben.

- In males

In the EOGRTS (Registration dossier (study report, 2021)), males in the F0 generation exhibited a reduction of sperm motility at the highest dose, as the percentage of motile count was of 72.67 % at 1000 mg/kg bw/d vs 77.05 % in control group and the percentage of rapid sperm was of 55.75 % at 1000 mg/kg bw/d vs 60.85 % in control group. Furthermore, the total number of abnormal sperms was of 13.33 at the highest dose while in control it was of 8.25 (on 200 sperms examined). Sperm morphology was critically affected regarding the only tail sperm which reached a percentage of 8.17% at the highest dose compared to 2.96 % only in the control group, corresponding to an increase of approx. 276 %. Sperm was also consistently affected in animals of the cohort 1A. At the highest dose of cohort 1A, as for the F0 generation, the total number of abnormal sperms was greatly increased (19.06 at 1000 mg/kg bw/d vs 10.35 in control). In the same way, the percentage of motile sperm count was reduced at the highest dose (72.42 % at 1000 mg/kg bw/d vs 79.10 % in control group). The percentage of rapid sperm was lower (58.11 % at 1000 mg/kg bw/d vs 64.83 % in control group) and the percentage of static sperm count was greatly increased (27.58 % at 1000 mg/kg bw/d vs 20.90 %).

These modifications cannot be explained by general toxicity as, in the F0 generation, body weight was not affected and the necropsy did not reveal treatment-related effects. In the F1 generation, body weight was significantly lower at the end of the exposure period, however body weight was already significantly reduced at the day 1. Despite these effects on sperm, the fertility index was not affected neither in the P0 nor the F1 generation.

Male fertility parameters were also examined in the Oishi's article (Oishi S., 2012). Oishi demonstrated that the sperm counts in the cauda epididymis (reserves and concentration) were severely and significantly affected at the mid and high doses (0.10 and 1.0 %, corresponding to 125 and 1290 mg/kg bw/d). Furthermore, the sperm production in tested (DSP and efficiency) was also significantly modified and already at the lowest dose (0.01 %, corresponding to 12.4 mg/kg bw/d). The mean testosterone concentration in the serum was also examined and revealed a significant dose-dependent decrease (9.08, 8.20, 7.17 and 5.86* ng/ml, resp. at 0, 0.01, 0.10 and 1.00 %).

The Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Registration dossier (study report, 2012)) did not reveal effects on sperm. However, 11 males per group were exposed to propylparaben and the sperm of only 5 males per group were examined. In the combined repeated dose toxicity study with the reproduction/developmental screening test, males were exposed to propylparaben during 4 weeks, whereas males were exposed during 10 weeks in EOGRTS (Registration dossier (study report, 2021)). Furthermore, animals received 0, 1500, 4500 and 15000 ppm (corresponding approx. to 0, 98.0, 305.1 and 980.9 mg/kg bw/d during the pre-pairing period and 0, 59.3, 178.3 and 605 mg/kg bw/d during the post-pairing period). The highest dose during the post-pairing period was less than the highest dose of the EOGRTS which was of 1000 mg/kg bw/d and than those of the Oishi's article which was of 1290 mg/kg bw/d.

As the fertility index in the EOGRTS (Registration dossier (study report, 2021)) was unaffected, classification as Repr. 1B for fertility is not appropriate.

However, two different studies (EOGRTS, 2021 and Effects of propylparaben on the male reproductive system (Oishi S., 2012)) demonstrated that sperm is greatly affected by exposure to propylparaben. A classification as Repr. 2 is warranted.

Table 34 : Summary table of adverse effects on male reproductive system

Dose (in mg/kg bw/d)		0	12.4	100	125	300	1000	1290
Sperm motility (% motile count)								
EOGRTS (Registration dossier (study report, 2021))	F0	77.05	/	77.60	/	77.98	72.67	/
	C1A	79.10	/	/	/	/	72.42	/
Cauda epididymal sperm conc. ($x10^7$/g)								
Effects of propylparaben on the male reproductive system (Oishi S., 2012)		108	70.8	/	63.1*	/	/	48.8*
Sperm production in testis (DSP : $x10^6$)								
Effects of propylparaben on the male reproductive system (Oishi S., 2012)		37.5	26.2*	/	27.0*	/	/	25.9*
Total nb of abnormal sperms (on 200 sperms examined)								
EOGRTS (Registration dossier (study report, 2021))	F0	8.25	/	/	/	/	13.33	/
	C1A	10.35	/	/	/	/	19.06	/
Sperm morphology (in %) : tail only								
EOGRTS (Registration dossier (study report, 2021))	F0	2.96	/	/	/	/	8.17	/
Testis weight (in g or %)								
EOGRTS (Registration dossier (study report, 2021)) : C1A	Abs	1.817	/	1.782	/	1.839	1.677	/
Prostate weight (in mg or %)								
EOGRTS (Registration dossier (study report, 2021)) : F0	Abs	3.281	/	3.226	/	3.008	2.856**	/
	Rela	0.727	/	0.716	/	0.678	0.643*	/
Testosterone conc. (ng/ml)								
Effects of propylparaben on the male reproductive system (Oishi S., 2012)		9.08	8.20	/	7.17	/	/	5.86*

- In females

The available studies did not demonstrate fertility effects in females which warrant a classification for the fertility.

- Conclusion

Based on the available information which demonstred severe effects in sperm in the absence of clear general toxicity general toxicity, a classification as **Repr. 2 H361 f**.

10.10.4 Adverse effects on development

Table 35: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study Rat (Wistar) 25 pregnant females per group OECD TG 414 GLP Reliability 1	Propyparaben Purity: 99.7 % Vehicle: 1 % hydroxyethyl-cellulose Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: GD 5 to GD 19	<u>Parental:</u> No mortality observed Bw: unaffected Pre- and post-implantation loss, resorptions: not modified Necropsy: no treatment-related findings nor histopathological changes observed <u>Pups:</u> Nb of live pups similar in all groups Litter and foetus weights unaffected Necropsy: no treatment-related effects observed NOAEL (maternal toxicity) : 1000 mg/kg bw/d NOAEL (development) : 1000 mg/kg bw/d	Registration dossier (study report, 2019)
EOGRTS with DNT and DIT Oral, gavage Rat (Wistar) F0: 30/sex in control and high dose and 25/sex in low and mid doses Cohorts 1A and 1B: 20/sex/dose Cohorts 2A, 2B, 3 and 4: 10/sex/dose OECD TG 443 GLP Reliability 1	Propylparaben Purity: 99.7 % Vehicle: 1 % of hydroxyethyl-cellulose Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: F0: min. 10w in males and 14d of pre-mating, max 14d of mating, gestation and through weaning in females F1: from weaning (PND 22) to terminal sacrifice and the respective cohorts	<u>F0 parental:</u> Clinical signs: increased salivation and moving bedding at mid dose in females and in both sexes at the highest dose BW: no significant change Post-implantation loss : increased at the highest dose Necropsy: few organ weight changes (liver and prostate in males and only thymus in females). No microscopic changes observed <u>F1 pups:</u> Viability index not modified AGD and nipple retention sign. changed (see table 45) Thyroid hormone exhibited variations <u>Cohort 1A:</u> Clinical signs: moving bedding observed at the highest dose Bw: changed at the highest dose in males at D64 Balano-preputial separation slightly reduced <u>Cohort 1B and F2 pups:</u> Moving bedding in female of the mid dose and in both sexes of the highest dose BW: sign. higher during gestation and lactation	Registration dossier (study report, 2021)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Pups: AGD and nipple retention sign. Affected NOAEL (general toxicity) : > 1000 mg/kg bw/d NOAEL (development) : > 1000 mg/kg bw/d (according to registration dossier) ; However, AGD was sign lower in all tested groups)	
Dose range finding study for reproduction/developmental toxicity screening test Oral, gavage Rat (Wistar) 10/sex/dose (except for control group: 5/sex) Equivalent or similar to OECD TG 421 Non-GLP Reliability 1 (according to registration dossier. But not GLP) Reliability 2 (according to DS)	Propylparaben Purity: 99.7 % Vehicle: 1 % hydroxyethyl-cellulose Doses: 0, 500 and 1000 mg/kg bw/d Duration of exposure: min 35d for males and during 21d for pre-mating, max 14d for mating, through gestation and up to PND 21 (except one dam of each treated group which was dosed up to GD 20). Surviving pups of one litter from each group were treated from PND 13 to PND 21	<u>Parental</u> BW: unaffected Female reproductive parameters: percentage of pre- and post-implantation loss increased. Other parameters unaffected Necropsy: no treatment related change <u>Pups:</u> Mean nb of pups at birth, mean nb of live pups and viability index unaffected NOAEL (general toxicity) : > 1000 mg/kg bw/d NOAEL (development) : > 1000 mg/kg bw/d (according to registration dossier) ; 500 mg/kg bw/d (according to DS as percentage of post-implantation loss was increased at the highest dose (+34% compared to control group)	Registration dossier (study report, 2018)
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Oral, feed Rat (Wistar) 11/sex/group OECD TG 422 GLP Reliability 1	Propylparaben Purity: 99.7% Vehicle: not specified Doses: 0, 1500, 4500 and 15000 ppm Duration of exposure: min 4w for males and approx. 7w for females (14d of premating, max 14d of mating, gestation and through lactation day 3)	<u>Parental:</u> BW: unaffected Male reproduction parameters: not sign. affected Female reproduction parameters: percentage of post-implantation loss severely higher at the highest dose (12.4 % vs 5.9 %) Necropsy: kidney and epididymide (right) weights sign. modified. No microscopic treatment-related changes observed <u>Pups:</u> Mean nb of living pups lower at the low and high dose groups, birth index decreased at the highest dose. Pup bw at PND 1 and 4 unaffected No treatment-related abnormalities observed NOAEL (parental toxicity) : 15000 ppm NOAEL (reproduction/developmental toxicity :	Registration dossier (study report, 2012)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		15000 ppm mentioned in the registration dossier. However, based on the higher percentage of post-implantation loss observed at the highest dose, a NOAEL of 4500 ppm (mid dose) is proposed by DS.	
Assessment of propylparaben in juvenile rats 2 separate studies were conducted to assess the potential estrogen-mimetic effects on 1) reproductive developmental and function examination when animals exposed from PND 4 to 90, and 2) examination of uterus weight in immature females when exposed from PND 4 to PND 7 or 21 Oral, gavage Rat (SD) 25/sex/dose in phase 1 and 15 or 30/sex/dose in phase 2 Reliability 2 (according to registration dossier, but only summary available, only few parameters examined, no individual data, no info about GLP conform) Reliability 3 (according to the DS)	Propylparaben Purity: 99.7 % Vehicle: 1 % hydroxyethylcellulose Doses: 0, 10, 100 and 1000 mg/kg bw/d Duration of exposure: from PND 4 to PND 90 in phase 1 and from PND 4 to PND 7 or 21 in phase 2	Mean age of vaginal patency sign. lower at the highest dose (within HCD) Preputial separation similar in all groups Necropsy: no treatment-related change observed <u>Pups:</u> Litter weight and viability index not modified No malformed pups observed NOAEL : 1000 mg/kg bw/d	Sivaraman *et al.*, 2018
Assessment on the impact of parabens on early pregnancy Subcutaneous injection Mouse Reliability 2 (according to registration dossier, but only summary available, only few parameters examined, no individual data, no info about GLP conform) Reliability 3 (according to the DS)	Propylparaben and butylparaben Purity : unknown Vehicle : depending of the experiment Dose : 35 or 45 mg of propylparaben Duration of exposure : GD 1 to GD 4 (euthanised at GD 6)	Mean nb of implantation sites unaffected NOAEL (parental toxicity) : 45 mg of propylparaben NOAEL (developmental toxicity) : 45 mg of propylparaben	Shaw and deCantazaro, 2009

No human data or other studies available

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a prenatal developmental toxicity study (Registration dossier (study report, 2019)), following OECD TG 414, groups of 25 pregnant female rats were exposed orally to propylparaben at a concentration of either 0, 100, 300 or 1000 mg/kg/d. Animals received test substance to GD 5 to 19, and were killed and necropsied at GD 20.

All females survived during the study period and no body weight modification was noted (see Table 36). Furthermore, food consumption was only slightly reduced at the mid and high dose groups. In the highest dose group, clinical signs such as moving bedding was observed in 16 females and increased salivation in 5 females. These clinical signs were noted immediately after exposure and were observed only during a short period.

Table 36 : body weight data (in g)

Dose level (in mg/kg bw/d)	0	100	300	1000
GD 0	235.20 (20)	232.87 (23)	231.52 (21)	231.79 (24)
GD 5	251.55 (20)	246.04 (23)	246.35 (20)	247.35 (23)
GD 11	268.85 (20)	264.74 (23)	262.35 (20)	263.35 (23)
GD 20	340.45 (20)	335.57 (23)	332.71 (21)	334.17 (24)

( ) : number of animals examined

Reproductive and developmental parameters were assessed and did not demonstrate significant changes (see Table 37).

Table 37 : Reproductive parameters

Dose level (in mg/kg bw/d)	0	100	300	1000
Mean % of pre-implantation loss	12.57	5.93	6.95	8.71
Mean % of post-implantation loss	6.23	8.95	5.36	8.07
Mean % of early resorptions	0.75	1.00	0.62	1.00
Mean % of late resorptions	0.00	0.04	0.00	0.00
Mean % of total resorptions	0.75	1.04	0.62	1.00

At necropsy, nor treatment-related macroscopic findings nor treatment-related histopathological changes were observed. Furthermore, terminal body weight, gravid uterus weight and adjusted maternal weight were unaffected (see Table 38).

Table 38 : uterus and adjusted maternal weight (in g)

Dose level (in mg/kg bw/d)	0	100	300	1000
Terminal bw	340.45	335.57	332.71	334.17
Gravid uterus weight	58.84	60.24	61.63	61.78
Adjusted maternal weight	281.61	275.32	271.08	272.39

Regarding pups, the mean number of live pups at birth was of 10.70, 10.91, 11.14 and 11.08, respectively at 0, 100, 300 and 1000 mg/kg bw/d, and no dead foetuses was observed. Furthermore, foetus and litter weights were not significantly modified. The mean foetus weight was of 3.64, 3.59, 3.69 and 3.67 g, respectively at 0, 100, 300 and 1000 mg/kg bw/d and the mean litter weight was of 38.24, 39.26, 40.94 and 40.56 g, respectively

at 0, 100, 300 and 1000 mg/kg bw/d. Pups were examined externally, and after that, visceral, craniofacial and skeletal examinations were performed. All these examinations did not reveal treatment-related effects.

In an extended-one generation reproductive toxicity study (Registration dossier (study report, 2021)), performed following OECD TG 443, groups of male and female Wistar rats were given, by gavage, propylparaben (purity of 99.7 %) at a concentration of either 0, 100, 300 or 1000 mg/kg bw/d. (See 10.10.2 for explanations of test method)

F0 parental and F1 pups (before weaning) :

Regarding the F0 parental generation, 3 females of low dose group were sacrificed at PM 6, GD 21 and PND 4 and 1 female of the mid dose was euthanized at PND 18. All these animals were sacrificed for animal welfare reasons. At the highest dose, 22 males exhibited increased salivation and all males exhibited moving bedding. While in females, clinical signs were already observed at the mid dose group. 4 females at the mid dose and 24 females at the highest dose exhibited excessive salivation, and moving bedding was observed in 5 females at the mid dose and 30 females at the highest dose. No significant and treatment-related bw change was observed (see 10.10.2 Table 9).

Regarding female developmental parameters, percentage of post-implantation loss was increased at the highest dose (approx. of 149 % compared to control group) (see Table 39). At the end of gestation, the mean number of live births was of 10.50, 11.18, 10.70 and 10.89, respectively at 0, 100, 300 and 1000 mg/kg bw/d.

Table 39 : Implantation data

Dose level (in mg/kg bw/d)	0	100	300	1000
Mean nb of implantation sites	11.12	12.14	11.70	11.67
Mean pre-implantation loss (in %)	4.88	3.30	5.42	3.20
Mean post-implantation loss (in %)	5.99	7.79	4.76	8.98

At necropsy, no treatment related gross pathological findings was observed and final body weight was not significantly changed. (see section 10.10.2)

Concerning pups examination, viability index was not modified (Table 40) and mean pup body weight was only significantly lower at PND 14 in the highest dose group (see Table 41**Error! Reference source not found.**). However, anogenital distance and nipple retention were significantly changed. In males, modification was noted at the highest dose, whereas, in females, change was observed in all treated groups and was dose-related (see Table 42). Thyroid hormones analysis exhibited also variations (at PND 21, T4 was of 82.70, 79.01, 74.55 and 74.03 nmol/l in males and 65.20, 79.44, 75.69 and 75.63 nmol/l in females, respectively at 0, 100, 300 and 1000 m/kg bw/d and TSH was of 971.70, 687.24, 751.43 and 711.05 pg/ml in males and 1152.33, 1537.38, 968.41 and 672.30 pg/ml in females, respectively at 0, 100, 300 and 1000 mg/kg bw/d). Necropsy did not reveal treatment-related macroscopic findings. Brain, spleen and thymus were weighed and did not show any modification.

Table 40 : litter data

Dose level (in mg/kg bw/d)	0	100	300	1000
Number of litter examined	26	22	23	28
PND 4 (before interim sacrifice)				
Mean nb of live pups/litter	10.31	11.29	10.65	10.79
Mean nb of males	5.31	5.62	5.96	5.54

Mean nb of females	5.00	5.67	4.70	5.25
Sex ratio (m/f)	1.36	1.19	1.47	1.23
PND 4 (after interim sacrifice)				
Mean nb of live pups	8.77	9.38	8.91	9.36
PND 7				
Mean nb of live pups	8.77	9.38	8.91	9.36
Mean nb of males	4.50	4.67	5.09	4.82
Mean nb of females	4.27	4.71	3.83	4.54
Sex ratio (m/f)	1.45	1.26	1.83	1.33
PND 13 (before interim sacrifice)				
Mean nb of live pups	8.77	9.38	8.91	9.32
Mean nb of males	4.50	4.67	5.09	4.79
Mean nb of females	4.27	4.71	3.83	4.54
Sex ratio (m/f)	1.45	1.26	1.70	1.32
PND 13 (after interim sacrifice)				
Mean nb of live pups	8.38	9.38	8.91	8.96
PND 21				
Mean nb of live pups	8.35	9.38	8.95	8.93
Mean nb of males	4.31	4.67	5.05	4.57
Mean nb of females	4.04	4.71	3.91	4.36
Sex ratio (m/f)	1.45	1.26	1.58	1.34
Viability index				
PND 0 – 4	98.34	98.92	99.59	99.01
PND 4 – 13 (after interim sacrifice)	100.00	100.00	100.00	99.63
PND 13 (after interim sacrifice) - 21	99.62	100.00	98.50	99.69

Table 41: Mean pup and litter weight (in g)

	Mean pup bw				Mean litter weight			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
PND 0	6.54 (N=26)	6.49 (N=22)	6.46 (N=22)	6.31 (N=27)	67.54 (N=26)	72.11 (N=22)	71.78 (N=22)	71.01 (N=27)
PND 4	11.60 (N=26)	11.29 (N=21)	11.27 (N=22)	11.37 (N=27)	116.95 (N=26)	125.35 (N=22)	123.73 (N=22)	126.37 (N=27)
PND 7	17.61 (N=26)	16.94 (N=21)	16.94 (N=22)	16.62 (N=27)	150.32 (N=26)	156.04 (N=21)	155.22 (N=22)	159.47 (N=27)

PND 14	32.81 (N=26)	31.21 (N=21)	31.07 (N=22)	29.68** (N=27)	268.02 (N=0)	286.36 (N=21)	280.82 (N=22)	271.98 (N=27)
PND 21	52.27 (N=26)	49.87 (N=21)	49.88 (N=21)	49.12 (N=27)	427.05 (N=26)	459.07 (N=21)	461.31 (N=21)	446.27 (N=27)

** : p<0.01

Table 42 : AGD and nipple retention

Dose level (in mg/kg bw/d)	0	100	300	1000
Males				
N examined pups	142	123	137	159
AGD (in mm)	2.84	2.78	2.73	2.71*
Relative AGD	1.51	1.48	1.46	1.46
Nb of pup nipple retention on PND 12	0.23	0.35	0.21	0.04*
Females				
N examined pups	132	126	109	149
AGD (in mm)	1.26	1.15***	1.13***	1.12***
Relative AGD	0.68	0.62***	0.61***	0.61***

* : p < 0.05 ; *** : p < 0.001

Cohort 1A :

Three females, exposed to 100 mg/kg bw/d, were sacrificed at PMD 6, GD 21 and PND 4, and one female, exposed to 300 mg/kg bw/d was euthanized at PND 18. All these animals were sacrificed for animal welfare reasons. Clinical signs, such as moving the bedding, was observed in 17 males and 7 females of the highest dose group. Furthermore, at this highest dose, body weight was significantly lower in males at day 64 (see 10.10.2 **Error! Reference source not found.**). Mean balano-preputial separation was slightly reduced (32.32, 31.75, 31.75 and 31.45 days, respectively at 0, 100, 300 and 1000 mg/kg bw/d) while mean vaginal opening was of 30.20, 30.55, 30.75 and 30.50 days, respectively at 0, 100, 300 and 1000 mg/kg bw/d. (see section 10.10.2 for more information)

In this cohort, immunological parameters were examined and revealed severe modifications (see 10.10.2 Table 15).

At the end of the exposure period, at approx. 13 weeks of age, animals were sacrificed and necropsied. No dose-related or significant necropsy findings were observed. FBW and organ weight were modified in males (for more information see section 10.10.2).

Cohort 1B and F2 pups :

During the study period, 1 male and 1 female of the control group were found dead and 1 male of the mid dose group. The clinical sign “moving the bedding” was observed in all animals exposed to 1000 mg/kg bw/d and also in 1 female of the low dose and 6 females of the mid dose. Furthermore, significant body weight changes were observed in females during gestation and lactation periods (see 10.10.2 Table 17).

Regarding female reproduction parameters, mean number of corpora lutea, mean number of implantation sites, mean percentage of pre-implantation loss, mean percentage of post-implantation loss and mean number of duration of gestation did not exhibit this same trend (See 10.10.2 Table 18).

Shortly before weaning, parental animals were sacrificed. Necropsy did not reveal significant organ weight change or treatment-related histopathological effects.

Regarding offspring examination, the mean number of pups (dead and alive) did not show variation (9.32, 10.75, 10.61 and 11.37, resp. at 0, 100, 300 and 1000 mg/kg bw/d). Furthermore, between birth and weaning, the mean number of live pups was unaffected (see Table 43). In the same way, mean pup body weight examination did not show significant change (see Table 44). However, as observed in F1 pups (produced from parental animals), anogenital distance and pup nipple retention was significantly affected (see Table 45).

Table 43 : mean number of live pups

Dose level (in mg/kg bw/d)	0	100	300	1000
PND 0	9.26	10.60	10.56	11.16
PND 4 (before interim sacrifice)	9.26	10.55	10.50	11.11
Alive pups after interim sacrifice	8.21	9.55	9.33	10.05
PND 7	8.21	9.50	9.33	10.05
PND 14	8.16	9.50	9.33	10.05
PND 21	8.11	9.50	9.33	10.05

Table 44 : pups body weight data (in g)

Dose level (in mg/kg bw/d)	0	100	300	1000
Nb examined	18	20	18	18[A]
PND 0	6.26	6.39	6.36	6.11
PND 4	11.33	11.33	11.39	10.94
PND 7	16.86	16.72	16.97	16.30
PND 14	31.38	30.27	31.13	29.93
PND 21	50.62	49.62	49.94	47.09

[A] : n = 17 at D0

Table 45 : AGD and nipple retention

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
Pup weight (g)	6.39	6.51	6.39	6.09**	6.10	6.17	6.24	5.99
AGD (mm)	2.98	2.89	2.87	2.77***	1.05	1.01	1.00	1.06
Relative AGD	1.61	1.55	1.55	1.52**	0.58	0.55	0.54	0.59
Pup nipple retention on PND 12	0.33	0.20	0.42	0.68**	-	-	-	-

** : $p < 0.01$; *** : $p < 0.001$

Cohort 2A :

Only one female exposed to 300 mg/kg bw/d was euthanized during the study period. Clinical observation showed that moving the bedding was observed in all females of the highest dose and increased salivation was

noted in 4 males and 3 females of this dose group. Furthermore, body weight was unaffected. In this cohort, neurotoxicity was examined and revealed some modifications (see 10.10.2 **Error! Reference source not found.**19). At necropsy, only one female of the mid dose group showed an uterus dilataion, and the final body weight and the brain weight were unaffected.

Cohort 2B :

No abnormalities were observed during the necropsy.

Cohort 3 :

During the study period, three animals were found dead (one female of the low dose, one male of the mid dose and one male of the highest dose). Clinical observation revealed signs such as moving the bedding in all males and in 7 females of the highest dose and excessive salivation in 2 males and 1 female of this highest dose. No significant body weight change was noted. In this cohort, mean IgG and IgM serum levels were examined. Few modifications were observed (see 10.10.2 **Error! Reference source not found.** and **Error! Reference source not found.**).

Cohort 4 :

All animals survived during the study period. As in the other cohort, clinical signs "moving the bedding" was observed in all males and in 2 females of the highest dose group. Body weight and gross pathology examination were unaffected by treatment. In this cohort, mean escape latency during learning and memory phases was examined and revealed a reduction during the memory phase which was significant in females (See 10.10.2 Table 22).

In a reproduction/developmental toxicity screening test (Registration dossier (study report, 2018)), performed as a range finding study preceding the EOGRTS, groups of male and female Wistar rats were given by gavage propylparaben (purity 99.7 %) at a concentration of either 0, 500 or 1000 mg/kg bw/d. Groups were composed of 5 males and 5 females in the control groups and 10 males and 10 females in the 500 and 1000 mg/kg bw/d groups. Males were exposed during minimum 35 days (21 days of premating period and maximum 14 days of mating period). Females were exposed during 21 days of premating period and up to 14 days. Thereafter, one dam of each group was dosed up to GD 20, the other dams received test substance during gestation and up to PND 21. The surviving pups of one litter from each group were treated from PND 13 to PND 21.

During the study period, 2 animals were found dead (one male of the low dose on PMD 8 and one female of the highest dose on PND 5). Body weight was unaffected by the treatment, as observed in Table 23 .

Regarding reproductive parameters, percentage of pre and post-implantation loss were increased. Other parameters such as duration of gestation, mean number of corpora lutea and mean number of implantations sites were unaffected (See Table 46).

Table 46 : reproductive parameters

Dose level (in mg/kg bw/d)		0	500	1000
Implantation sites	Nb examined	1	1	1
	Mean nb	12.0	11.0	14.0
	N examined	4	9	8
	Mean nb	13.75	13.11	13.38
Pre- and post-implantation loss	Nb examined	4	9	8

	% pre-	0.00	0.79	1.74
	% post-	6.47	6.74	8.72

At necropsy, one female exposed to 500 mg/kg bw/d had fluid-filled uterus and a uterus horn dilatation and 1 female exposed to 1000 mg/kg bw/d exhibited dark lung accompanied by congestion and atelectasis. No other modification was observed.

Pups were recorded and examined. The mean number of pups at birth was not modified (12.75, 12.44 and 12.56, respectively at 0, 500 and 1000 mg/kg bw/d). Furthermore, mean number of live pups did not significantly decrease at PND4 (PND4 : 12.75, 12.00 and 11.22, resp. at 0, 500 and 1000 mg/kg bw/d) and viability index was unaffected by treatment (PND 0-4 : 100.0, 98.29 and 96.64 % ; PND 4-13 : 100.0, 100.0 and 100.0 % ; PND 13-21 : 100.0, 100.0 and 98.61 %, respectively at 0, 500 and 1000 mg/kg bw/d). No modifications were noted during the body weight examination (see Table 47).

Table 47 : pup body weight and litter weight (in g)

	Pup bw			Litter weight		
Dose level (in mg/kg bw/d)	0	500	1000	0	500	1000
Nb examined	4	9	9	4	9	9
D0	5.84	6.39	5.86	74.33	77.82	71.67
D4	10.23	10.82	10.19	130.10	125.68	113.78
D7	25.48	25.58	25.50	294.40	275.48	265.09
D14	33.35	33.05	30.55	342.98	312.44	284.06
D21	45.45	44.53	42.12	467.70	422.61	373.57

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous, 2012), groups of 11 male and 11 female Wistar rats received, in the feed, propylparaben (99.7 %) at a concentration of either 0, 1500, 4500 or 15000 ppm (See **Error! Reference source not found.** for the mean achieved dose level in mg/kg bw/d in section 10.10.2). Animals were exposed during minimum 4 weeks in males and approximately 7 weeks in females.

All animals survived during the study period. At the highest dose, only one female exhibited malpositioned hind leg during the gestation period. No other abnormalities were recorded and the body weight examination did not reveal significant change (see **Error! Reference source not found.**).

Concerning female reproductive parameters, oestrous cycle, mean number of corpora lutea and mean number of implantation sites were unaffected. However, the percentage of post-implantation loss was severely higher in the highest dose group (5.9, 6.7, 5.2 and 12.4 %, respectively at 0, 1500, 4500 and 15000 ppm). And the mean living pups at the first litter check was lower at the low and the highest dose (11.2, 9.8, 11.6 and 9.9, respectively at 0, 1500, 4500 and 15000 ppm).

At necropsy, macroscopic findings were observed, however only enlarged liver was observed dose dependently (1, 1, 2 and 4 males, respectively at 0, 1500, 4500 and 15000 ppm). Final body weight was not significantly affected in both sexes. And the microscopic examination did not reveal effects (See table 28).

At birth, mean number of living pups was lower at the low and high dose group (11.2, 9.8, 11.6 and 9.9, respectively at 0, 1500, 4500 and 15000 ppm). The birth index was of 94.1, 93.3, 94.8 and 87.6 %, respectively at 0, 1500, 4500 and 15000 ppm. All pups of control and high dose groups survived until the end of the study (PND 4), whereas 2 pups in the low dose group died (1 male at PND 2 and 1 female at PND 4) and 1 pups of

the mid dose group died at PND 3. Body weight was examined at PND 1 and 4 and did not reveal significant changes (see Table 48). No treatment-related abnormalities were observed during the external examination. Soft tissue and skeletal examination were not performed.

Table 48 : Pup body weight data (in g)

Dose level (in ppm)		0	1500	4500	15000
D1	M+F	6.3	6.3	6.0	6.1
	M	6.4	6.4	6.1	6.0
	F	6.1	6.3	5.9	6.0
D4	M+F	8.7	8.9	8.1	8.5
	M	8.9	8.9	8.2	8.2
	F	8.5	9.0	8.0	8.4

Stat : Dunnett-test

An article "Safety assessment of propylparaben in juvenile rats" (Sivaraman *et al.*, 2018) described a study which exposed male and female rats (F1 generation) to propylparaben on PND 4 through PND 90. Groups of male and female SD rats were exposed by gavage to propylparaben at a oncentration of either 0, 10, 100 or 1000 mg/kg bw/d. The study design is explained in the Figure 1.

The F1 generation was observed. At the highest dose group, an increased incidence of abdominal distention during the pre-weaning period was noted as well as an increased incidence of excessive salivation immediately after dosing. Developmental landmarks were examined. In females, mean age of vaginal patency was significantly lower at the highest dose (33.9, 32.4, 32.7 and 31.2** PND, respectively at 0, 10, 100 and 1000 mg/kg bw/d). The article's authors explained that this modification was within the range of the HCD (29.0 to 33.9 days) and that, in their study, 7 control females out of 25 had late development (35 to 43 days) resulting in a high control value. In males, preputial separation was similar in all groups (42.1, 42.3, 42.3 and 43.2 PND, respectively at 0, 10, 100 and 1000 mg/kg bw/d).

Regarding female reproductive performance (treated female mated with non-treated male), the mean number of implantation sites was significantly increased at the low dose (14.3, 17.4**, 16.1 and 15.6, respectively at 0, 10, 100 and 1000 mg/kg bw/d). Other parameters such as mean duration of oestrous cycle, mating index, fertility index, duration of gestation, were unaffected (see Table 29 in section 10.10.2)

Additional groups were used to examine reproductive performance. Untreated females were mated with treated males and females were examined at GD 13 after caesarean. Slight increase of the percentage of pre-implantation loss was observed. Other parameters did not show difference (see **Error! Reference source not found.** in section 10.10.2).

At necropsy, no treatment-related macroscopic and microscopic findings were noted. Higher absolute and relative uterus weight was observed (+ 36 % and + 43 % compared to control, respectively).

Concerning the second generation, pups did not exhibit clinical signs or litter weight change (no more information available). Percentage of male decreased slightly at the highest dose level (49.33, 48.22, 48.06 and 43.69 %, respectively at 0, 100, 100 and 1000 mg/kg bw/d). Viability index at day 4 was unaffected by treatment (100.0, 99.56, 98.59 and 99.04 %, respectively at 0, 10, 100 and 1000 mg/kg bw/d). No malformed pups were observed at any dose level (nor more information available).

An article, “Estrogenicity of parabens revisited : Impact of parabens on early pregnancy and an uterotrophic assay in mice” (Shaw and deCantazaro, 2009), describes two different studies. The first experiment tested butylparaben and the second experiment examined propylparaben. After these 2 parts, an uterotrophic assay was additionally performed with butylparaben.

In the second experiment which tested propylparaben, mouse were exposed by a subcutaneous injection during a period of 4 days (GD 1 to GD 4). Animals received 35 or 45 mg of the test substance.

On gestation day 6, animals were euthanized and examined. The mean number of implantation sites was unaffected by treatment.

10.10.6 Comparison with the CLP criteria

Criteria for Category 1	Criteria for category 2
“Known or presumed human reproductive toxicant Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human (category 1A) or from animal data (category 1B). Category 1A : known human reproductive toxicant. The classification is largely based on evidence from humans Category 1B : presumed human reproductive toxicant. The classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate.”	“Suspected human reproductive toxicant Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

Since no human studies are available for effects on development, classification in Repr. 1A for development is not appropriate.

Developmental effects were reported as follow:

- Post-implantation loss

In the EOGRTS (Registration dossier (study report, 2021)), in the F0 generation, the percentage of post-implantation loss was increased at the highest dose, but the modification was not dose-related (5.99, 7.79, 4.76 and 8.98 %). This effect was not confirmed in the cohort 1B.

A slight but not dose-related increase of the post-implantation loss was also observed in the prenatal developmental toxicity study (Registration dossier (study report, 2019)) at the low and high doses (6.23, 8.95, 5.36 and 8.07 %, respectively at 0, 100, 300 and 1000 mg/kg bw/d).

In the dose range finding study for reproduction/developmental toxicity screening (Registration dossier (study report, 2018)), the post-implantation loss increased slightly, but here in a dose-dependent manner (6.47, 6.74 and 8.72 %, respectively at 0, 500 and 1000 mg/kg bw/d).

Furthermore, in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Registration dossier (study report, 2012)), the percentage of post-implantation loss was severely higher at the highest dose, corresponding to a more than two-fold increase (5.9, 6.7, 5.2 and 12.4 %, respectively at 0, 1500, 4500 and 15000 ppm (corresponding approx. to 0, 98.0, 305.1 and 980.9 mg/kg bw/d during the pre-pairing period and 0, 59.3, 178.3 and 605 mg/kg bw/d during the post-pairing period)).

Table 49 : Summary table of the Percentage of Post-implantation loss

Dose level (in mg/kg bw/d)	0	100	116-137.3	300	341.9-431.8	500	1000	1076.4-1380.0
Prenatal developmental toxicity study (Registration dossier (study report, 2019))	6.23	8.95	-	5.36	-	-	8.07	-
EOGRTS (Registration dossier (study report, 2021)) : F0	5.99	7.79	-	4.76	-	-	8.98	-
Dose range finding study for reproduction/developmental toxicity screening test (Registration dossier (study report, 2018))	6.47	-	-	-	-	6.74	8.72	-
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Registration dossier (study report, 2012))	5.9	-	6.7	-	5.2	-	-	12.4

- Anogenital distance

In the EOGRTS (Registration dossier (study report, 2021)), in the F1 male pups, the anogenital distance was significantly lower at the highest dose (2.71 mm at 1000 mg/kg bw/d vs 2.84 mm in control group). Considering the weight of the pups, the relative anogenital distance still decreased, but not significantly (1.51, 1.48, 1.48 and 1.46 at 0, 100, 300 and 1000 mg/kg bw/d, respectively). In F2 male pups, the anogenital distance was also significantly lower at the highest dose (2.77 mm at 1000 mg/kg bw/d vs 2.98 in control group) and the relative anogenital distance decreased in a significant dose-dependent manner (1.61, 1.55, 1.55 and 1.52** at 0, 100, 300 and 1000 mg/kg bw/d, respectively).

Interestingly in F1 females pups, a dose-dependent decrease, already significant at the lowest dose, was observed for the anogenital distance (1.26, 1.15***, 1.13*** and 1.12*** mm at 0, 100, 300 and 1000 mg/kg bw/d, respectively) and the relative anogenital distance (0.68, 0.62***, 0.61*** and 0.61*** at 0, 100, 300 and 1000 mg/kg bw/d, respectively). This decrease was not confirmed in F2 female pups.

Anogenital distance was not assessed in the other available studies.

- Conclusion

A classification as **Repr. 2, H361d** is warranted based on AGD and post-implantation loss's modifications.

10.10.7 Adverse effects on or via lactation

No data available

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Based on the available information, a classification as **Repr. 2, H361fd** is warranted.

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 below.

Additional key elements

In addition to the studies reported by the DS, European Commission's Scientific Committee on Consumer Safety (SCCS) described several other studies with propyl paraben (SCCS opinion on propyl paraben, 2021), amongst others:

- Vo *et al*. (2010). Immature female Sprague-Dawley rats were exposed orally by gavage with 62.5, 250, and 1000 mg/kg bw/day propyl paraben from PND21-40. Effects reported in the high dose group were myometrial hypertrophy and increased adrenal weight. SCCS noted that the animals were not necropsied at specific stages. It is very likely that a number of females were in proestrus or

[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.*

estrus, which could explain the unexpected observation of myometrial hypertrophy.
- Ahn *et al*. (2012). Neonatal female Sprague-Dawley rats (N=5) were administered subcutaneously with 0, 62.5, 250 or 1000 mg/kg bw/day for 7 days (PND1-7). In the highest dose group, an increased number of primordial follicles and a decreased number of early primary follicles were reported. So, propyl paraben inhibited the early phase of folliculogenesis in the ovaries of the neonatal female rat.

Further, SCCS noted limitations in the Oishi (2002) study:
- control values were outside the normal range, not consistent with literature data and other publications from this research group,
- absence of dose-response for daily sperm production,
- small group size, and
- full study protocol and raw data not available.

SCCS concluded that the available data on propyl paraben provide some indication for potential endocrine effect, however not yet sufficient to identify propyl paraben as an endocrine disrupting substance.

European Medicines Agency (EMA) published a Reflection paper on methyl- and propyl paraben in 2015. Based on results by Oishi (2002), Gazin *et al*. (2013), and Pouliot (2013; later published as Sivaraman *et al*., 2018), it was concluded that propyl paraben does not cause any effect on male reproduction parameters following daily oral administration of doses up to 1000 mg/kg to male rats from 4 to 90 days of age. With regard to the female reproductive system development, the EMA noted that propyl paraben seemed to induce myometrial hypertrophy at 1000 mg/kg bw/day in a juvenile study (Vo *et al*., 2010). This finding was not confirmed in a GLP compliant, 3-month juvenile toxicity study (Pouliot, 2013; same as Sivaraman et al., 2018). However, propyl paraben-related changes suggestive of an estrogenic effect were observed at the high dose level, i.e., earlier onset of puberty and increased weight of uterus, without any concomitant effect on the histology of reproductive tissues, oestrous cyclicity, mating and fertility, and maternal performance. The NOEL was determined at 100 mg/kg/day.

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 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.

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

No relevant effects were found in *Cohort 2A, 2B, 3 and 4*.

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 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.

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

- 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 for reproductive toxicity, RAC concludes **no classification for Reproductive Toxicity for fertility and developmental toxicity, and no classification for effects on or via lactation.**

Supplemental information - In depth analyses by RAC

***Table**: Overview on adverse effects of exposure to propyl paraben on male reproductive system*

Dose (in mg/kg bw/day)		**0**	**3**	**10**	**12.4**	**100**	**125**	**300**	**1000**	**1290**
Sperm motility (% motile count)										
EOGRTS (2021) [2]	F0	77.05				77.60		77.98	72.67	
	C1A	79.10				-		-	72.42	
OECD TG 422 screening study (2012)[1]: percentage *not* motile sperm count		13.4				12.2		13.9	10.1	
Gazin *et al*. (2013)		81.1	88.2	71.4				85.5	85.8	
Sperm counts										
Oishi (2002): in cauda epididymis (x10^7/g)[4]		108			70.8		63.1*			48.8*
EOGRTS (2021)[2]:	P, testicular sperm count in million sperms/g	113.5				115.5		124.0	114.9	
	F1, mean testicular sperm count[3]	127.6				126.8		131.5	137.2	
OECD TG 422 screening study (2012): mean testis sperm count in mio/g		123.7							130.0	

Gazin *et al*. (2013):	million sperm/g testis	153.9	144.4	145.2		151.3			162.3	
	Epididymal sperm count	428	501	449		473			547	
Sperm production in testis										
Oishi, 2002 (DSP : $x10^6$) [4]		37.5			26.2*		27.0*			25.9*
Total nb of abnormal sperms (on 200 sperms examined)										
EOGRTS (2021)	F0	8.25				n.d.		n.d.	13.33	
	C1A	10.35				n.d.		n.d.	19.06	
Sperm morphology (in %)										
EOGRTS (2021): in F0	Amorphous head	0.0				n.d.		n.d.	0.0	
	Head only	2.46				n.d.		n.d.	2.63	
	Bent tail	2.17				n.d.		n.d.	2.38	
	Broken tail	0.42				n.d.		n.d.	0.08	
	Coiled tail	0.25				n.d.		n.d.	0.08	
	Tail only	2.96				n.d.		n.d.	8.17	
Testis weight (in g or %)										
EOGRTS (2021): in F1 cohort A	Abs	1.817				1.782		1.839	1.677	
OECD TG 422 screening study (2012)										
Gazin *et al*. (2013)	Abs	1.81				1.76		1.74	1.77	
Oishi (2002)	Abs	2.65			2.67		2.60			2.60
	Rel	0.961			0.955		0.950			0.999
Prostate weight (in mg or %)										
EOGRTS (2021): F0	Abs	3.281				3.226		3.008	2.856**	
	Rel	0.727				0.716		0.678	0.643*	
Gazin *et al*. (2013)			Not affected							
Testosterone concentration										
Oishi (2002): in ng/ml [4]		9.08			8.20		7.17			5.86*
Gazin *et al*. (2013): in nmol/l		16.9				21.2		22.9	18.9	

* : p<0.05

1. Concentration in feed of 0, 1500, 4500 and 15000 ppm (mean achieved dose levels were 98.0, 305.1 and 980.9 mg/kg bw/day respectively in males in pre-pairing period).
2. Historical control data on sperm motility for Wistar rats (years 2010-2017/2016-2020/2019-2020) provided by a company in the RCOM:
   - Motile count (%): mean 81.71, SD 8.23, N=114, range 44.5-95.5
   - Million sperms/g: mean 114.45, SD 28.51, N=123, range 28-196.8
3. Data on mean testicular sperm count as reported in RCOM document by a company for Cohort 1A – F1 generation.
4. Control data from Oishi, 2001 and 2004 respectively:
   - Sperm counts - Cauda epididymides – concentration: 169.8 ±91.4 (in $x10^7$/g) and 39.8 ±7.32 (in $x10^6$/g)
   - Testis – DSP (in $x10^6$): 40.0±5.86 and 12.8 ±3.28
   - Testosterone concentration (in ng/ml) in control group: about 9 and 11.9 ±2.09 ng/ml

***Table**: Overview of post-implantation loss (%) from available rat studies with propyl paraben*

Dose (in mg/kg bw/day)	**0**	**10**	**100**	**116 - 137.3**	**300**	**341.9-431.8**	**500**	**1000**	**1076.4-1380.0**
PNDT (2019)	6.23		8.95		5.36			8.07	
EOGRTS range finding study (2018)	6.47						6.74	8.72	
EOGRTS (2021)[1]: F0	5.99		7.79		4.76			8.98	
EOGRTS (2021): C1B	9.05		4.11		4.90			7.61	
OECD TG 422 screening study[2] (2012)	5.9			6.7		5.2			12.4
1-generation like study (Sivaraman *et al*., 2018)	5.15	8.15	5.10					3.68	

1. Historical control data on percentage post-implantation loss for Wistar rats (years 2010-2017/2016-2020/2019-2020) provided by a company in the RCOM:
   - Mean 10.26%, SD 20.8, N=507, range 0.0-100
2. HCD for the combined study (provided in the RCOM by DS): 5.1-12.6% (11 studies, from 05-2009 till 09-2010)

***Table**: AGD and nipple retention on PND12 from EOGRT study (2021) with propyl paraben*

Dose (in mg/kg bw/day)	**0**	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
	males				*females*			
AGD in mm								
F1 pups	2.84	2.78	2.73	2.71*[1)]	1.26	1.15***	1.13***	1.12***
F2 pups	2.98	2.89	2.87	2.77***[1)]	1.05	1.01	1.00	1.06
Relative AGD								
F1 pups	1.51	1.48	1.46	1.46	0.68	0.62***	0.61***	0.61***
F2 pups	1.61	1.55	1.55	1.52**[1)]	0.58	0.55	0.54	0.59
Nipple retention								
F1 pups	0.23	0.35	0.21	0.04*	-	-	-	-
F2 pups	0.33	0.20	0.42	0.68**	-	-	-	-

** : p < 0.01 ; *** : p < 0.001

1. In the registration dossier, it was noted that values were within the normal range of historical control data, thus not considered toxicologically relevant.

Historical control data for Wistar rats (years 2010-2017/2016-2020/2019-2020) provided by a company in the RCOM:

- AGD male pups (PND0): Mean 2.6 mm, SD 0.4, N=2073, range 0.96-4.21

- Relative AGD male pups (PND0): Mean 1.4, SD 0.2, N=2073, range 0.53-2.18
- AGD female pups (PND0): Mean 0.9 mm, SD 0.3, N=2021, range 0.27-2.65
- Relative AGD female pups (PND0): Mean 0.5, SD 0.2, N=2021, range 0.16-1.43

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier

10.13 Aspiration hazard

Hazard class not assessed in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard class not assessed in this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Hazard class not assessed in this dossier

13 ABREVIATIONS

* : $p < 0.05$

** : $p < 0.01$

*** : $p<0.001$

Abs : absolute

AGD : anogenital distance

ALH : amplitude of lateral head displacement

Approx. : approximately

APTT : activated partial thromboplastin time

Bw : body weight

Conc. : concentration

DMSO : dimethyl sulfoxide

DIT : developmental immunotoxicity

DNT : developmental neurotoxicity

DSP : daily sperm production

EOGRTS : Extended one-generation reproductive toxicity study

F : female

FBW : final body weight

FM : fast movements

FR : fast rearings

FSH : follicle stimuling hormone

GD : gestational day

GLP : good laboratory practice

HCD : historical control data

Hg : hemoglobin

HPLC : high performance liquid chromatography

Ht : hematocrit

IgG : immunoglobulin G

IgM : Immunoglobulin M

KLH : keyhole limpet haemocyanin

L. : left

LIN : linearity (VSL/VCL *100)

LH : luteinizing hormone

LOAEL : low observed adverse effect level

M : male

Max : maximum

MCV : mean corpuscular volume

MCHC : mean corpuscular haemoglobin concentration

Min : minimum

Mio : million

NA : not applicable

Nb : number

NK : natural killer

NOAEL : No observed adverse effect level

NT : not tested

OECD : Organisation for Economic Cooperation and Development

PC : positive control

Plt : platelet

PMD : post mating day

PND : post natal day

PP : post-partum

PT : prothrombin time

R. : right

RBC : red blood cell

Rel. : reliability

Rela : relative

Resp. : respectively

SD : Sprague Dawley

Sign. : significant

SM : slow movemements

SR : slow rearings

St. De. : standard deviation

Stat : statistical

STR : straightness (VSL/VAP *100)

T3 : triiodothyronine

T4 : L-thyroxine

TG : test guideline

Tot : total

TSH : thyroid-stimulating hormone

VAP : average path velocity

VCL : curvilinear velocity

VSL : straight line velocity

WBC : with blood cell

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Full study report

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15 ANNEXES

- Annex I to the CLH report
- Confidential Annex to the CLH report