SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Propyl 4-hydroxybenzoate (propylparaben)

EC No. 202-307-7

CAS RN 94-13-3

Evaluating Member State(s): Belgium

Dated: 13 July 2023

Evaluating Member State Competent Authority

MSCA name
Belgian Federal Public Service Health, Food Chain Safety and Environment, Risk Management Service
Galileelaan 5/2
1210 Bruxelles
Belgium
Email: evaluation.reach@health.fgov.be

Year of evaluation in CoRAP: 2015

Before concluding the substance evaluation, a Decision to request further information was issued on: 8 September 2016

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B, the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Propyl 4-hydroxybenzoate, referred to hereinafter as propylparaben or PPB was originally selected for substance evaluation to clarify concerns about:

- Suspected Reprotoxic
- Potential endocrine disruptor
- Consumer use
- Exposure of environment
- Exposure of sensitive populations
- Wide dispersive use

During the evaluation another concern was identified. The additional concern was:

- ❖ Toxicity to the environment.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Based on all available data for the endpoint reproductive toxicity, including the studies requested via SEv decision, BE CA decided to submit a CLH proposal for this endpoint: Repr. 2, H361fd. This proposal was launched for public consultation[2] by ECHA on 11/4/2022 and the commenting period ended on 10/6/2022. The Committee for Risk Assessment (RAC) adopted in their opinion that propylparaben should not be classified for reproductive toxicity[3].

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1: Conclusion of substance evaluation

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	√
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	√
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

[2] https://echa.europa.eu/harmonised-classification-and-labelling-previous-consultations/-/substance-rev/69402/term

[3] RAC opinion of propyl 4- hydroxybenzoate adopted on 16 March 2023: https://echa.europa.eu/documents/10162/f1124e2a-2fe2-badd-57b3-37bbcece8034

4. FOLLOW-UP AT EU LEVEL

4.1 Need for follow-up regulatory action at EU level

4.1.1 Harmonised Classification and Labelling

Not applicable.

4.1.2 Identification as a substance of very high concern, SVHC (first step towards authorisation)

The substance has endocrine disrupting properties and can be identified as substance of equivalent level of concern to CMR/PBT substances in accordance with article 57(f) of REACH for Environment.

Based on all available scientific evidence, it can be concluded that Propylparaben fulfils the WHO/IPCS (2002)[4] definition of an endocrine disruptor:
- It has endocrine modes of action: clear estrogenic and anti-androgenic mode of action.
- It shows clear reproductive adverse effect in fish (changed sex ratio).
- The adverse effects, including the recognised EAS-mediated effects (e.g., sex ratio) and effects sensitive, but not diagnostic of EAS (e.g. oocyte maturation, hatching success and time to hatch), are a consequence of the endocrine modes of action.

4.1.3 Restriction

Not applicable.

4.1.4 Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1 No need for regulatory follow-up at EU level

Not applicable, see section 4.

5.2 Other actions

Not applicable, see section 4.

[4] An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny or (sub)populations.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2: Follow-up

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
RMOA	January 2024	BE CA
SVHC identification	August 2024	BE CA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1 Overview of the substance evaluation performed

Propylparaben was originally selected for substance evaluation to clarify concerns about:

- Suspected Reprotoxic
- Potential endocrine disruptor
- Consumer use
- Exposure of environment
- Exposure of sensitive populations
- Wide dispersive use

During the evaluation also another concern was identified. The additional concern was:

- Toxicity to the environment.

Table 3: Evaluated endpoints

EVALUATED ENDPOINTS	
Endpoint evaluated	Outcome/conclusion
Reprotoxicity	**No concern**: No classification for reproductive and developmental toxicity (RAC opinion adopted on 16 March 2023)[5]
Endocrine disruption – human health	**No ED concern for human health,** based on all available data on propylparaben including requested data: - Prenatal Developmental Toxicity Study (OECD TG 414) under dossier evaluation (compliance check) - Extended One-Generation Reproductive Toxicity Study (EOGRTS - OECD TG 443) under substance evaluation
Endocrine disruption - environment	**ED concern for the environment confirmed**: based on the results of all available data on propylparaben (including the Fish Sexual Development Test (FSDT - OECD TG 234) requested under substance evaluation propylparaben can be identified as an endocrine disruptor for the environment (ENV).
Consumer use	As the initial concerns for human health (HH) were refuted, an exposure assessment for consumers was not undertaken by the eMSCA in the context of this substance evaluation.
Wide dispersive use	Data on occurrence demonstrate the wide dispersive use in Europe and beyond: use in cosmetics, personal care products, pharmaceuticals, veterinary products, medical devices, detergents, …).
Exposure of environment	A full exposure assessment was not undertaken by the eMSCA in the context of this substance evaluation. Data on occurrence in the environment indicate that, due to its widespread uses in cosmetics and personal care products, and thus continuous

[5] RAC opinion of propyl 4- hydroxybenzoate adopted on 16 March 2023: https://echa.europa.eu/documents/10162/f1124e2a-2fe2-badd-57b3-37bbcece8034

	introduction in the environment, propylparaben is found with increasing concentrations in water, soil, sludge, and indoor dust.
Exposure of sensitive populations	As the initial concerns for HH were refuted, an exposure assessment for the sensitive populations was not undertaken by the eMSCA in the context of this substance evaluation.
Other endpoints	Other endpoints were checked and following conclusion were issued. **Aquatic chronic toxicity**: **Concern confirmed**. The results of the Daphnia magna Reproduction Test (OECD TG 211), requested under substance evaluation, support the self-classification in the REACH registration dossier: Aquatic Chronic 3, H412.
	Genotoxicity: No concern. An AMES Test (Bacterial Reverse Mutation Test) (OECD TG 471) and an In Vitro Mammalian Cell Micronucleus Test (OECD TG 487) were conducted following a compliance check. Based on all available data, it can be concluded that there is no concern.

7.2 Procedure

Substance Evaluation and follow up:

Based on an opinion of the ECHA Member State Committee and due to initial grounds for concern related to Human health (suspected reprotoxicant); potential endocrine disruptor and wide dispersive use, consumer use and exposure of environment and sensitive populations, propylparaben was included in the Community Rolling Action Plan (CoRAP) for substance evaluation (article 44(2) of the REACH Regulation) to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015.

Pursuant to Article 45(4) of the REACH Regulation, the REACH registration dossier of propylparaben and all other relevant and available information (fi. *in vitro* and *in vivo* scientific literature) were used as a basis for the evaluation. eMSCA considered that further information was required to clarify the concerns by means of an Extended One-Generation Reproductive Toxicity Study (OECD TG 443), Daphnia magna Reproduction Test (OECD TG 211) and a Fish Sexual Development Test (OECD TG 234). The draft decision prepared by the eMSCA was unanimously agreed upon after discussion by the Member State Committee (MSC meeting of 12-16 December 2016) and the SEv decision was notified to the Registrant(s) by ECHA on 8 March 2017 with a deadline to submit the information (including the robust study summaries, full study report and updated Chemical Safety Report) by 17 June 2019.

The registrant(s) submitted their updated registration dossier containing all requested information on 28 May 2020. In accordance with Article 46(3) of REACH, the evaluating Member State (eMSCA) started the second round of the evaluation without undue delay.

Classification and labelling:

After receipt of the requested information concerning reprotoxicity and assessment of all available study results, BE CA decided to submit a CLH report to classify in Repr. 2 (H361fd) as there was no self-classification nor an entry in annex VI of CLP for reprotoxicity. This CLH report[6] was published by ECHA on 11 April 2022 for public consultation and commenting period ended on 10 June 2022. In their opinion adopted on 16 March 2023,

[6] https://echa.europa.eu/harmonised-classification-and-labelling-previous-consultations/-/substance-rev/69402/term

the Committee for Risk Assessment (RAC) concluded that propylparaben should not be classified for reproductive toxicity[7].

Identification as substance of very high concern:

The eMSCA presented the results of the requested studies (HH and ENV) for propylparaben during the open session of the 19th meeting of the Endocrine Disruptor Expert Group (EDEG) (18 November 2020) where a representative of the Registrants was also present. EDEG experts agreed that the substance is an ED for ENV acting via estrogen Mode of action (MoA) and that further refinement of the assessment was needed to clarify the concerns for ED HH.

eMSCA concluded that based on all available scientific evidence, the substance can be identified as a substance of very high concern due to its endocrine properties for the environment.

7.3 Identity of the substance

Table 4: Substance identity

SUBSTANCE IDENTITY	
Public name:	Propyl 4-hydroxybenzoate
EC number:	202-307-7
CAS number:	94-13-3
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	C10H12O3
Molecular weight range:	180.21 g/mol
Synonyms:	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

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

[7] RAC opinion of propyl 4- hydroxybenzoate adopted on 16 March 2023: https://echa.europa.eu/documents/10162/f1124e2a-2fe2-badd-57b3-37bbcece8034

Structural formula:

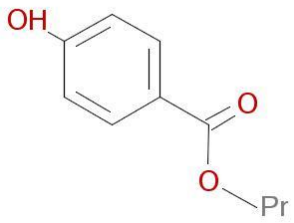


Table 5: Constituent

Constituents	Typical concentration	Concentration range	Remarks
propyl 4-hydroxybenzoate (EC N° 202-307-7)	-	-	Mono constituent substance

7.4 Physico-chemical properties

Table 6: Physicochemical properties of propylparaben

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES			
Property	**Value**	**Reference**	**Reliability**
Physical state at 20 °C and 101.3 kPa	White crystalline solid	*REACH registration dossier, Unpublished study report, 2012*	*1*
Melting point	97 °C (mean value of 2 peer reviewed article reports and 1 peer reviewed handbook report. No experimental details are given)	*REACH registration dossier, Publications: Nikitakis and McEwen, 1990; Bergfeld et al., 2006 and O'Neil M.J, 2001*	*4*
Boiling point	301 °C ± 16 °C (The sample shows a broad endothermic boiling peak in the 290 - 350 °C region)	*REACH registration dossier, Unpublished study report, 2012 (REACH registration dossier)*	*1*
Density at 20 °C	1.287 g/cm3	*REACH registration dossier, Publications: Giordano et al., 1999 and Feng and Grant, 2006*	*2*
Vapour pressure	0.00034 Pa at 20 °C (OECD TG 104) by vapour pressure balance (effusion method)	*REACH registration dossier, Unpublished study report, 2011*	*1*
Water solubility	500 mg/L at 25 °C 579.6 mg/L at 25 °C (EPI Suite v4.0)	*REACH registration dossier: Publication: Yalkowsky and* He, 2003 *REACH registration dossier: EPI suite v4.0 (US EPA, 2008)*	*4* *2*
Partition coefficient n-octanol/water (Log Kow)	2.8 (mean of results of the most reliable studies)	*REACH Registration dossier: Publications:*	

	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)	*Angelov et al., 2008* *Hansch et al., 1995* *Seki et al., 2003* *Terasaki et al., 2009* *Peer reviewed article, 1981 (author not mentioned)* *Hayward et al., 1990* *Golden et al., 2005*	*2* *4* *4* *1* *2* *2* *4*
Flammability	No flammable solid (EU Method A.10) No pyrophoricity No flammability on contact with water	*REACH registration dossier: Unpublished study report, 2011*	*1*
Explosive properties	No	*REACH registration dossier*	*/*
Oxidising properties	No	*REACH registration dossier*	*/*
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 %	*REACH registration dossier: Unpublished study report, 2011*	*1*
Stability in organic solvents and identity of relevant degradation products	The functional groups of the substance indicate no instabilities in common organic solvents.	*REACH registration dossier*	*/*
Dissociation constant	pKa= 8.87 (HPLC) pKa= 8.16 at 25 °C (potentiometric titration) pKa= 8.35	*REACH registration dossier: Publications: Angelov et al., 2008* *Shoghi et al., 2009* *Dymicky and Huhtanen, 1979 and Bergfeld et al., 2006*	*2* *2* *4*

7.5 Manufacture and uses

7.5.1 Quantities

Table 7: Aggregated tonnage (per year)

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000 - 10,000 t	☐ 10,000 - 50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2 Overview of uses

Formulation of preparations is covered in the registration dossier, together with consumer uses (see table 8). There is no reference in the registration dossier to manufacture, industrial uses or uses by professional workers.

Table 8: Overview of uses

USES	Use(s)
Uses as intermediate	-
Formulation	Manufacturing of cosmetic products and pharmaceutical preparations (ointments)
Uses at industrial sites	-
Uses by professional workers	-
Consumer Uses	Consumer End Use of cosmetic products or pharmaceuticals
Article service life	-

7.6 Classification and Labelling

7.6.1 Harmonised Classification (Annex VI of CLP)

In their opinion of 16 March 2023, the Committee for Risk Assessment (RAC) concluded that propylparaben should not be classified for reproductive toxicity[8].

No entry in Annex VI of CLP (harmonised classification) is available.

7.6.2 Self-classification

- In the registration(s) (consulted on 16/9/2022):

Aquatic chronic 3, H412

[8] RAC opinion of propyl 4- hydroxybenzoate adopted on 16 March 2023: https://echa.europa.eu/documents/10162/f1124e2a-2fe2-badd-57b3-37bbcece8034

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory (consulted on 16/9/2022):

Number of aggregated notifications: 27
Not classified
Acute Tox. 4, H302
Acute Tox. 4, H332
Skin Irrit. 2, H315
Skin Sens. 1, H317
Skin Sens. 1B, H317
Eye Irrit. 2, H319
Eye Dam. 1, H318
Resp. Sens. 1, H334
STOT SE 3, H335 (central nervous, lung, respiratory tract or not specified)
STOT RE 2, H373 (lungs)
Aquatic Chronic 3, H412
Aquatic Chronic 4, H413
Aquatic Acute 1, H400
Aquatic Acute 2, H411

7.7 Environmental fate properties

7.7.1 Degradation

Table 9: Overview degradation data

	Conclusion	Study Reference	Reliability
Abiotic degradation			
Hydrolysis	Not relevant due to rapid biodegradability	REACH registration dossier	/
Aerobic degradation in soil (abiotic)	After 28 d 54 % of PPB was detected.	Samarasinghe *et al.*, 2021	1 Well documented non-guideline study
Biotic degradation			
OECD TG 301 F (Ready Biodegradability: Manometric Respirometry Test)	91.5 % (ThOD) degradation within 28 d	REACH registration dossier: peer review in Danish EPA report, 2001	2
Anaerobic biodegradability Guideline: ISO 11734 (measurement of gas production at 35 °C and 90 days)	18 % (ThGP) degradation within 90 d	REACH registration dossier: peer review in Danish EPA report, 2001	2
Aerobic and anaerobic degradation in activated sludge of a municipal WWTP in	Aerobic T1/2: At 1 mg/L: 16.9 min	Wu *et al.*, 2017b	1 Well documented

Xiamen (China)	At 10 mg/L: 19.8 min Anaerobic T1/2: At 1 mg/L: 9.5 h		non-guideline study
Degradation in untreated agricultural soil and agricultural soil treated with compost from WWTP	Untreated soil: PPB conc. (meas): 14.3 ng/g, decreased to 0.6 ng/g after 845.5 h Half life: 167.4 h Treated soil: PPB conc. (meas): 13.5 ng/g and decreased to 1.2 ng/g after 845.5 h Half life: 188.3 h	Camino-Sánchez *et al*., 2016	2 Well documented non-guideline study but not clear if disappearance is due to biotic or abiotic degradation
Aerobic degradation in soil (biotic)	Concentrations were degraded with 90.3 %, 98.3 %, 98.6 % and 100 % resp. at day 3, 7, 14 and 28 d.	Samarasinghe *et al*., 2021	1 Well documented non-guideline study

From data in the REACH registration dossier, it can be concluded that propylparaben is readily biodegradable.

Furthermore, literature studies demonstrate also rapid degradation in soil:

Camino-Sánchez *et al*. (2016) examined degradation of propylparaben in untreated agricultural soil and agricultural soil treated with compost from WWTP. The concentration of propylparaben measured in the untreated soil was 14.3 ng/g and it decreased over time (845.5 h) to 0.6 ng/g. In the treated soil the measured concentration of propylparaben was 13.5 ng/g and decreased to 1.2 ng/g after 845.5 h. A half-life of respectively 167.4 h (± 6.98 d) and 188.3 h (± 7.85 d) was determined for the untreated and treated soil.

Samarasinghe *et al*. (2021) examined the abiotic and biotic degradation of methyl-, propyl- and butylparaben in soil. After 7 days already 98 % of propylparaben disappeared in the soil while only 54 % degraded in frozen soil (abiotic), demonstrating rapid degradation under biotic conditions.

7.7.2 Environmental distribution

Table 10: Transport and distribution

Endpoint	Conclusion	Study reference	Reliability
Adsorption/desorption	Log Koc= 2.4573 at 25 °C Koc= 286.6 at 25 °C	REACH registration dossier: Calculation based on MCI (KOCWIN v2.00), 2013	2

Henry's law constant	4.25E-009 atm-m3/mole	REACH registration dossier: estimated by Group SAR Method (HENRYWIN v3.20), 2015	2

Level III Fugacity Model (Episuite v4.1,2015, by eMSCA):

The following results are obtained with this model:

Table 11: When equal emission to all compartment is considered

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)
Air	0.0757	18.2	1000
Water	17.7	360	1000
Soil	80	720	1000
Sediment	0.222	3.24×10^3	0

Table 12: When only emission to air is assumed

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)
Air	0.194	18.2	1000
Water	4.73	360	0
Soil	95	720	0
Sediment	0.0595	3.24×10^3	0

Table 13: When only emission to water is assumed

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)
Air	7.25×10^{-6}	18.2	0
Water	98.8	360	1000
Soil	0.00356	720	0
Sediment	1.24	3.24×10^3	0

Table 14: When only emission to soil is assumed

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)
Air	9.31×10^{-5}	18.2	0
Water	1.18	360	0
Soil	98.8	720	1000
Sediment	0.0148	3.24×10^3	0

Table 15: When emission to air and water is considered

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)

Air	0.139	18.2	1000
Water	31.4	360	1000
Soil	68	720	0
Sediment	0.395	3.24 x 10^3	0

Table 16: when emission to air and soil is considered

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)
Air	0.0896	18.2	1000
Water	2.82	360	0
Soil	97.1	720	1000
Sediment	0.0355	3.24 x 10^3	0

Table 17: When emission to water and soil is considered

	Mass Amount (%)	Half Life (h)	Emissions (kg/h)
Air	7.13 x 10^{-5}	18.2	0
Water	26	360	1000
Soil	73.7	720	1000
Sediment	0.327	3.24 x 10^3	0

With evenly distributed emissions, the substance seems to partition mainly to soil (82 %) and in a lesser extent to water (17.7 %). It can however be expected that in view of the use of the substance as an additive in consumer pharmaceutical and cosmetic products, such as ointments, shampoos and conditioners, the majority of the substance will be emitted to waste water. In formulation stage, it can be expected that the substance will mainly be discharged via wastewater during cleaning processes. When only emission to the water compartment is assumed, it is estimated that the majority of the substance (98.8 %) will remain in that compartment. In view of this, it can be expected that water and soil are the most affected environmental compartments.

7.7.3 Bioaccumulation

Table 18: Overview data on bioaccumulation

	Conclusion	**Study reference**	**Reliability**
Log Kow (Key study)	2.34 at 20 °C	REACH registration dossier: Terasaki, *et al*., 2009	1
BCF	NA	/	/

Based on the log Kow, the substance is expected to have low potential to bioaccumulate.

This is supported by the study of Bjerregaard *et al*. (2003). Authors demonstrated in a feeding experiment with rainbow trout (*Oncorhynchus mykiss*) that <1 % of 1830 mg/kg/2d propylparaben was bioaccumulated in muscle and liver after 10 days of exposure. In the waterborne experiment resp. 6700 and 870 µg/kg propylparaben was detected in liver and muscle after 12 days exposure to 225 µg/L propylparaben showing low uptake of the substance (BCF of resp. 29 and 3.8, not lipid normalized).

7.8 Environmental hazard assessment

7.8.1 Aquatic compartment (including sediment)

Table 19: Overview aquatic acute toxicity

Species/test method	Result	Study reference	Reliability
Fish			
OECD TG 203 *Danio rerio* Test conc.: 0, 1.0, 2.2, 4.6, 10 and 22 mg/L Static GLP	96h LC50= 6.4 mg/L (geom. mean)	REACH registration dossier: Unpublished study report, 2012	1
Non-standard assay Exposure: Larval fish fathead minnow, *Pimephales promelas* (24hpf), exposure to 7 parabens for 48 h and 7 days Test conc.: Acute toxicity: 5 test conc., water, and solvent control, 2 replicates Subchronic toxicity: 5 test conc., highest test conc. is half of LC50, dilution factor of 0.5, 4 replicates Effect measurement: Acute toxicity: mortality (LC50) Subchronic toxicity: growth in fish GLP: not specified	Acute toxicity: Benzylparaben (BZPB) < Butylparaben (BPB) < isobutylparaben (IBPB) < Isopropylparaben (IPPB) < LC50 fish 9.7 mg/L Propylparaben (PPB) < Ethylparaben (EPB) < Methylparaben (MPB) Subchronic toxicity: growth LOEC values were related to log P value, range for 7 parabens between 1.0 and 25 mg/L (MPB least toxic). LOEC of PPB was 2.5 mg/L; NOEC: 1.25 mg/L *(NOECs confirmed by the authors).*	Dobbins *et al.*, 2009	2 (Test duration for determining NOEC was 7 days instead of 21 days as recommended by OECD TG 210)
According to OECD TG 203 *Oryzias latipes* 10 d old, n= 10 5 test conc. (not specified), blank control, 3 replicates Static renewal (renewal every 24 h of half of the solution)	96h LC50= 4.9 mg/L (meas.), IC95 range: 3600 – 6700	Yamamoto *et al.*, 2011	3 (According to an OECD guideline, test conditions well described, but no dose-response curves given, no analytical monitoring reported)

GLP: not specified			
According to OECD TG 236 (Fish embryo Acute Toxicity (FET) test) Freshly fertilised eggs of zebrafish (*Danio rerio*) Test conc.: solvent control, 0, 10, 100, 1000, 3500, 6000, 8500, 10 000 µg/L, 8 replicates/treatment Test duration: until 80 hpf Water and solvent control were grouped GLP: not specified	Zebrafish: - Sign. mortality at highest conc. tested (100 %, p<0.05) compared to control (3.13 %); no LC50 determined. - Sign. difference at 80 hpf in hatching rate (dose-dependent) at 6000 (18.75 %, p<0.05), 8500 (2.5 %, p<0.05) and 10000 (0.0 %, p<0.05) µg/L compared to control (92.19 %). - Sign. difference in total abnormalities at 3500 (100** %), 6000 (100** %) and 8500 (100** %) µg/L (p<0.05).	Torres *et al.*, 2016	2 (According to an OECD guideline, but considered supportive as some exposure conditions are not described, no data reported on analytical monitoring)
Invertebrates			
ISO 6341 *Daphnia magna* Test conc.: control, 0.5, 1, 2, 5, 10, 20, 50 and 100 mg/L, control: 6 replicates, test conc.: 4 replicates Test type: no data GLP: not specified	48h EC50= 15.4 mg/L (no data on nom./meas.)	REACH registration dossier: peer review in Danish EPA report, 2001	2
Non-standard assay Exposure: Larvae of water flea, *Daphnia magna,* exposure to 7 parabens for 48 h and 10 days Test conc.: Acute toxicity: 5 test conc., 4 replicates Subchronic toxicity: 5 test conc., highest test conc. is half of LC50, dilution factor of 0.5, 10 replicates. Effect measurement: Acute toxicity: mortality (LC50) Subchronic toxicity:	Acute toxicity: Benzylparaben (BZPB)< Butylparaben (BPB) < Isopropylparaben (IBPB) < LC50 waterflea 12.3 mg/L Propylparaben (PPB) < IPPB < Ethylparaben (EPB) < Methylparaben (MPB) Subchronic toxicity: growth LOEC values for both species were related to log P value, range for 7 parabens for waterflea between 0.1 and 9 mg/L LOEC of PPB for waterflea growth was 0.4 mg/L, NOEC <0.375 mg/L *(NOECs confirmed by the authors).* Waterflea **reproduction** with LOEC between 1.5 mg/L for MPB and 6.0 mg/L for PPB (NOEC= 3 mg/L)	Dobbins *et al.*, 2009	2 (Test duration for determining NOEC was 10 days instead of 21 days as recommended by OECD TG 211)

growth and reproduction in waterflea GLP: not specified			
According to OECD TG 202 *Daphnia magna* <24 h old, n= 20, 5 neonates/beaker 5 test conc. (not specified) Static GLP: not specified	**48h EC50= 2 mg/L** (meas.), IC95 Range: 770 - 2900	Yamamoto *et al*., 2011	3 (According to an OECD guideline, test conditions well described, but no dose-response curves given, no analytical monitoring reported)
Non-standard assay *Ceriodaphnia dubia* 24 h old Test conc.: 0, 0.63, 1.25, 2.50, 5.00 and 10 mg/L (nom), replicates/treatment Semi-static Non-GLP Test duration: 6 to 7 d	Survival: EC50= 1.3 mg/L NOEC: not obtained Number of offspring: EC50= 2.5 mg/L NOEC: not obtained Time to first brood: EC50= 5.7 mg/L NOEC: not obtained	Terasaki *et al*., 2015	1 Well documented non-guideline study, with analytical analysis of test concentrations
Fertilised eggs of sea urchin (*Paracentrotus lividus*), max. 30 min pf Test conc.: solvent control, 0, 10, 64, 100, 160, 400, 1000, 10000 µg/L, 8 replicates/treatment Test duration: 48 h Water and solvent control were grouped GLP: not specified	Sign. effect on total abnormalities (increase) and larval length at 400**, 1000** and 10000** µg/L ($p<0.05$).	Torres *et al*., 2016	2 Well documented non-guideline study, but no analytical monitoring of test concentrations reported
Algae			
OECD TG 201 *Pseudokirchnerella subcapitata* Test conc.: 2.1, 4.6, 10, 21 and 46 mg/L, control: 6 replicates, 3 replicates/test conc. Static GLP	72h ErC50= 16 mg/L (nom.)	REACH registration dossier: Unpublished study report, 2012	1

ISO 8692 *Pseudokirchnerella subcapitata* Test conc. and test type: no data GLP: not specified	72h EC50= 15 mg/L (no data on nom./meas.)	REACH registration dossier: Madsen *et al*., 2001 (peer review in Danish EPS report)	2
According to OECD TG 201 *Pseudokirchneriella subcapitata* 5 test conc. (not specified), 3 replicates. GLP: not specified	72h EC50= 36 mg/L (meas.), IC95 range could not be determined.	Yamamoto *et al.*, 2011	3 (According to an OECD guideline, test conditions well described, test concentrations not specified, and no dose-response curves given, no analytical monitoring reported)

Table 20: Overview aquatic chronic toxicity

Species/test method	Result	Study reference	Reliability
Fish			
OECD TG 234 (FSDT) *Danio rerio* Test conc.: 0, 7.66, 25.4, 59.9, 165 and 518 µg/L (mm), 4 replicates Flow through GLP: yes	70 dpf NOEC= 0.165 mg/L (mean meas., sex ratio)	REACH registration dossier: study report, 2020 Deviation from OECD TG 234: - Test duration prolonged until 70 dpf due to the size of the fish at 63 dpf not guaranteeing a proper sex determination.	1
Invertebrates			
OECD TG 211 *Daphnia magna* Test conc.: control, 0.07, 0.25, 0.80, 3.10 and 10.8 mg/L (Time weighted Mean concentration (TWM), 10 replicates/treatment Semi-static GLP	21d NOEC= 0.07 mg/L (time weighted mean concentration (TWM), length) 21d NOEC= 0.25 mg/L (TWM) (repro, mortality, age of first brood, developmental rate, intrinsic rate) 21d EC10= **0.16 mg/L** (TWM, repro of introduced parents) 21d EC10= 0.22 mg/L (TWM, repro survival parents) 21d LC10= 0.44 mg/L (TWM,	REACH registration dossier: Unpublished study report, 2019	1

	mortality) 21d EC10= 0.69 mg/L (TWM, length) 21d EC10= 0.58 mg/L (TWM, developmental rate) 21d EC10= 0.20 mg/L (TWM, intrinsic rate)		
Algae			
OECD TG 201 *Pseudokirchnerella subcapitata* Test conc.: 2.1, 4.6, 10, 21 and 46 mg/L, control: 6 replicates, 3 replicates/test conc. Static GLP	72h NOErC= 2.1 mg/L (nom.)	REACH registration dossier: Unpublished study report, 2012	1
ISO 8692 *Pseudokirchnerella subcapitata* Test conc. And test type: no data GLP: not specified	72h NOEC= 5 mg/L (no data on nom./meas.)	REACH registration dossier: peer review in Danish EPS report, 2001	2
According to OECD TG 201 *Pseudokirchneriella subcapitata* 5 test conc. (not specified), 3 replicates GLP: not specified	72h NOEC= 7,4 mg/L (meas.)	Yamamoto *et al.*, 2011	3 (According to an OECD guideline, test conditions well described, test concentration not specified, and no dose-response curves given, no analytical monitoring reported)

7.8.1.1 Fish

A 96h LC50 of 6.4 mg/L was reported for zebrafish in an unpublished study of 2012 (REACH registration dossier). Although no LC50 was determined by Torres *et al*. (2016) high mortality (100 %) was seen after exposure to 10 mg/L of propylparaben.

A **Fish Sexual Development Test** (FSDT) was performed according to OECD TG 234 as requested in the SEv decision. A 70 dpf **NOEC** of **0.165 mg/L** (mm) was determined for sex ratio.

For detailed information see chapter 7.10.2 Endocrine disruption – Environmental data

7.8.1.2 Invertebrates

In the acute toxicity study of Yamamoto *et al*. (2011) following median effect concentrations were measured: 96h LC50= 4.9 mg/L (meas.) for fish, 48h EC50= 2 mg/L (meas.) for invertebrates and 72h EC50= 36 mg/L (meas.) for algae.

The study of Dobbins *et al*. (2009) gives information both about acute (LC50= 9.7 mg/L for fish and 12.3 mg/L for Daphnia) and subchronic toxicity (LOEC= 2.5 mg/L for fish and 0.4 mg/L for Daphnia) of propylparaben. Subchronic data show that invertebrates are more sensitive than fish.

Instead of 21 days as for chronic toxicity, fish were exposed for 7 days to propylparaben, while *D. magna* was exposed for 10 days. Only LOECs are reported in this study, but authors confirmed NOECs for growth of 1.25 mg/L for fish and <0.375 mg/L for Daphnia, and NOECreproduction for Daphnia of 3 mg/L.

The exposure duration of 10 days is not long enough to conclude on sexually maturation in *D. magna* as they start to release neonates from their brood chamber at day 8-10, followed by a release of a second brood at day 10-12, a third by day 12-14 and a fourth by day 14-17. Therefore a *D. magna* reproduction study was requested in the SEv decision.

In an ISO 6341 study (peer reviewed in the Danish EPA report of 2001) a 48h EC50 of 15.4 mg/L was reported for *D. Magna*, however Yamamoto *et al*. (2011) measured a lower LC50 (2 mg/L) in a study performed according to OECD TG 202.

In the non-guideline literature study of Terasaki *et al*. (2015) *Ceriodaphnia dubia* 24 h old neonates were exposed to nominal concentrations of 0.63, 1.25, 2.50, 5.00 and 10 mg/L until day 6 or 7 (depending on the time to produce a third brood). The lowest determined EC50 was for survival: 1.3 mg/L. NOECs were not obtained.

Table 21: Parental survival, number of offspring and time to first brood in *C. dubia* after exposure to propylparaben (Terasaki *et al*., 2015)

Concentration (mg/L)		Parental survival (%)	Number of offspring	Time to first brood
Nom.	Meas.			
0	0	100	28.3	5.16
0.63	0.59	50	17.1****	4.64**
1.25	1.18	60	21.8	4.77
2.50	2.60	70	21.4	4.91
5.00	4.96	0	4.0****	4.33**
10.0	11.0	0	No viable offspring	-

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

Table 22: EC50 and NOEC for *C. dubia* after exposure to propylparaben (Terasaki *et al*., 2015)

Endpoint	EC50 (mg/L)	NOEC (mg/L)
Survival	1.3	Not obtained
Number of offspring	2.5	Not obtained
Time to first brood	5.7	Not obtained

Torres *et al*. (2016) also investigated the impact of propylparaben exposure on the development of freshly fertilised eggs of the sea urchin. This resulted in a statistically significant increase in total abnormalities and decrease in larval length at ≥400 µg/L ($p<0.05$).

In 2019, a chronic (21 d) *Daphnia magna* Reproduction Study was performed according to OECD TG 211 in which Daphnids were exposed to nominal concentration of 0, 0.10, 0.32, 1.00, 3.16 and 10.0 mg/L [TWM 0.07, 0.25, 0.80, 3.10 and 10.8 mg/L (66.3 – 108 % of nom. conc.)].

For reproduction, mortality, age of first brood, developmental rate, and intrinsic rate a NOEC of 0.25 mg/L (time weighted mean) was determined. For length a NOEC of 0.07 mg/L (TWM) was calculated (statistically significant effect at 0.25 mg/L with 9.1 % decrease in length).

The lowest calculated EC10 was for reproduction of introduced parents (survived and death): 0.16 mg/L (TWM).

Table 23: NOEC and EC10 for *D. magna* after 21 d exposure to propylparaben (OECD TG 211)

Endpoint	NOEC	EC10
Length	0.25 mg/L was assigned by the registrant. However, stat. sign. inhibition was observed at 0.25 mg/L (9.1 % decrease). Therefore, eMSCA prefers a NOEC of 0.07 mg/L	0.69 mg/L
Reproduction (fecundity/fertility)	0.25 mg/L (per introduced parent: 17.2 % decrease, per survived parent 8 %, but not stat. sign)	**0.16 mg/L** (per introduced parent) 0.22 mg/L (per survived parent)
Development	0.25 mg/L	0.58 mg/L
Immobility	0.25 mg/L	0.44 mg/L
Age at first reproduction	0.25 mg/L	/

7.8.1.3 Algae

The studies with the **algae** *Pseudokirchnerella subcapitata* performed according to OECD TG 201 and ISO 8692 reported an 72h NOErC of 2.1 and 5 mg/L resp.

7.8.1.4 Sediment organisms

No data available.

Conclusion on the aquatic compartment:

- Acute toxicity: the most sensitive species is *Ceriodaphnia dubia* with a 7d EC50 of 1.3 mg/L.

- Chronic toxicity: Fish and invertebrates are equally sensitive with a 70d NOEC of 0.165 mg/L (sex ratio) for fish (*Danio rerio*) and a 21d EC10 of 0.16 mg/L (reproduction) for invertebrates (*Daphnia magna*).

7.8.2 Terrestrial compartment

No data are available in the REACH registration dossier. However, in a non-guideline literature study (García-Espiñeira *et al*., 2018), the toxic effects (growth and fertility) and gene expression (heat shock, antioxidant enzymes, biotransformation enzymes and transcription factor) of BPA, propylparaben and triclosan on *Caenorhabditis elegans* were examined. Nematodes were exposed to 0.05, 0.5, 5, 50, 500, 5000 and 50000 µM propylparaben. Stat. sign. mortality was seen from 0.5 µM and increased in a concentration-dependent manner. The 24h LC50 was determined to be 261.7 µM.

After 48 h, width and width-length ratio were sign. increased at all concentrations ($p<0.05$), while body length stat. sign. decreased at 0.05**, 0.5**, 5** and 50** µM.

Until 0.5 µM propylparaben increased the brood size (only stat. sign. at 0.05** µM), followed by a concentration-dependent decrease (only stat. sign. at 5** µM).

Propylparaben caused upregulation of mRNA expression of heat shock (hsp-4, hsp-16.2), antioxidant enzymes (sod-1 and sod-4), biotransformation (CYP-34A9) and transcription factor (skn-1) genes. Upregulation was found to be the highest for sod-4 after exposure to

500 µM (5-fold). Transcription of genes encoding hsp-3 and hsp-70 (heat shock) and CYP-29A2 (biotransformation enzymes) changed the least (<2-fold).

Kim *et al.* (2020) investigated the effect of propylparaben on plants, earthworm, Collembola, soil nematodes and soil algae. In the soil nematode assay performed according to the procedure of Kim *et al*. (2018), *Caenorhabditis elegans* was exposed to 100, 200, 300, 400, 500 mg/kg soil (meas. conc. resp. 97, 193, 290, 386, 483 mg/kg soil) for 1 day. A sign. reduction in reproduction rate (number off offspring) was observed at 300**, 400** and 500** mg/kg dry soil.

The acute and chronic study on plants was performed according to OECD TG 208 with modifications. Seeds from cherry tomato (*Lycopersicon esculentum*), mung bean (*Vigna radiata*), barley (*Hordeum vulgare*) and rice (*Oryza sativa*) were exposed to propylparaben for resp. 14 and 21 days and impact on shoot growth was measured. Results were compared to a solvent control (50 mL of acetone/kg dry soil).

Table 24: Results of the plant assay: shoot growth inhibition

Species	Test conc. (nom. – mg/kg soil)	Test conc. (meas. – mg/kg soil)	14d EC50 (mg/kg soil)	21d NOEC/EC10 (mg/kg soil)
Lycopersicon esculentum	50, 100, 200, 300, 400	46, 93, 185, 278, 370	>370	278/185
Vigna radiata	100, 200, 300, 400, 500	97, 193, 290, 386, 483	386	193/204
Hordeum vulgare	50, 100, 200, 300, 400	46, 93, 185, 278, 370	222	185/NC
Oryza sativa	100, 200, 300, 400, 500	97, 193, 290, 386, 483	228	193/NC

NC= not calculated

In the study of Nagar *et al*. (2020), the soil nematode *Caenorhabditis elegans* was exposed to a solvent control, 0, 36.04, 72.08, 144.16, 180.2 and 216.24 mg/L propylparaben. Results were compared to the solvent control (0.1 % DMSO, except for mortality where 0.8 % DMSO was used).

A 72h LC50 of 169.2 µg/mL was determined. Further testing of the endpoints was therefore performed with 1/50th (3.38 mg/L) and 1/5th of the LC50 (33.81 mg/L) of propylparaben.

The mean recovery of propylparaben in spiked samples of *C. elegans* detected through UHPLC were between 99.08 and 111.44 %. When nematodes were exposed to 1/5th of the LC50 for 72h, 8.367 % (2.83 % mg/L) propylparaben was recovered.

Mean length significantly decreased with 13.2 % after exposure to 1/5th of the LC50 ($p<0.05$). The average progeny significantly decreased at 3.38**** (with 11.7 %) ($p<0.01$) and 33.8***** mg/L (with 28.19 %) ($p<0.001$).

Head trash, the change in the direction of bending the mid-body, was significantly affected at 3.38 ***** (reduced with 16.326 %) and 33.8***** mg/L (7 - 8 %).

Feeding ability of the nematodes were not affected after treatment with 1/50th and 1/5th of the LC50 of propylparaben.

Exposure to propylparaben significantly downregulated the expression of vitellogenin genes *vit-2* and *vit-6*. *Daf-16*, however, was not affected.

2.5-fold increase of ROS generation was observed when exposed to 1/5th of the LC50 ($p<0.01$) together with a significant upregulation of the expression of oxidative stress response genes sod-3, ctl-2, hsf-1, skn-1, gcs-1, gst-4, hsp-16.2 and hsp-70.

Exposure to 1/50th and 1/5th of LC50 of propylparaben did not provoke toxicity in *Escherichia coli*, indicating that the effects seen in *C. elegans* are not attributable to a depletion of bacteria.

In the earthworm assay performed according to Kwak *et al*. (2014), *Eisenia andrei* adults were exposed for 14 days to nominal test concentration ranging from 0 - 500 mg/kg dry soil and impact on normal functioning and mortality were measured (50 mL of acetone/kg dry soil).

Table 25: Results of the earthworm assay with *Eisenia andrei*

Test conc. (nom. – mg/kg soil)	Test conc. (meas. – mg/kg soil)	Adverse effect	Result (after 14 d of exposure)
100, 200, 300, 400, 500	97, 193, 290, 386, 483	Survival rate	>80 % survival at all conc. tested
		Normal functioning	EC50 >483 mg/kg dry weight

The collembola assay was performed with *Folsomia candida* (according to OECD TG 232) and *Lobella sokamensis* (according to An *et al*., 2013) (Kim *et al*., 2020). Both species were exposed to a range of 0 - 500 mg/kg propylparaben and results were compared to the solvent control (50 mL acetone/kg soil).

Table 26: Results of the Collembola assay

	Test conc. (nom. – mg/kg soil)	Test conc. (meas. – mg/kg soil)	Survival rate (after 14 d of exposure)	Reproduction rate (after 28 days of exposure)
Folsomia candida (9-12 d old)	100, 200, 300, 400, 500	97, 193, 290, 386, 483	Sign. decrease at 386** and 483** mg/kg soil	Conc.-dependent decrease, sign. at 300**, 400** and 500** mg/kg soil
Lobella sokamensis (15-20 d old)	50, 100, 200, 300, 400	55, 109, 218, 327, 436	Conc.-dependent decrease, sign. at 218**, 327** and 436** mg/kg soil	ND

The soil algae assay, performed according to Nam and An (2015 and 2016), examined the growth inhibition of 3 algae species. Results were compared to the solvent control (50 mL acetone/kg dry soil) (Kim *et al*., 2020).

Table 27: Results of the algae assay

	Test conc. (nom. – mg/kg soil)	Test conc. (meas. – mg/kg soil)	Growth inhibition (after 6 d of exposure)
Chlamydomonas reinhardtii	10, 20, 30, 35, 40, 50	6, 13, 19, 23, 26, 32	Sign. increased at 6** and 13** mg/kg soil while sign. decreased at 23**, 26** and 32** mg/kg soil in a conc.-dependent manner.
Chlorococcum infusionum	10, 15, 20, 25, 30, 40	6, 9, 12, 15, 18, 23	Sign. decreased at 15**, 18** and 23** mg/kg soil in a conc.-dependent manner.

Chlorella sorokiniana	25, 50, 100, 150, 200, 300, 400	16, 32, 64, 97, 129, 193, 258	Conc.-dependent decrease (all conc.), sign. decreased at 64**, 97**, 129**, 193* and 258** mg/kg soil.

Samarasinghe *et al*. (2021) performed an acute toxicity guideline study (according to OECD TG 207) and a reproduction guideline study (according to OECD TG 222) with adult *Eisenia fetida*. In the acute study earthworms were exposed to 0.1, 1, 10, 20, 50 and 100 mg/L while in the chronic study to 0, 0.1, 1, 10, 100 and 1000 mg/kg propylparaben. Acetone was used as solvent in both assays.

Measured concentrations in the spiked soils were within 20 % of nominal concentrations at the beginning of the study. However, after 28 days no propylparaben was found in spiked soils at 0.1, 1 and 10 mg/kg and very low concentrations at 100 and 1000 mg/kg (≤ 1 - 2 %).

No mortality was observed neither in the acute nor in the chronic study. Furthermore, no effect on weight and reproduction was observed after 28 days of exposure to propylparaben.

Conclusion on the terrestrial compartment:

Depending on the species tested, propylparaben caused mortality, reduced the number of offspring, increased growth inhibition and impacted expression of heat shock (hsp-4, hsp-16.2), antioxidant enzymes (sod-1 and sod-4), biotransformation (CYP-34A9), transcription factor (skn-1) and vitellogenin vit-2 and vit-6 genes.

7.8.3 Microbiological activity in sewage treatment systems

Table 28: Overview microbiological activity in sewage treatment systems

Species/test method	Result	Study reference
Microorganisms		
OECD TG 301 F (Ready Biodegradability: Manometric Respirometry Test) Static	28d NOEC= ≥20 mg/L (nom.)	REACH registration dossier: Unpublished study report, 2001

Since no studies on the toxicity to aquatic microorganisms are available, the ready biodegradability study is used to derive a NOEC for the toxicity to aquatic microorganisms: within 28 days, the substance attained 91.5 % degradation based on O_2 consumption.

Conclusion microbiological activity:

The NOEC for toxicity to aquatic microorganisms is stated as ≥ 20 mg/L.

7.8.4 PNEC derivation and other hazard conclusions

Not assessed.

7.8.5 Conclusions for classification and labelling

Environmental classification:

- Propylparaben is rapidly biodegradable: 91.5 % degradation in a Ready Biodegradability Study (OECD TG 301 F - Manometric Respirometry).

- The substance does not meet the classification criteria for acute aquatic toxicity. All LC50 >1 mg/L (6.4 mg/L for fish, the most sensitive species).

- In the Fish Sexual Development Test a 70 dpf NOEC of 0.165 mg/L was reported for sex ratio. In the *Daphnia magna* Reproduction Study, a 21d EC10 of 0.16 mg/L (repro of introduced parents) was determined. The lowest NOEC for algae (*Psedokirchnerella subcapitata*) was 2.1 mg/L (nom.).

Based on the lowest NOEC/EC10 for the three trophic levels and the fact that the substance is rapidly degradable, classification as Aquatic Chronic 3, H412 is warranted for propylparaben.

7.9 Human Health hazard assessment

7.9.1 Toxicokinetics

Table 29: Toxicokinetics

Test method	Conclusion	Study reference	Reliability
Metabolism			
In vitro / Ex vivo			
Investigation of enzymatic hydrolysis (parabens) Extracts from Differing Layers of Human Skin (abdominal cutis, subcutaneous fat tissue and stratum basal/stratum spinosum), total blood and extract from a human keratinocyte cell line HaCaT-cells Dose: 0.5 mM	Hydrolysis of the test substance was observed in all investigated extracts.	REACH registration dossier: Publication: Lobemeier *et al.*, 1996	2
In vivo			
n-propyl 4-hydroxybenzoate-2,3,5,6-d4 (PP-d4, 98.8 %) and p-hydroxybenzoic-2,3,5,6-d4 (pHBA-d4, 99 %) 12 healthy adult	PPB is rapidly absorbed (within 2 h) and systemically distributed in humans after oral ingestion. PPB is likely to be metabolized prior to excretion, and PPB is rarely present in its free form in the human body.	REACH registration dossier: Publication: Shin *et al.*, 2019	4

volunteers Dose: 0.6 or 2.5 mg/kg bw	Elimination: quickly (terminal half-life= 2.9 h).		
Metabolism of ethyl and n-propyl-p-hydroxybenzoate Cat (4/sex/dose) Oral: feed Dose: 168 mg/kg bw (single application) Urine was collected at 24, 48 and 72 h intervals; total faeces were collected over 72 h	Absorption: rapid (± 86.7 % of the test substance excreted within 24 h via urine). Metabolism: after 24 h, 2 metabolites were identified in urine (p-hydroxyhippuric acid (54 - 69 % of the radioactivity) and p-hydroxybenzoic acid (31 - 46 %). Excretion: rapid via urine (95.6 % excreted within 72 h)	REACH registration dossier: Publication: Phillips *et al*., 1978	2
p-hydroxybenzoic acid and its esters Rat Wistar Oral: gavage Dose: ± 500 mg/kg bw Observation period: 6 h	Metabolism: metabolites: p-hydroxybenzoic acid, p-hydroxyhippuric acid, conjugates with glucuronic acid, ethereal sulphate, and an unidentified metabolite. Blood concentration of p-hydroxybenzoic acid reaches a maximum after 1 h exposure. Excretion: rapid via urine.	REACH registration dossier: Publication: Derache and Gourdon, 1963	2
Toxicokinetic study of [14C]-(methyl-), propyl- (and butyl)paraben SD rats (male and female) mean BW 231 g for males, 168 g for females (age?) Oral, dermal (SC) Single dose: 100 mg/kg Duration of exposure: 6 h	<u>Oral administration:</u> Elimination of PPB in urine and faeces accounted for 84 % in males and 71 % in females. Excretion in faeces accounted for 1 - 3 %. <u>Dermal administration:</u> Elimination of PPB in urine and faeces accounted for 17 % in males and 20 % in females. Excretion in faeces accounted for 1 - 2 %. 58/47 % (M/F) of the dose remained unabsorbed. 21/37 % (M/F) was retained in the remaining carcass.	REACH registration dossier: Publication: Aubert *et al*., 2012	Reliability 1 (study of very good quality, even if not GLP)

	(See Table 30)		
Propyl-p-hydroxybenzoate Rat Wistar Oral: gavage Doses: 3, 10, 100 and 1000 mg/kg	**Preliminary pharmacokinetic study:** At PND 31: - PPB is rapidly absorbed (Tmax between 5 and 30 min). - Plasma PPB conc.: the increase in systemic exposure between 3 and 1000 mg/kg was less than dose proportional. - No PPB was detected beyond 4 h after dosing, except at 1000 mg/kg. - PPB is eliminated quite rapidly (half-lives: 47 min (10 mg/kg) and 58 min (100 mg/kg)). **Satellite toxicokinetics study:** PND 21 -> PND 77 Total PPB were sign. lower on PND 77 (dosing day 56) compared with PND 21 (dosing day 1).	REACH registration dossier: Publication: Gazin *et al*., 2013	4 (for the registrant as only abstract available) Based on the literature study, eMSCA support a reliability of 2.
Propyl-p-hydroxybenzoate Dogs (3) Oral: capsule Dose: 1000 mg/kg bw	Metabolism: the major part of the test substance was recovered in urine as free p-hydroxybenzoic acid (12.7 %) and as conjugate with glucuronic acid (16.5 %). Excretion: 57.6 % of the test substance was recovered in urine after 48 h.	REACH registration dossier: Publication: Jones *et al*., 1956	2
Propyl-p-hydroxybenzoate Dogs (3) IV Dose: 50 mg/kg bw	Metabolism: the major part of the test substance was recovered in urine as free p-hydroxybenzoic acid (26 %) and as conjugate with glucuronic acid (22.6 %). Excretion: 94 % of the test substance was recovered in urine after 48 h.	REACH registration dossier: Publication: Jones *et al*., 1956	2

Table 30: Recovery of radioactivity (% of administrated dose) in groups treated orally and dermally with 100 mg/kg [14C*-labelled propylparaben (Aubert *et al*., 2012)

Admin. route	Sex	Urine	Faeces	Cage wash	Swabs	Stripping/biopsies	Tissues	Carcass	Total recovery
Oral	M	83.7 ± 9.0	1.22 ± 0.65	7.52 ± 5.24	NA	NA	0.08 ± 0.07	BLQ	92.5 ± 4.1
Oral	F	71.1 ± 9.9	3.30 ± 2.17	14.0 ± 6.9	NA	NA	0.11 ± 0.06	BLQ	88.7 ± 3.6
Dermal	M	17.4 ± 5.2	1.02 ± 0.39	6.58 ± 3.84	57.8 ± 8.9	0.04 ± 0.02	0.57 ± 0.19	20.7 ± 4.7	104.0 ± 1.0
Dermal	F	20.4 ± 8.3	1.66 ± 0.79	9.14 ± 0.79	47.0 ± 10.2	0.06 ± 0.02	0.72 ± 0.05	37.4 ± 4.3	116.0 ± 6.0

NA: not applicable; BLQ: below limit of quantification.

In the registration dossier, 4 other studies provided information about permeability which were not consider further by the eMSCA for the evaluation of the dermal absorption.

<u>Additional information</u>:

Human data:

In humans, the parent, free and predominantly conjugated (glucuronides and sulphate esters) parabens have been detected in human serum or urine, in biomonitoring studies (reviewed in SCCS/1446/11, Annex 4; Buttke *et al*., 2012) and in experimental human studies after dermal application (Janjua *et al*., 2007 and 2008). Moreover, it has been detected in human milk samples in free unconjugated form (Schlumph *et al*., 2010). So in humans, parent (un-metabolised) parabens become systematically available, even if in limited amounts.

Different expression of metabolic enzymes between adults and children:

In the first post-natal months (neonates/newborns and infants) immaturity of drug metabolising enzymes involved in the metabolism of parabens in human (carboxylesterases, UDP-glucuronosyltransferases, and sulfotransferases) may influence the level of free, unconjugated parabens.

The UDP-glucuronosyltransferase enzyme family is not fully developed until the age of 6 months and data suggest reduced carboxylesterase expression in children below 1 year (SCCS/1446/11).

Consistent with a reduced metabolic capacity in very young children, 3- to 5-fold higher proportions of free methyl- and propylparaben were found in urine samples of hospitalized preterm neonates/newborns (about 10 - 15 % of the total (free and conjugated) fraction) compared to 2 - 5 % in adults (Calafat *et al*., 2009).

Comparison of the rat and human data

In its opinion on Parabens (propyl- and butylparaben) (SCCS/1514/13), the SCCS highlighted differences between rats and humans for the toxicokinetics of parabens. In conclusion, the metabolism of propylparaben in rats seems to be faster than in humans even if uncertainties remain.

<u>Conclusion on ADME of Propylparaben</u>:

<u>Absorption</u>: Propylparaben is rapidly absorbed systemically after oral administration and partially absorbed after dermal uptake.

Distribution: The available data support distribution of propylparaben with data from dermal exposure suggesting a potential of the skin to sequester the substance (carcass).

Metabolism: After oral uptake, propylparaben was rapidly metabolized to p-hydroxybenzoic acid, p-hydroxihippuric acid and conjugates with glucuronic acid. After dermal application, p-hydroxybenzoic acid was detected as metabolite. The differences in propylparaben systemic concentration between PND 21 and PND 77 may be attributable to an increase in carboxylesterase activity at PND 77 compared to PND 21.

Excretion: The data indicate a rapid elimination of the test substance and its metabolites in different species. The main route of excretion is via urine.

7.9.2 Acute toxicity and Corrosion/Irritation

7.9.2.1 Acute toxicity:

By oral route

Table 31: Acute toxicity studies

Test method	Results	Study reference	Reliability
OECD TG 401 (Acute Oral Toxicity Study) Rat (Wistar): 5/sex/dose By gavage: 5000 mg/kg bw Observation period: 14 d Not GLP	No mortality and no clinical signs observed. Necropsy: no substance related findings LD50: >5000 mg/kg bw	REACH registration dossier: Unpublished study report, 1982	2 (necropsy not performed in all surviving animals, not GLP, no analytical purity)
OECD TG 401 (Acute Oral Toxicity Study) Rat (albino): 5 females/dose By gavage: 15000 mg/kg bw Observation period: 7 d Not GLP	No mortality and no clinical signs observed. Necropsy: no substance related findings LD50: >15000 mg/kg bw	REACH registration dossier: Unpublished study summary, 1977	2 (observation period of 7 d, no data on bw during observation period)
OECD TG 401 (Acute Oral Toxicity Study) Mouse (albino) By gavage: up to 8000 mg/kg bw	LD50: >8000 mg/kg bw	REACH registration dossier: Publication: Matthews *et al*., 1956	2 for REG (basic data given) eMSCA support a reliability of 3 (no data available in the registration dossier only the LD50)

Observation period: 7 d Not GLP			
No guideline followed Rabbit Oral	LD50: 6000 mg/kg bw	REACH registration dossier: Publication: Sabalitschaka and Neufled, 1954	4
No guideline followed Dog Oral	LD50: 6000 mg/kg bw	REACH registration dossier: Publication: Sabalitschaka and Neufled, 1954	4

The registrant concludes that propylparaben is not acutely toxic via the oral route (LD50 >5000 mg/kg bw), and based on the available information, the eMSCA can support this conclusion.

7.9.2.2 Irritation/Corrosion:

Skin irritation

In the REACH registration dossier, different studies are provided for this endpoint:

- One study with propylparaben (with a reliability 4).

- 2 other studies with methyl- and ethylparaben.

Table 32: Skin irritation studies

Test method	Results	Study reference	Reliability
OECD TG 404 (Acute Dermal Irritation/Corrosion) Rabbit Unspecified information: coverage, vehicle, nb of animals, duration of observation Conc.: 10 % Duration of treatment: 48 h Not GLP	No noticeable irritation.	REACH registration dossier: Publication: Lehman *et al*., 1949	4 (limited data given)
OECD TG 404 (Acute Dermal Irritation/Corrosion)	Erythema: mean score (24, 48 and 72 h): 0/4	REACH registration dossier: Unpublished	2 Read across performed by

Rabbit (HC:NZW): 3 males Semi-occlusive 0.5 g of test substance Duration of treatment: 4 h Observation period: 7 d (reading time: 1 h, 24 h, 48 h, 72 h, 7 d) No GLP Test material: **EC n° 204-399-4 (ethylparaben)**	Edema: mean score (24, 48 and 72 h): 0/4 Not irritating	study report, 1983	REG **READ ACROSS**
No guideline followed Rabbit (albino): 9 females Occlusive: 0.1 mL, conc. Test 100 % Primary skin irritation test design (= modification of the Draize test technique and is similar to the technique used in grading skin irritation in the Human patch test) Duration of treatment: 24 h Observation period: 24 and 72 h Not GLP Test material: **EC n° 202-785-7 (methylparaben)**	Primary dermal irritation index (PDII) of test sample (mean 24/72 h): 0.67/4 (vs 0.44/4 in control group)	REACH registration dossier: Unpublished study report, 1976	2 (not according to OECD guideline) **READ ACROSS**

Eye irritation

Table 33: Eye irritation studies

Test method	Results	Study reference	Reliability
OECD TG 405 (Acute Eye Irritation/Corrosion) Rabbit (NZW) (3 animals) 0.1 g/animal applied Observation period: 1 h, 24 h, 48 h, 72 h and 7 d GLP	Corneal opacity: mean score over 24, 48 and 72 h: 0/4 for all animals. Iritis: mean score over 24, 48 and 72 h: 0/2 for all animals. Conjunctival redness: mean score over 24, 48 and 72 h: score/max. score: 1/3, 2/3 and 1.67/3 resp. for the 3	REACH registration dossier: Unpublished study report, 2012	1

	animals (fully reversible within 7 d). Chemosis: mean score over 24, 48 and 72 h: 0/4 for all animals.		
In vitro OECD TG 437 (Bovine Corneal Opacity and Permeability test Method) 0.75 mL applied to 3 bovine corneas 20 % suspension of test item Exposure period: 240 min GLP	*In vitro* irritation score: 13.03 (slight increase of the corneal opacity) Neg. control: *in vitro* irritation score: 1.92 Pos. control: *in vitro* irritation score: 186.99	REACH registration dossier: Unpublished study report, 2012	1

The registrant concludes that the substance is not eye irritating, and based on the available information, the eMSCA can support this conclusion.

7.9.3 Sensitisation

Skin sensitisation:

Table 34: Skin sensitisation studies

Test method	Results	Study reference	Reliability
OECD TG 429 Mouse (4/dose) Mouse Local Lymph Node Assay (LLNA) Conc.: 5, 10 and 25 %	SI: 1.3, 1.6 and 1.3 resp. at 5, 10 and 25 % For pos. control group: SI of 4.5 and 4.6 resp. at 10 and 25 % Not sensitising	REACH registration dossier: Publication: Basketter and Scholes, 1992	2
OECD TG 406 Guinea Pig Maximisation Test (GPMT) Guinea pig (Dunkin-Hartley) Induction: ID (0.5 % test substance in physiological saline) and epicutaneous (25 % test substance in acetone/polyethylene glycol) Challenge: epicutaneous, occlusive: 10 % test substance in	80 % reaction in pos. control group 0 % of pos. reaction in the test group Not sensitising	REACH registration dossier: Publication: Basketter and Scholes, 1992	2 (basic data given)

acetone/polyethylene glycol			
OECD TG 429 Mouse (4/dose) LLNA Conc.: 5, 10 and 25 %	SI: 1.4, 1.0 and 1.3 resp. at 5, 10 and 25 % of test substance Pos. control: SI of 4.5 and 4.6 % resp. at 10 and 25 % Not sensitising	REACH registration dossier: Publication: Basketter *et al*., 1994	2
OECD TG 429 Mouse (4/dose) LLNA Conc. 5, 10 and 25 %	SI at 5, 10 and 25 %: Laboratory A: 1.3, 1.3 and 1.3 Laboratory B: 1.9, 2.2 and 1.3 Laboratory C: 1.0, 1.2 and 1.5 Laboratory D: 1.2, 0.5 and 2.0 Not sensitising	REACH registration dossier: Publication: Basketter *et al*., 1991	2 (basic data given)
OECD TG 406 (Skin Sensitisation) Guinea pig (23/dose) Draize test Conc. 3% Induction: ID Challenge: ID and epicutaneous Not GLP	Pos. reactions in 0/23 animals Not sensitising	REACH registration dossier: Publication: Marzulli *et al*., 1968	2 (basic data given)
Similar to OECD TG 406 (Skin Sensitisation adopted in 1981) (no epicutaneous challenge) Guinea pig Induction and challenge: ID 0.1 % Similar to Maurer optimisation test Not GLP	No allergic response in any case after 48 h Not sensitising	REACH registration dossier: Publication: Lehman *et al*., 1949	4

The registrant concludes that the substance is not skin sensitising, and based on the available information, the eMSCA can support this conclusion.

7.9.4 Repeated dose toxicity

Oral route:

Table 35: Summary of repeated dose toxicity studies (oral route)

Test method	Conclusion	Study reference	Reliability
OECD TG 408 90-Day Repeated Dose Oral Toxicity Study Duration of treatment: 90 days Rat (Wistar) (10/sex/group + 5/sex/group for control and high dose as recovery groups) Oral Conc.: 0, 100, 300 and 1000 mg/kg bw/d GLP	NOAEL= 1000 mg/kg bw/d	REACH registration dossier: Unpublished study report, 2018	1
OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test Duration of treatment: 4 w for M and approx. 7 w for F Rat (Wistar) (11/sex/dose) Oral: feed Conc.: 0, 1500, 4500 and 15000 ppm GLP	NOAEL for general toxicity= 15000 ppm (corresp. to 980.9 mg/kg bw/d in M and 1076.4 mg/kg bw/d in F)	REACH registration dossier: unpublished study report, 2012	1
Short-term repeated dose toxicity study Exposure: 14 days range-finding study prior to the study OECD TG 422 Rat (RccHan:WIST) (20/sex/dose) Oral: feed Conc.: 0, 1200, 3600 and 12000 ppm Not GLP	NOAEL= 12000 ppm in M and F (corresp. to 745.4 mg/kg bw/d in M and 760.5 mg/kg bw/d in F)	REACH registration dossier: unpublished study report, 2012	2

Chronic toxicity study Exposure period: 96 weeks Rat (Wistar) Oral: feed Conc.: 0, 2 and 8 % in diet Not GLP	NOAEL= 8 % (corresp. to 5500 mg/kg bw/d in M/F)	REACH registration dossier: Publication: Matthews *et al*., 1956	2 for the registrant However, eMSCA supports a reliability of 3 (only 4 animals/sex/dose or even less. Difficult to get the right info, not GLP, a lot of parameters not examined, …)
Chronic toxicity study Exposure period: 195 and 422 D for neg. control (2 animals), 394 D for low dose group (1 animal), 313 - 394 D for the high dose group (3 animals) Dog Oral: capsule (6 times per week) Conc.: 0, 0.5 and 1 g/kg bw/d Not GLP	NOAEL (dog)= 1000 mg/kg bw/d	REACH registration dossier: Publication: Matthews *et al*., 1956	2 for the registrant However, eMSCA supports a reliability of 3 (only few animals, not same exposure for all groups, …)
Short-term repeated dose toxicity study (7 days) Dog (1 male) Oral: feed Conc.: Increasing doses (1000, 2000, 3000 and 4000 mg/kg bw resp. on D 1, 2, 3 and 4; no treatment on D 5; treatment with 4000 mg/kg bw on D 6 and with 3000 mg/kg bw on D 7) Not GLP	NOAEL > 1000 mg/kg bw/d	REACH registration dossier: Publication: Schübel and Manger, 1929	4
Short-term repeated dose toxicity study (23 days) Rabbit (1 animal)	The animal was found dead on D 24. Before its death on D 24, the rabbit was anorectic and somnolent and its	REACH registration dossier: Publication: Schübel and	4

Oral: unspecified Conc.: Increasing dose (D 1 - 6: 500 mg/kg bw/d, D 7: 1000 mg/kg bw, D 8: 2000 mg/kg bw, D 9: 3000 mg/kg bw, D 12: 3000 mg/kg bw, D 17: 3000 mg/kg bw, D 19: 4000 mg/kg bw, D 22: 5000 mg/kg bw and D 23: 6000 mg/kg bw) Not GLP	respiration was slowed down. At necropsy, two stomach ulcerations were found surrounded by submucosal bleeding.	Manger, 1929	

90-Day Repeated Dose Oral Toxicity Study (REACH registration dossier: Unpublished study report, 2018)

Guideline: OECD TG 408

GLP: yes

Reliability: 1 (reliable without restriction)

Materials and methods:

Test type: repeated dose

Test substance: Propylparaben (EC n° 202-307-7)

Test animals: Rat / Wistar / male and female: 10/sex/group + 5/sex/group for recovery group (control and high dose groups)

Age and weight at the study initiation: approx. 7 – 8 w, 155 to 190 g (males) and 127 to 146 g (females)

Administration/exposure: route of administration: oral

Duration and frequency of test: 90 days, daily

Recovery period: 28 days after the last administration

Doses: 0, 100, 300 and 1000 mg/kg bw/d

Vehicle: 1 % hydroxyethyl-cellulose

Control group and treatment: negative control

Results and discussion:

NOAEL: 1000 mg/kg bw/d

LOAEL: /

During the study period, all animals survived (except one female exposed to 100 mg/kg bw/d) and no clinical signs of systemic toxicity were observed. Parameters of functional observation battery, of haematological and blood coagulation and of clinical biochemistry did not exhibit treatment-related modifications. T3, T4 as well as TSH were analysed and no statistically significant effect was observed. At necropsy, no treatment-related gross lesions, organ weight modification, nor histopathological change were noted.

Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (REACH registration dossier: Unpublished study report, 2012)

Guideline: OECD TG 422

GLP: yes

Reliability: 1 (reliable without restriction)

Materials and methods:

Test type: repeated dose

Test substance: Propylparaben (EC n° 202-307-7)

Test animals: Rat / Wistar / male and female: 11/sex/dose

- Age and weight at the study initiation: 11 weeks, 344 to 381 g (males) and 184 to 209 g (females)

Administration/exposure: route of administration: oral: feed

- Duration and frequency of test: 4 weeks for males and approx. 7 weeks for females, daily
- Doses: 0, 1500, 4500 and 15000 ppm corresp. in males to 98.0, 305.1 and 980.9 (pre-pairing period) and 59.3, 178.3 and 605.0 mg/kg bw/d (after pairing period) and in females to 116.0, 341.9 and 1076.4 (pre-pairing period), 121.6, 349.2 and 1124.6 (gestation) and 137.3, 431.8 and 1380.0 mg/kg bw/d (lactation)
- vehicle: no vehicle
- Control group and treatment: negative control

Results and discussion:

NOAEL: 15000 ppm for general toxicity in males and females

LOAEL: /

During the study, all animals survived and no clinical signs were observed. The body weight and the food consumption were not modified however the body weight gain was slightly decreased in males at the high dose (3 % at D 8 of pairing period vs 4 % in control group). The biochemistry evaluation revealed a statistically significant increase in triglycerides concentration at the highest dose (in males: 1.03 vs 0.71 mmol/L in control). No significant changes were observed on the hematology parameters. The necropsy revealed no test item-related effects, only an enlarged liver was noted in 1, 1, 2 and 4 animals resp. at 0, 1500, 4500 and 15000 ppm. Some modifications in organ weight were observed such as higher absolute weight of right epididymides at the 2 highest doses (0.705**** and 0.695** g resp. at the mid and high doses vs 0.642 g in control), lower kidney weight to body weight ratio at the low and mid-dose group (resp. 0.54** and 0.55** g vs 0.59 g in control) however none effects at the histopathology evaluation were observed.

Short-term repeated dose toxicity study (REACH registration dossier: Unpublished study report, 2012)

Guideline: /

GLP: no

Reliability: 2 (reliable with restrictions)

Materials and methods:

Test type: repeated dose

Test substance: Propylparaben (EC n° 202-307-7)

Test animals: Rat / RCCHan:WIST / male and female: 20/sex/dose

Age and weight at the study initiation: 11 weeks, 323 to 331 g (males) and 196 to 246 g (females)

Administration/exposure: route of administration: oral: feed

Duration and frequency of test: 14 days, dietary administration

Doses: 0, 1200, 3600 and 12000 ppm corresp. in males to 77.0, 234.1 and 745.4 mg/kg bw/d and in females to 83.2, 252.5 and 760.5 mg/kg bw/d

Vehicle: no vehicle

Control group and treatment: negative control

Results and discussion:

NOAEL: 12000 ppm (corresp. to > 745.4 mg/kg bw/d in males and > 760.5 mg/kg bw/d in females

LOAEL: /

During the study, all animals survived and no clinical signs were observed. The body weight and the food consumption were not statistically significantly modified, a decrease was only observed in females at the highest dose. Mean body weight gain was of 15, 14, 13 and 13 % in males and 9, 8, 8 and 3 % in females, resp. at 0, 1200, 3600 and 12000 ppm. The necropsy revealed no gross pathology findings and the analysis of the terminal organ weight revealed higher absolute weight of right epididymides at the 2 highest doses, lower kidney weight to body weight ratio at the low and mid-dose groups. Some changes were observed at the histopathological evaluation such as foci in lungs in 1 male/5 of the control group, hardened liver in 1 male/5 of the mid dose group, pelvic dilatation of the kidneys in 1 male/5 of the low and mid-dose groups and in 1 female/5 of the highest dose and discoloration of the seminal vesicles in 1 male/5 of the highest dose.

Chronic toxicity study (REACH registration dossier: Publication: Matthews *et al*., 1956)

Guideline: /

GLP: no

Reliability: 2 for the registrant (However, eMSCA supports a reliability of 3 (only 4 animals/sex/dose or even less. Difficult to get the right info, not GLP, a lot of parameters not examined, …))

Materials and methods:

Test type: repeated dose

Test substance: Propylparaben (EC n° 202-307-7)

Test animals: Rat / Wistar / male and female: 6/sex/dose

Age and weight at the study initiation: weaning age

Administration/exposure: route of administration: oral: feed

Duration and frequency of test: 96 weeks, dietary administration

Doses: 0, 2 and 8 % corresp. to 0, 0.9 - 1.2 and 5.5 - 5.9 g/kg bw/d

Vehicle: no vehicle

Control group and treatment: negative control

Results and discussion:

NOAEL: 8 % (corresp. to ≥ 5500 mg/kg bw/d)

LOAEL: /

The animals showed a slight decrease of body weight gain at the high dose group (this modification was much more striking in the early part of the study, and tended to disappear at the end of the study). The food consumption was not affected. Some information is missing such as clinical signs, mortality, gross pathology findings, terminal organ weight. The article mentioned that tissues from all rats surviving the chronic test were entirely normal and noted that the only change was observed in the died rats (extensive consolidation of the lung and pneumonia) (however no more information available in the article).

Chronic toxicity study (REACH registration dossier: Publication: Matthews *et al*., 1956)

Guideline: /

GLP: no

Reliability: 2 for the registrant (However, eMSCA supports a reliability of 3 (only few animals, not same exposure for all group, …))

Materials and methods:

Test type: repeated dose

Test substance: Propylparaben (EC n° 202-307-7)

Test animals: Dog / Mongrel / male and female: 2 animals for control group, 1 for the low dose group and 3 for the high dose group

Age and weight at the study initiation: weaning age, 2.3 - 6.4 kg

Administration/exposure: route of administration: oral: capsule

Duration and frequency of test: 195 and 422 days for control group, 394 days for the low dose group and 313 - 394 days for the high dose group, 6 times per week

Doses: 0, 0.5 and 1 g/kg bw/d

Vehicle: no vehicle

Control group and treatment: negative control

Results and discussion:

NOAEL: 1000 mg/kg bw/d

LOAEL: /

The study revealed no effects during the evaluation of the body weight, the food consumption, the clinical signs, the mortality, the hematology, the gross pathology findings, and the histopathology findings. In the summary, there was no information about the terminal organ weight.

Dermal route:

Table 36: Summary of repeated dose toxicity studies (dermal route)

Test method	Results	Study reference	Reliability
Similar to OECD TG 411 Subchronic Dermal Toxicity Study (13 weeks) Rat (SD) (10/sex/dose) Coverage: open (coverage of 10 - 15 %) Conc.: 0, 12.4 mg/kg bw/d Not GLP Test material: EC n° 202-307-7 (composition of the test material: contains **0.3 % propylparaben**)	NOAEL: 12.4 mg/kg bw/d	REACH registration dossier: Unpublished study report, 1981	4 (test substance was one ingredient of a formulation)

eMSCA comment: The provided 90-day study (dermal exposure) has a reliability of 4. Propylparaben (in a concentration of 12.4 mg/kg bw/day) was one ingredient of the tested formulation ("Medicated Lotion, 13824-32"). The eMSCA cannot conclude based on this study.

Conclusion for repeated dose toxicity:

The registrant concludes that the substance is not toxic after repeated dose via oral route. Based on the available information, the eMSCA can support this conclusion.

7.9.5 Mutagenicity

Table 37: Summary of mutagenicity studies

Test method	Conclusion	Study reference	Reliability
In vitro			
In Vitro Mammalian Cell Micronucleus Test: OECD TG 487 *In vitro* mammalian cell micronucleus test of in human lymphocytes GLP compliant Test Substance: Propyl 4-hydroxybenzoate Vehicle: DMSO	No increase in the induction of micronuclei Clastogenicity: negative Aneugenicity: negative With and without met. act. Moderate precipitation observed at 2 mg/mL Cytotoxicity tested: yes; observed with 45	REACH registration dossier: Clariant, 2018	1

Conc.: 0.5, 1 and 2 mg/mL Short term (3-6 h) with and without met. act. and long term (20-24 h) without met. act. Positive controls: 2-nitrofluorene, sodium azide, 9-Aminoacridine and Mitomycin C for trials "without metabolic activation" and 2-Aminoanthracene for trials "with metabolic activation"	% at 2 mg/mL		
In vitro Gene Mutation Test: OECD TG 471 Bacterial Reverse Mutation Assay Target gene: his GLP compliant Test Substance: Propyl 4-hydroxybenzoate Vehicle: DMSO Conc.: 0.01, 0.03, 0.1, 0.32, 1 mg/plate Strain: *S. Typh.* (*Salmonella typhimurium)* TA98, TA1537, TA100, TA1535 and TA102	Genotoxicity: negative Cytotoxicity: yes, at 1 mg/plate With and without met. act. Precipitation: 5 mg/plate	REACH registration dossier: Clariant, 2018	1
In Vitro Gene Mutation Test: OECD TG 471 Bacterial Reverse Mutation Assay Target gene: his *S. typh.* TA 1535, TA 1537, TA 1538 With and without met. act. Met. act. : co-factor supplemented post-mitochondrial fraction (S9 mix) (livers of Sprague-dawley (SD) rats, ICr mice and Macaca mulatta primates) Conc.: 0.075% Vehicle: DMSO Pos. control: yes	Genotoxicity: negative with and without met. act. Cytotoxicity: negative	REACH registration dossier: unpublished study report, 1975	2

Preincubation period: 1 hour Exposure period: 96 h (plate incorporation); 48 h (preincubation) No GLP			
In Vitro Gene Mutation Test: Similar to OECD TG 471 Bacterial Reverse Mutation Assay Target gene: his *S. typh.* TA 1535, TA 1537, TA 98 and TA 100 With and without met. act. Met. act.: Cofactor supplemented post-mitochondrial fraction (S9 mix), (livers of rats) Conc.: up to 2000 µg/plate No GLP	Genotoxicity: negative with and without met. act. Cytotoxicity: no data	REACH registration dossier : McCann *et al*., 1975	4 (only secondary literature)
In Vitro Gene Mutation Test: OECD TG 471 Bacterial Reverse Mutation Assay Target gene: his, trp *S. typh.* TA 100, TA 98 (with and without met. act.) E. coli WP2 (without met. act.) Met. act.: Cofactor supplemented post-mitochondrial fraction (S9 mix) (livers of SD rats) No data about test concentration, vehicle, controls, cytotoxicity Preincubation period: 20 min Exposure duration: 48 hours No GLP	*S. typh.* TA 100 and TA 98: genotoxicity : negative with and without met. act. E. coli WP2: genotoxicity : negative without met. act.	Sugimura, *et al*., 1976	3
In Vitro Mammalian Cell Gene Mutation Test:	Genotoxicity : negative with and without met.	REACH registration dossier:	1

OECD TG 476 Target gene: HPRT Chinese hamster lung fibroblasts (V79) With and without met. act. Met. act. system: Phenobarbital/Beta-Naphtoflavone induced Rat liver Experiment I/ without met. act./ 4 h treatment: 7.0, 14.0, 28.0, 56.0, 93.0, 112.0 µg/mL Experiment I/ with met. act./ 4 h treatment: 28.0, 56.0, 112.0, 224.0, 336.0, 448.0 µg/mL Experiment II/ without met. act./ 24 h treatment: 14.0, 28.0, 56.0, 112.0, 168.0, 224.0 µg/mL Experiment II/ with met. act./ 4 h treatment: 28.0, 56.0, 112.0, 224.0, 336.0, 448.0 µg/mL In experiment I with and without met. act. and in experiment II with met. act. the cultures at the maximum concentration were not continued due to exceedingly severe cytotoxicity. In the second experiment without met. act. the cultures at the two highest concentrations were not continued for the same reason. Pos. control: yes Exposure duration: Experiment I: 4 h with and without met. act. Experiment II: 24 h without met. act., 4 h with met. act. Number of replications: 6 Number cells evaluated: >1.5x10^6 GLP	act. Cytotoxicity : yes No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration. The mutation frequency remained within the historical range of solvent controls. The induction factor did not reach or exceed the threshold of 3 time the mutation frequency of the corresponding solvent control.	unpublished study report, 2012	
In vitro Mammalian Chromosome Aberration Test No guideline followed Chinese hamster fibroblast cells	Genotoxicity : negative After 48 h, 3 % of the cells chromosome aberration was noticed. The aberration types	Ishidate *et al*., 1978	4

Conc.: up to 0.125 mg/mL (maximum tolerated conc. on cells) Vehicle: Ethanol No data about pos. control, cytotoxicity, met. act. Not GLP	observed were breaks.		
Bacterial Reverse Mutation Assay OECD TG 471 *S. typh.* TA 1535, TA 1537, TA 98, TA 100 and TA 102 With and without met. act. Target gene : His Experiment I (plate incorporation): 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Experiment II (preincubation): 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate Vehicle: DMSO Met. act. system: rat S9 liver microsomal fraction Neg. and pos. control : yes Preincubation period: 60 min at 37 °C Exposure duration: 48 h at 37 °C in the dark For each strain and dose level, including the controls, three plates each in two independent experiments were used. No precipitation of the test item was observed in experiment I and II (with and without met. act.). GLP Test material : **EC n° 204-399-4 (ethylparaben)**	Genotoxicity : negative with and without met. act. Cytotoxicity : yes During the test, ethylparaben did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used	REACH registration dossier: unpublished study report, 2012	2 **READ ACROSS**
In vivo			

Genetic Toxicology: Rodent Dominant Lethal Test OECD TG 478 Chromosome aberration. Dominant lethal assay Rat, SD (10 males/dose) Oral: gavage Doses: acute (single dose): 5, 50, 500, 5000 mg/kg bw subacute (5 doses on 5 consecutive days): 5, 50, 500, 5000 mg/kg bw Vehicle: 0.85 % saline Post exposure: 8 weeks Neg. and pos. control: yes Following treatment. Males were sequentially mated to 2 females per week for 8 weeks (7 weeks in subacute study). Females were killed at 14 days after separating from the male, and at necropsy the uterus was examined for deciduodimata, late fetal deaths and total implantations No GLP Test material : **EC n° 202-785-7 (methylparaben)**	Genotoxicity : negative Toxicity : no effects Methylparaben is considered to be non-mutagenic in rats in the Dominant Lethal Assay applying dosages up to 5000 mg/kg bw	Litton Bionetics Inc, 1974	3 for the eMSCA (2 for the registrant) **READ ACROSS**
Mammalian Bone Marrow Chromosome Aberration Test OECD TG 475 Rat, SD (male) Acute study: 15 males/dose (9 males for neg and 5 for pos control) Subacute study: 5 males/dose (3 males for neg and 5 for pos control) Oral: gavage Doses: 5, 50, 500 mg/kg bw	Genotoxicity : negative Toxicity : no effects Methylparaben did not produce detectable significant aberration of the bone marrow metaphase chromosomes of rats when administered orally at 5, 50 and 500 mg/kg.	Litton Bionetics Inc. 1974	2 **READ ACROSS**

Acute: single administration Subacute: once a day for 5 consecutive days Post exposure period: 6, 24, 48 h for acute study and only 6 h after for subacute study No GLP Test material : **CAS : 99-76-3 (methylparaben)**			

Based on the available study results in 2015, the registrant concluded that the substance is not mutagenic. However, the only reliable AMES test available for the substance (REACH registration dossier: unpublished study report, 1975) was performed on 3 strains (instead of 5). The registrant provided a supporting study for *in vitro* chromosome aberration with the registered substance (Ishidate *et al*., 1978) of reliability 4, non-GLP and no guideline followed. According to the Registrant there is no genotoxicity: "After 48 h, in 3 % of the cells chromosome aberration was noticed. The aberration types observed were breaks." There is no other information reported and the robust study summary is missing.

The data on ethyl- and methylparaben provided by the registrant for read-across are not considered appropriate by the eMSCA (see discussion in section 7.3).

Bacterial Reverse Mutation Test (AMES test) (OECD TG 471) and an *In vitro* Mammalian Chromosome Aberration Test (OECD TG 473) [or an *In Vitro* Mammalian Cell Micronucleus Study (test method: OECD TG 487)] were requested under dossier evaluation (compliance check). The chromosome aberration test should be preferred to the *in vitro* micronucleus study as Pérez Martín *et al*. (2014) showed the ability of propyl 4-hydroxybenzoate to cause oxidative stress and a significant increase in the fraction of gamma-H2AX positive cells (assay to detect DNA double-strand breaks) was reported.

Following the above stated requests, a Bacterial Reverse Mutation Test (OECD TG 471; 2018) and an *In Vitro* Mammalian Cell Micronucleus Study (OECD TG 487; 2018) were conducted.

Propylparaben was evaluated for mutagenicity in Bacterial Reverse Mutation Test using *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 according to OECD TG 471 "Bacterial Reverse Mutation Test" in a GLP compliant study. In a first step the solubility of propylparaben was tested and at a concentration of 50 mg/mL a minimal precipitation was found. Based on these results the highest concentration selected for the initial cytotoxicity test was 5 mg/plate. The *salmonella typhimurium* TA100 tester strain was exposed to vehicle control, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4 and 5 mg/plate in the presence and absence of metabolic activation. This treatment resulted in cytotoxicity and those concentrations are graded as 0 (Absent lawn) for 5 mg/plate, 1+ (extremely reduced lawn) for 3 and 4 mg/plate, 2+ (moderately reduced lawn) for 2 mg/plate and 3+ (Slightly reduced lawn) for 1 mg/plate when compared to vehicle control. On the basis of these results, 1 mg/plate was considered as the highest test concentration for the mutation assay. In mutation assay propylparaben was tested at the concentrations of 0.01, 0.03, 0.10, 0.32 and 1 mg/plate. Two independent trials were conducted by plate incorporation method and pre incubation method in the presence and absence of metabolic activation system. The vehicle control (dimethyl sulphoxide) and the positive controls were tested simultaneously.

Based on the experimental setup the study can be regarded as a key study, and is reliable without restrictions. The experimental results showed in both the trials, and in the presence

and absence of metabolic activation, that the mean numbers of revertant colonies at the tested concentrations were comparable to those of the vehicle control. There was no noticeable increase in number of revertant colonies. The number of revertant colonies in the positive controls resulted in 2.2- to 18.8-fold increase.

Therefore, Propyl 4-hydroxybenzoate is "non-mutagenic" in the Bacterial Reverse Mutation Test up to the highest tested concentration of 1 mg/plate in the described test conditions.

Further, the requested *In Vitro* Mammalian Cell Micronucleus Study (OECD TG 487) was conducted. Propylparaben was tested for the formation of micronuclei in human lymphocytes. Propylparaben was evaluated for the formation of micronuclei in the cytoplasm of interphase cells using human lymphocytes. The GLP compliant experiments were conducted according to the OECD TG 487 "*In vitro* Mammalian Cell Micronucleus Test".

Initial solubility test revealed a moderate and mild precipitation at 2 and 1 mg/mL respectively. No precipitation was observed in any other concentrations tested. No change in pH was observed in any of the concentrations tested. Hence, 2 mg/mL was selected as the highest concentration for initial cytotoxicity test using dimethyl sulphoxide as vehicle. The experiment was conducted with and without metabolic activation. The test item was assessed in proliferated lymphocytes in duplicates after a short-term treatment (3 to 6 hours, with and without metabolic activation) and a long-term treatment (20 to 24 hours, without metabolic activation). In order to assess the cytotoxicity of propylparaben, the Cytokinesis-Block Proliferation Index (CBPI) was calculated for cultures treated with propylparaben at concentrations of 0.125, 0.25, 0.50, 1 and 2 mg/mL and the vehicle control. The maximal percentage reduction of the Cytokinesis-Block Proliferation Index (CBPI) was 30 % at 2 mg/mL and therefore not greater than 45 ± 5 %. Hence, 2 mg/mL was selected as highest concentration for the micronucleus test.

Thereafter, propylparaben was tested in the micronucleus test at the concentrations of 0.5, 1 and 2 mg/mL for short-term treatment in presence and absence of metabolic activation and long-term treatment in the absence of metabolic activation.

The experiment showed no statistically significant increase in the percentage of micronuclei in binucleated cells in any of the tested concentration when compared to the vehicle control. In contrast, the positive controls showed an increase in the micronuclei frequency with statistical significance at 95 % level of confidence ($p<0.05$), when compared with the vehicle control. Thus, the test conditions were adequate and within the range of historical control. Based on the experimental setup the study can be regarded as a key study, and is reliable without restrictions.

In conclusion, Propylparaben showed no evidence of increase in the induction of micronuclei under the test conditions. Hence, it is non clastogenic and/or non aneugenic in cultured human lymphocytes at concentrations of 0.5, 1 and 2 mg/mL, in short term and long-term treatment and in presence and absence of metabolic activation.

Overall, Propylparaben was not mutagenic in a guideline compliant Ames test (OECD TG 471; 2018), and also not mutagenic in a guideline compliant *In Vitro* Mammalian Cell Gene (HPRT) Mutation Assay (OECD TG 476; 2012) in V79 Chinese hamster cells, as there was no induction of chromosome aberrations or clastogenic effects. Furthermore, the guideline compliant *In Vitro* Mammalian Cell Micronucleus Test (OECD TG 487; 2018) showed no clastogenic and/or no aneugenic effects. Hence a mutagenic potential of the test item can most probably be excluded, as the available data from three *in vitro* assays showed no mutagenicity.

7.9.6 Carcinogenicity

Table 38: Carcinogenicity studies

Test method	Results	Study reference	Reliability
No data about test guideline Hamsters, 15 males Oral: feed No vehicle used Duration: 20 weeks No post exposure period Doses: 0 and 3 % nom. diet (corresp. to 0, 1009.6 - 2163.5 mg/kg bw/d) No data about GLP	No mortality noted No bw changes (208 g vs 203 g in control) Liver weight were slightly increased compared to control (4.4 g vs 3.8 g) Histopathology: in 5/15 treated hamsters, mild hyperplasia of the forestomach with an epithelium thickness of <0.1 mm was observed. No papillomatous lesions were observed. In the control group, 7/15 hamsters showed mild hyperplasia and 1/15 hamsters showed moderate hyperplasia with epithelium thickness of 0.1 - 0.5 mm. No inflammation, hyperplasia or tumorous lesions were observed in the urinary bladder. Changes in DNA synthesis were measured in the auto radiographic study by determining the labelling index: Forestomach: 12.5 g vs 12.5 g in control) Pyloric region: 6.9 g vs 7.3 g) Urinary bladder: 0.52** g vs 0.08 g) No histopathological changes were noted in the urinary bladders.	REACH registration dossier: Publication: Hirose *et al*., 1986	2
No data about test guideline Rat, Fischer 344 (5males)	No mortality observed No bw changes (293 g vs 306 g in control) Food and water	REACH registration dossier: Publication: Shibata *et al*.,	2

Oral: feed No vehicle Exposure: 8 weeks No post exposure period Doses: 0, 3 % nom. diet (corresp. to 0, 1883.96 - 4150.38 mg/kg No GLP	consumption was also comparable to the control group. No histopathological changes and effects on labelling indices were observed in forestomach epithelial cells. Furthermore, no proliferative response of the pyloric gland epithelium was noted.	1990	
No data about test guideline Rat, Fischer 344 (20 males) Oral: feed No vehicle Duration of treatment: 36 weeks No post exposure treatment Doses: 3 % nom. diet (corresp. to 785.3 - 1570.7 mg/kg bw/d) 3 Groups: Group 1: pre-treated with BBN for 4 weeks and then treated with 3 % of test substance until end of study. Group 2: treated with BBN for 4 weeks and tapwater thereafter. Group 3: treated with test substance (no pre-treatment) No data about GLP	Bw was 382 ± 16 g, 420 ± 18 g and 377 ± 13 g resp. in group 1, 2 and 3 Bladder weight was 0.14 ± 0.04 g, 0.14 ± 0.04 g and 0.15 ± 0.005 g resp. in group 1, 2 and 3 Necropsy: in group 1: small nb of nodules were observed in urinary bladder	REACH registration dossier: Publication: Kurata *et al*., 1990	3
No data about test guideline Mouse, female Duration of treatment/ exposure: 3 times on GD 14, 16 and 18 or on GD	PPB was found to be non-carcinogenic in offspring of treated mice.	REACH registration dossier: Publication: Odashima S., 1976	4

15, 17 and 19 Post-exposure period: 1 year No data about route, vehicle, dose, animals/sex/dose, control animals No data about GLP			
No data about test guideline Mouse, male/female SC Exposure: 4 weeks, once a week on PND 1, 8, 15 and 22 Post-exposure period: 1 year No data about vehicle, doses, animals/sex/dose, controls No GLP	PPB was found to be non-carcinogenic in treated mice.	REACH registration dossier: Publication: Odashima S., 1976	4

The registrant concludes that there is no concern for carcinogenicity. The available information does however not allow to assess this endpoint. No definite conclusion can be taken for carcinogenicity. However, as the available data doesn't point to a specific concern for carcinogenicity, no additional information was requested by the eMSCA for this endpoint in their SEv decision.

7.9.7 Toxicity to reproduction (effects on fertility and developmental toxicity)

7.9.7.1 Fertility

Table 39: Summary table of reproductive toxicity studies (Fertility)

Test method	Results	Study reference	Reliability
EOGRTS with DNT and DIT OECD TG 443 GLP 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 Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: F0: min. 10 w in males and 14 d of premating, max 14 d of mating, gestation and through weaning in females F1: from weaning (PND 22) to terminal sacrifice and the respective cohorts	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, eMSCA is in favour of a NOAEL of 300 mg/kg bw/d based on the sperm effects **F0 parental:** Clinical signs: increased salivation and moving bedding at mid dose in F 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 M and only thymus in F). No microscopic changes observed. **F1 pups:**	REACH registration dossier: Unpublished report, 2021	1

	Viability index not modified. **Cohort 1A:** Clinical signs: moving bedding observed at the highest dose. Bw: changed at the highest dose in M at D 64. Balano-preputial separation slightly reduced. Thyroid hormone: sign. higher in F of the mid and high doses. Male reproduction parameters: reduced testes weight, percentage of motile sperm count and higher percentage of abnormal sperms. Immunological parameters affected. Necropsy: few organ weight changed. **Cohort 1B and F2 pups:** Moving bedding in F 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. **Cohort 2A:**		

	Moving bedding at the highest dose. Neurotoxicity parameters: few modifications. **Cohort 2B:** No abnormalities observed. **Cohort 3:** Immunological parameters affected. **Cohort 4:** Mean escape latency sign reduced in F during memory phase.		
Dose Range-Finding Study for Reproduction/ Developmental Toxicity Screening Test OECD TG 421 Not GLP Oral, gavage Rat (Wistar) 10/sex/dose (except for control group: 5/sex) Doses: 0, 500 and 1000 mg/kg bw/d Duration of exposure: min. 35 d for M and during 21 d for premating, max 14 d 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	NOAEL (general toxicity): > 1000 mg/kg bw/d NOAEL (fertility): 1000 mg/kg bw/d **Parental:** 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 **Pups:** Mean nb of pups at birth, mean nb of live pups and viability index unaffected	REACH registration dossier: Unpublished report, 2018	1 However, eMSCA disagrees and considers a reliability of 2 (low number of animals tested and not equivalent between treatment groups, only 2 doses tested, non-GLP)

litter from each group were treated from PND 13 to PND 21			
Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test OECD TG 422 GLP Rat (Wistar) 11/sex/dose Oral: feed Duration of exposure: min. 4 weeks for males and approx. 7 weeks for females (Day 1 of pre-pairing to post-partum day 3) Doses: 0, 1500, 4500 and 15000 ppm (corresp. in males to 98.0, 305.1 and 980.9 (pre-pairing period) and 59.3, 178.3 and 605.0 mg/kg bw/d (after pairing period) and in females to 116.0, 341.9 and 1076.4 (pre-pairing period), 121.6, 349.2 and 1124.6 (gestation) and 137.3, 431.8 and 1380.0 mg/kg bw/d (lactation))	NOAEL (general toxicity): 15000 ppm NOAEL (reproductive F1): 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 eMSCA **Parental**: 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. **Offspring**: 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.	REACH registration dossier: Unpublished study report, 2012	1
Assessment of propylparaben in juvenile rats 2 separate studies were conducted to assess the	NOAEL (parental toxicity): 1000 mg/kg bw/d	REACH registration dossier:	2

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 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	NOAEL (fertility): 1000 mg/kg bw/d 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. **Pups:** Litter weight and viability index not modified. No malformed pups observed.	Publication: Sivaraman *et al.*, 2018	
Assessment of propylparaben on juvenile male rats GLP Oral, gavage Rat (Wistar) Preliminary study: 3 M in control group and 18 M in treated groups Main study: 20 M/group Doses: 0, 3, 10, 100 and 1000 mg/kg bw/d	NOAEL (general toxicity): 1000 mg/kg bw/d NOAEL (male fertility): 1000 mg/kg bw/d 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	REACH registration dossier: Publication: Gazin *et al.*, 2013	4 However, eMSCA disagrees and considers a reliability of 2 (No guideline followed, but GLP, 4 tested doses and control group, Well documented non-guideline study)

Duration of exposure: single exposure at PND 31 for preliminary study, 8 weeks for main study (divided into 2 subgroups: one euthanized at the end of exposure period and a second after a 26 weeks of recovery period)	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.		
No data about test guideline Effects of propylparaben on the male reproductive system Oral, feed Rat (Wistar) 8 males/doses Exposure: 4 weeks (from PND 19 - 21 to PND 46 - 48) Doses: 0, 0.01, 0.1 and 1 % corresp. to 0, 12.4, 125 and 1290 mg/kg bw/d No data about GLP	LOAEL (fertility): 12.4 mg/kg bw/d (based on sign. decreased mean sperm counts in testes) No NOAEL 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	REACH registration dossier: Publication: Oishi, 2002	3

Extended One-Generation Reproductive Toxicity Study with DNT and DIT (REACH registration dossier: Unpublished report, 2021)

OECD TG 443

GLP

Cohorts: 1A and 1B for reproductive and developmental toxicity testing

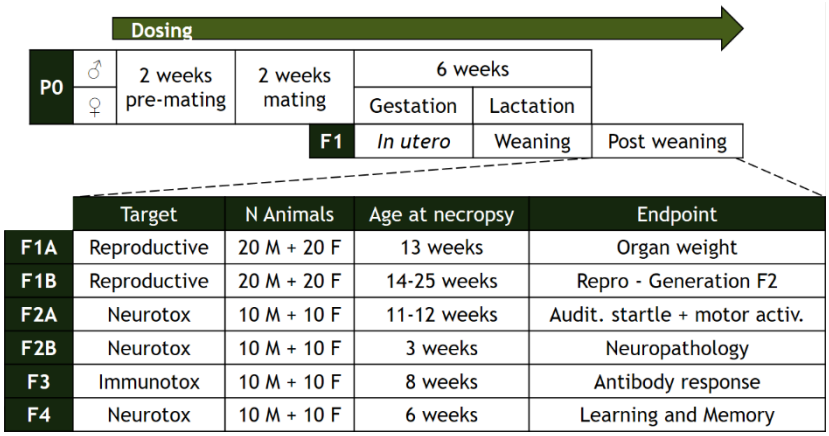
2A: neurobehaviour testing and neurohistopathology assessment

2B: neurohistopathology assessment at PND 21 or 22

3: for developmental immunotoxicity testing on PND 56

4: for learning and memory testing

Figure 1: Study design



	Target	N Animals	Age at necropsy	Endpoint
F1A	Reproductive	20 M + 20 F	13 weeks	Organ weight
F1B	Reproductive	20 M + 20 F	14-25 weeks	Repro - Generation F2
F2A	Neurotox	10 M + 10 F	11-12 weeks	Audit. startle + motor activ.
F2B	Neurotox	10 M + 10 F	3 weeks	Neuropathology
F3	Immunotox	10 M + 10 F	8 weeks	Antibody response
F4	Neurotox	10 M + 10 F	6 weeks	Learning and Memory

Test substance: Propylparaben

Degree of purity: 99.7 %

Test animals: Species/strain/sex: rats / Wistar / both sexes

Nb. of animals per sex per dose: parental generation: 30/sex/group for control and high dose and 25/sex/dose for low and mid doses

Cohort 1A: 20/sex/dose

Cohort 1B: 20/sex/dose

Cohort 2A: 10/sex/dose

Cohort 2B: 10/sex/dose

Cohort 3: 10/sex/dose

Cohort 4: 10/sex/dose

Age and weight at the study initiation: 13 – 14 weeks old (at study initiation), 306 – 358 g for males and 177 – 241 g for females (at allocation to the group).

Administration/exposure: Route of administration: oral, gavage

Duration and frequency of test/exposure period: daily

F0 parental: Males: min. 10 weeks (14 d of pre-mating, max 14 d of mating and until terminal sacrifice)

Females: 14 d of pre-mating, max 14 d of mating, during gestation and until weaning (PND 21)

F1 selected offspring: from weaning (PND 22) to terminal sacrifice of the respective cohorts:

Cohort 1A: sacrifice at week 13 of age (approx. 10 weeks of treatment)

Cohort 1B: exposure until weaning of F2 (sacrifice approx. to week 20 – 25 of age)

Cohort 2A: approx. to week 12 of age (approx. 9 weeks of treatment)

Cohort 2B: at weaning

Cohort 3: at 8 weeks of age (approx. 5 weeks of treatment)

Cohort 4: sacrifice at PND 35 - 42

Doses/concentration levels: 0, 100, 300 and 1000 mg/kg bw/d

Control group and treatment: positive control: cyclophosphamide and hemocyanin megathura crenulata

Vehicle: 1 % of hydroxyethyl-cellulose

Results and discussion:

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 40**). 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 40: 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 41**).

Table 41: 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
Nb of sperms evaluated		200.00	/	/	200.00
Tot. nb of abnormal sperms		8.25	/	/	13.33
Tot. nb 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 42**Table 42**). 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 42: 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 (Table 43) and mean pup body weight was only significantly lower at PND 14 in the highest dose group (see Table 44). 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 45). 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 43: Litter data

Dose level (in mg/kg bw/d)	0	100	300	1000
Nb 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 (in %)				
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 44: 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 45: AGD and nipple retention

Dose level (in mg/kg bw/d)	0	100	300	1000
Males				
Nb 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				
Nb 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 sign. Lower in males at day 64 (see **Table 46**). 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 47**), 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 48**).

Table 46: 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 47: 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 (10^{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 (10^9/L)	701.4	681.3	659.9	613.9**	669.2	794.2	678.6	679.5
WBC (10^9/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 48: Thyroid hormone data

	Males				Females			
Dose level (in mg/kg bw/d)	0	100	300	1000	0	100	300	1000
Nb 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 parameters, 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 modifications (see **Table 49**).

Table 49: 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 50**).

Table 50: Organ weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	100	300	1000	0	100	300	1000
Nb examined		20	20	19	18	20	20	20	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/	Abs	0.0299	0.0329	0.0344	0.0335	0.0246	0.0233	0.0233	0.0267
parathyroid	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 51**).

Table 51: 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 52: 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). Furthermore, between birth and weaning, the mean number of live pups was unaffected (see Table 53). In the same way, mean pup body weight examination did not show significant change (see Table 54). However, as observed in F1 pups (produced from parental animals), anogenital distance and pup nipple retention was significantly affected (see Table 55).

Table 53: 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 54: 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 55: 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 as mentioned in **Table 56**. 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 56: 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 57** and

Table 58).

Table 57: 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
Tot IgM	Baseline	50457	46246	53960	43916	44836	51163	44183	57456	46696	44951
	D6	85678	32436	65536	58650	61502	67790	37980	84141	80677	68915

Table 58: 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
Tot 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 59: 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**

**: $p<0.05$

Dose Range-Finding Study for Reproduction/Developmental Toxicity Screening Test (REACH registration dossier: Unpublished report, 2018)

OECD TG 421

Not GLP

Aim of this study: generate toxicological information on the maximum tolerated dose of propylparaben to select dose levels for scheduled EOGRTS study.

Test substance: Propylparaben

Degree of purity: 99.7 %

Test animals: Species/strain/sex: rat / Wistar / both sexes

Nb. of animals per sex per dose: 5/sex for control group and 10/sex for each treated group

Age and weight at the study initiation: 8 to 9 weeks old (at the start of the treatment period), 236 to 284 g for males and 155 to 188 g for females (at the allocation of the animals to the groups)

Administration/exposure: Route of administration: oral, gavage

Duration and frequency of test/exposure period: min. 35 days for males (21 days of pre-mating and max 14 days of mating). Females were exposed during 21 days

of pre-mating and up to 14 days of mating. One dam of each treated group was dosed up to GD 20, the other dams were dosed during gestation and up to PND 21. The surviving pups of one litter from each group were treated from PND 13 to PND 21. Daily

Doses/concentration levels: 0, 500 and 1000 mg/kg bw/d

Vehicle: 1 % hydroxyethyl-cellulose mittelviskos

Results and discussion:

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 60.

Table 60: 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 61).

Table 61: Reproductive parameters

Dose level (in mg/kg bw/d)		0	500	1000
Duration of gestation	Nb animals examined	4	9	9
	Mean days	22.25	22.33	22.11
Corpora lutea	Nb animals examined	1	1	1
	Mean nb	14.0	13.0	14.0
	Nb animals examined	4	9	8
	Mean nb	13.75	13.22	13.63
Implantation sites	Nb animals examined	1	1	1
	Mean nb	12.0	11.0	14.0

	N animals examined	4	9	8
	Mean nb	13.75	13.11	13.38
Pre- and post-implantation loss	Nb animals 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 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).

Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test (REACH registration dossier: Unpublished study report, 2012)

OECD TG 422

GLP

Test substance: Propylparaben

Degree of purity: 99.7 %

Test animals: Species/strain/sex: Rat / Wistar / Both sexes

Nb. of animals per sex per dose: 11 animals/sex/group

Age and weight at the study initiation: 11 weeks, 344 to 381 g for males and 184 to 209 g for females

Administration/exposure: Route of administration: oral (feed)

Duration and frequency of test/exposure period:

Males: min. 4 weeks (14 days of pre-mating period and 14 days of pairing period) (necropsy after treatment of at least 28 days)

Females: approx. 7 weeks (14 days of pre-mating period, max. 14 days of pairing period, gestation period (21 days) et until days 3 of lactation) (necropsy of females and pups on DPP 4)

Doses/concentration levels: 0, 1500, 4500 and 15000 ppm

Table 62: Mean achieved dose level (mg/kg bw/d)

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

Vehicle: not specified

Description of test design:

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 63 and Table 64).

Table 63: 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 64: 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 absolute 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 65).

Table 65: Incidence of 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	1/1	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.

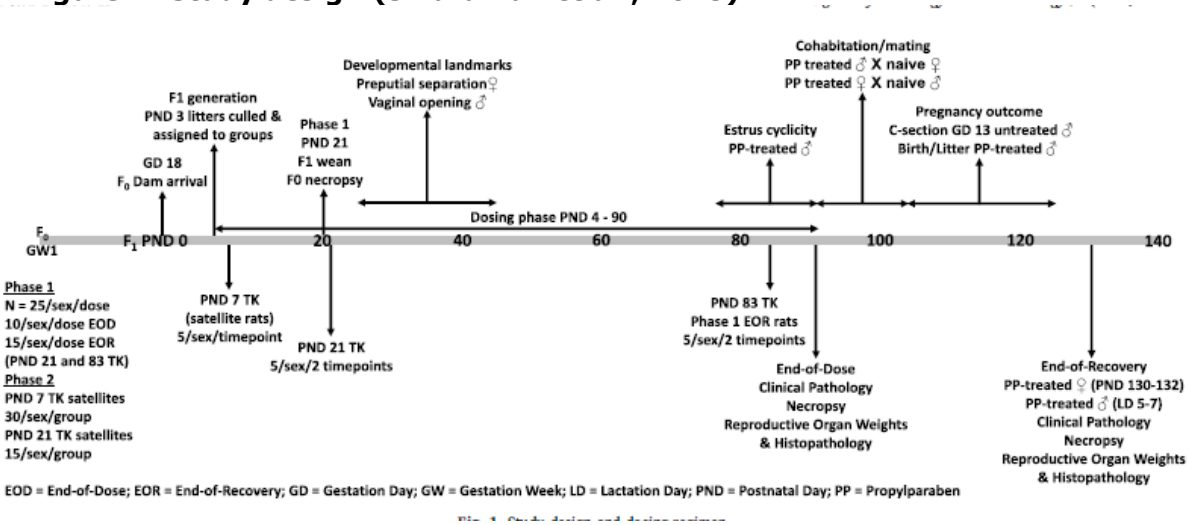
Assessment of propylparaben in juvenile rats (REACH registration dossier: Publication: Sivaraman *et al.*, 2018)

2 separate studies were assessed:

- Phase 1: Reproductive development and function in male and female rats when administered on PND 4 to PND 90
- Phase 2: Uterus weight in immature female rats when administered on PND 4 to PND 7 or 21

Pregnant dams were used to produce the F1-generation litters which were the experimental population

Figure 2: Study design (Sivaraman *et al.*, 2018)



Test substance: Propylparaben

Degree of purity: 99.7 %

Test animal: Species/strain/sex: Rat / SD / both sexes

Nb. of animals per sex per dose: Phase 1: 25/sex/dose (10/sex/dose end-of dose necropsy subset and 15/sex/dose reproduction/recovery subset)

Phase 2: 15 or 30/sex/dose for toxicokinetic assessment (PND 7 or 21)

Age and weight at the study initiation: Phase 1: 8.5 to 13.6 g for males and 8.7 to 13.6 g for females

Phase 2: 7.8 to 15.4 g for males and 6.5 to 14.6 g for females

Administration/exposure: Route of administration: oral, gavage

Duration and frequency of test/exposure period: phase 1: from PND 4 through PND 90.

Phase 2: from PND 4 through PND 7 or from PND 4 through PND 21

Doses/concentration levels: 0, 10, 100 and 1000 mg/kg bw/d

Vehicle: 1% hydroxyethylcellulose

Results and discussion:

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 bw/d 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 66)

Table 66: 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 67).

Table 67: 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, 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 (no more information available).

Assessment of propylparaben on juvenile male rats (REACH registration dossier: Publication: Gazin *et al*., 2013)

Preliminary study to assess pharmacokinetic parameters such as time and concentration at peak plasma concentration, distribution volume, elimination t1/2 and renal clearance.

Main study: study design based on other studies already published and performed to assess the effects of parabens on the male reproductive system (with male rats treated with similar oral doses, from weaning to adulthood)

GLP

Test substance: Propylparaben

Degree of purity: 100 %

Test animals: Species/strain/sex: rat / Wistar / male

Nb. of animals per sex per dose: Preliminary pharmacokinetic study: 18 males/dose (except for control group: 3 rats)

Main study: 20 male/group (divided into 2 subgroups: the first one was sacrificed and necropsied at the end of the 8-week treatment period and the second after a 26-week washout period (to cover 3 spermatogenic cycles) + Satellite groups (juvenile toxicity study): 17 male/group for treated group and 9 male for control group (for TK study)

Age and weight at the study initiation: 24 d old and 85 to 120 g on dosing day (PND 31) for preliminary study

Administration/exposure: Route of administration: oral, gavage

Duration and frequency of test/exposure period:

Preliminary study: single exposure on PND 31 (all animals sacrificed after time point H24)

Main study: start at PND 21 and lasted for 8 weeks (necropsy: first subgroup was sacrificed and necropsied at the end of the 8-week treatment period and the second subgroup after a 26-week washout period)

Doses/concentration levels: 0, 3, 10, 100 and 1000 mg/kg bw/d

Vehicle: 1 % hydroxyethylcellulose

Actual doses (mg/kg bw/day): on the first day of administration (preliminary study), routine analysis demonstrated that the actual doses for the 10 and 100 mg/kg bw were outside of the specifications (with actual doses of 5.71 and 47 mg/kg bw, resp). On PND 77, actual dose of the 10 mg/kg bw group was equivalent to 7.8 mg/kg bw and this level of exposure was taken into account when performing the pharmacokinetic study.

Results and discussion:

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 68). Furthermore, mean epididymal and testis sperm count were not affected. As observed in
Table 69, epididymal sperm motility parameters showed variations.

Table 68: 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 69: 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.

Effects of propylparaben on the male reproductive system (REACH registration dossier: Publication: Oishi, 2002)

No OECD guideline followed

Wistar rats exposed to propylparaben to examine the effects on the male reproductive system (male reproductive organ weight, sperm count and serum testosterone concentration)

Test substance: Propylparaben

Degree of purity: ≥ 99 %

Test animals: Species/strain/sex: rats / Wistar / immature male

Nb. of animals per sex per dose: 8 males/dose

Age and weight at the study initiation: 19 to 21 days and 52.5 ± 2.17 g

Administration/exposure: Route of administration: oral, feed

Duration and frequency of test/exposure period: 4 weeks

Doses/concentration levels, rationale for dose level selection: 0, 0.01, 0.10 and 1.0 % (corresp. approx. to 0, 12.4, 125 and 1290 mg/kg bw/d)

Post exposure observation period: no, animals were killed after 4 weeks of exposure

Vehicle: corn oil

Results and discussion:

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 70). The cauda epididymal sperm reserve and sperm concentration decreased in a dose-dependent manner and these reductions were significantly modified 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 70: 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**
Conc. ($x10^7$/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

In addition, two studies, not reported under the reproductive endpoint in the registration dossier (not following OECD test guidance) are looking at **female reproductive function**:

In **Vo *et al.* (2010)**, significant myometrial hypertrophy of the uterus was observed after oral exposure to 1000 mg/kg bw/day of propylparaben in Sprague-Dawley rats (exposure from PND 21 to PND 40).

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

For more details on these 2 studies, see section 7.10.3 Endocrine Disruption – rodent data

Other information: The substance has also been tested for spermicidal activity and assessed as anti-conceptive product, with direct activity on spermatozoa:

Table 71: Summary table of other information

Method description	Result	Description of results	Reference

Spermicidal activity in human semen species (Harris method): determine concentration to immobilize human spermatozoa	anti-conceptive product	Exposure to 4 parabens in concentration range 0.25 - 10 mg/mL Pass point concentration at 3 mg/mL for PPB, is much less compared to "international planned parenthood federation's" cut off point concentration being 90 mg/mL. PPB is thus considered as anti-conceptive product. MoA has been described for BPB (showing the stronger effect). BPB is a potent inhibitor of acrosin, a protein of spermatozoa essential for the penetration of the oocyte. Moreover, BPB has been shown to impair the sperm membrane function. Regarding the potency of other parabens in this assay: BPB > PPB > EPB > MPB.	Song *et al*., 1991 Reliability: 3 (lack of details)

<u>Conclusion on fertility</u>:

There is **no clear evidence of adverse effects on fertility**.

7.9.7.2 Developmental toxicity

Table 72: Summary table of developmental toxicity studies

Test method	Results	Study reference	Reliability
Prenatal Developmental Toxicity Study OECD TG 414 GLP Rat (Wistar) 25 pregnant females/group Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: GD 5 to GD 19	NOAEL (maternal toxicity): 1000 mg/kg bw/d NOAEL (development): 1000 mg/kg bw/d **Parental:** No mortality observed Bw: unaffected Pre- and post-implantation loss Resorptions: not modified Necropsy: no treatment-related findings nor histopathological changes observed **Pups:** Nb of live pups similar in all groups Litter and foetus weights unaffected Necropsy: no treatment-related effects observed	REACH registration dossier: Unpublished study report, 2018	1
EOGRTS with DNT and DIT OECD TG 443 GLP Oral, gavage	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)	REACH registration dossier: Unpublished report, 2021	1

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 Doses: 0, 100, 300 and 1000 mg/kg bw/d Duration of exposure: F0: min. 10 w in males and 14 d of premating, max 14 d of mating, gestation and through weaning in females F1: from weaning (PND 22) to terminal sacrifice and the respective cohorts	**F0 parental:** 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 **F1 pups:** Viability index not modified AGD and nipple retention sign. changed Thyroid hormone exhibited variations **Cohort 1A:** Clinical signs: moving bedding observed at the highest dose Bw: changed at the highest dose in males at D64 Balano-preputial separation slightly reduced **Cohort 1B and F2 pups:** Moving bedding in female of the mid dose and in both sexes of the highest dose BW: sign. higher during gestation and lactation		

	Pups: AGD and nipple retention sign. affected		
Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test OECD TG 422 GLP Rat (Wistar) 11/sex/dose Oral: feed Exposure: min. 4 weeks for males and approx. 7 weeks for females (Day 1 of pre-pairing to post-partum day 3) Conc.: 0, 1500, 4500 and 15000 ppm (corresp. in males to 98.0, 305.1 and 980.9 (pre-pairing period) and 59.3, 178.3 and 605.0 mg/kg bw/d (after pairing period) and in females to 116.0, 341.9 and 1076.4 (pre-pairing period), 121.6, 349.2 and 1124.6 (gestation) and 137.3, 431.8 and 1380.0 mg/kg bw/d (lactation))	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 eMSCA. **Parental:** 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 **Pups:** 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	REACH registration dossier: Unpublished study report, 2012	1
Assessment of propylparaben in juvenile rats 2 separate studies were conducted to assess the potential estrogen-mimetic effects on 1) reproductive	NOAEL: 1000 mg/kg bw/d Mean age of vaginal patency sign. lower at the	REACH registration dossier: Publication:	2

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 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	highest dose (within HCD) Preputial separation similar in all groups Necropsy: no treatment-related change observed **Pups:** Litter weight and viability index not modified No malformed pups observed	Sivaraman *et al.*, 2018	
Assessment on the impact of parabens on early pregnancy Subcutaneous injection Mouse Dose: 35 or 45 mg of propylparaben Duration of exposure: GD 1 to GD 4 (euthanised at GD 6)	NOAEL (parental toxicity): 45 mg of propylparaben NOAEL (developmental toxicity): 45 mg of propylparaben No modifications on the number of implantation sites at any dose group. No other information available	REACH registration dossier: Publication: Shaw and deCantazaro, 2009	2 (comment: information about nb of animal/group only mentioned in the table)

Prenatal Developmental Toxicity Study (REACH registration dossier: Unpublished study report, 2018)

OECD TG 414

GLP

*Test substance: P*ropylparaben

Degree of purity: 99.7 %

Test animals: Species/strain/sex: rats / Wistar / pregnant females

Nb. of animals per sex per dose: 25 pregnant females/dose

Age and weight at the study initiation: 11 - 12 weeks (at the arrival to the lab), 197 to 264 g

Administration/exposure: Route of administration: oral

Duration and frequency of test/exposure period: GD 5 to 19, daily (animals were killed and necropsied at GD 20).

Doses/concentration levels, rationale for dose level selection: 0, 100, 300 and 1000 mg/kg bw/d

Vehicle: 1 % hydroxyethyl-cellulose

Description of test design:

Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy): after an acclimation period of at least 5 days, females were paired with males (1 male to 2 females per cage)

Results and discussion:

All females survived during the study period and no body weight modification was noted (see Table 73). 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 73: 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 74).

Table 74: 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 75).

Table 75: 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.

Extended One-Generation Reproductive Toxicity Study (REACH registration dossier: Unpublished report, 2021)

Details of the study available in section 7.9.7.1 Fertility

Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test (REACH registration dossier: Unpublished study report, 2012)

Details of the study available in section 7.9.7.1 Fertility

Assessment of propylparaben in juvenile rats (REACH registration dossier: Publication: Sivaraman *et al.*, 2018)

Details of the study available in section 7.9.7.1 Fertility

Assessment on the impact of parabens on early pregnancy (REACH registration dossier: Publication: Shaw and deCantazaro, 2009)

Two different studies were explained in the article: one with butylparaben (Exp. 1) and the second one with propylparaben (Exp. 2). As these 2 studies did not induce the expected findings, an uterotrophic assay was additionally performed with butylparaben (Exp. 3)

Test substance: Propylparaben for Exp. 2 and butylparaben for Exp. 1 and 3

Degree of purity: not mentioned

Test animals: Species/strain/sex: mice / CF-1 and CD-1/ both sexes

Nb. of animals per sex per dose: not mentioned

Age and weight at the study initiation: average BW was 36.9 ± 0.26 and 35.0 ± 0.45 g for CF-1 and CD-1 mice (no sex distinction), respectively. All females were between 100 and 180 days old and males were between 150 - 420 days old.

Experiment 1:

Administration/exposure: Route of administration: subcutaneous injection

Duration and frequency of test/exposure period: once a day, for 4 days

Doses/concentration levels, rationale for dose level selection: 0, 0.05, 0.5, 5, 10, 15, 20, 30 and 35 mg butylparaben; other females were exposed to 17β-estradiol butylparaben and estradiol were dissolved in peanut oil: 0.05 and 0.5 mg butylparaben in 0.05 mL peanut oil; estradiol in 0.05 mL peanut oil; 5 mg butylparaben in 0.1 mL peanut oil; 10, 15, and 20 mg butylparaben in 0.3 mL peanut oil; 30 and 35 mg butylparaben in 0.45 mL peanut oil.

Vehicle: peanut oil

Description of test design:

CF-1 females were firstly weighed then separated (1 per cage) at the start of the experiment.

After 4 days, each female mice was paired with a previously proven fertile male in a new cage.

Females were then observed 3 times a day for sperm plug. When the sperm plug was seen, it was considered as day 0 of gestation (GD 1). Males were removed from the cage before the first injection.

Subcutaneous injection of butylparaben (purity unspecified) was administered daily from GD 1 to 4, starting 3 to 6 hours into the dark cycle. Each injection was given at four different sites to reduce the risk of local irritation (right and left flanks, rear middle area and scruff of the neck).

Females were placed in a new cage 14 to 16 days after mating than left without any disturbance until delivery.

After parturition, the number of pups born was reported, pups BW was recorded on PND 3 and remaining pups' survival rate was measured on PND 5. Due to an experimental error, no data was monitored in the groups exposed to 5 and 10 mg after PND 3.

An additional group was exposed to the highest dose in order to confirm the obtained results with a distinct source of butylparaben. Females received 35 mg butylparaben (purity > 99 %) in 0.45 mL peanut oil. A control group received the same volume of peanut oil at the same relevant site of injection depending of the day of treatment.

Experiment 2:

Administration/exposure: Route of administration: subcutaneous injection

Duration and frequency of test/exposure period: daily on GD 1 to 4

Doses/concentration levels, rationale for dose level selection: 35 or 40 mg of propylparaben

Vehicle: propylparaben was dissolved in DMSO (0.05 mL)

Description of test design:

Goal was to see if high doses of propylparaben would impact gestation and if the substance used direct measures of uterine implantation sites.
CF-1 females were inseminated as in experiment 1.
Females received either 0.05 mL DMSO or 35 mg propylparaben in 0.05 mL DMSO or 40 mg propylparaben in 0.05 mL DMSO.
Treatment occurred from GD 1 to 4. On GD 6, the mice were euthanized via CO2 asphyxiation and each uterus was removed after abdominal incision. The number of visible implantation sites in the uterus was counted as described by Berger *et al.* (2008) "Impact of acute bisphenol A exposure upon intrauterine implantation of fertilized ova and urinary 17β-estradiol and progesterone levels" in Reproductive toxicology 26:94-9.
The used definition of an implantation site was "a small, round swelling in an otherwise smooth, uninterrupted uterine horn".

Experiment 3:
Administration/exposure: Route of administration: subcutaneous injection

Duration and frequency of test/exposure period: daily injection, once a day, for 3 consecutive days

Doses/concentration levels, rationale for dose level selection: butylparaben: doses of either 0.735, 7.35 or 35 mg; selection rationale specified: included doses able to elicit an uterotrophic response according to Lemini *et al.* (2003) in "*In vivo and in vitro* estrogen bioactivities of alkyl parabens" in Toxicol. Ind. Health, 19, 69 - 79, and to include the highest dose of the Experiment 1.

Control group: positive control group received 500 ng 17β-estradiol

Vehicle: peanut oil; 0.45 mL per injection

Description of test design:
Adult CD-1 and CF-1 female mice were used. First they were bilaterally ovariectomized under sodium pentobarbital anaesthesia. Animals were caged per groups of 5 for a 24 h recovery period and then were isolated.
A randomization was performed to assign each animal to a treatment group (butylparaben – treatment group- or vehicle only – negative control group; or estradiol – positive control group).
Administration of treatment was made via subcutaneous injection, once a day, on three consecutive days starting from day 27 post-ovariectomy. A positive control group received subcutaneous injections of 17β-estradiol. Exposed mice receive either 0.735, 7.35 or 35 mg butylparaben or 500 ng 17β-estradiol. The control group received only peanut oil. Injection sites were changed every day to avoid any local irritation (right and left flank and scruff of the neck and administration occurred 3 - 6 h into the dark cycle. Butylparaben and 17β-estradiol were dissolved in 0.45 mL peanut oil.

Age and weight were counterbalanced across conditions. 24 h after the last injection (Day 30 post-ovariectomy), mice were weighed again and euthanized with CO_2. Hysterectomies were performed on each mouse via abdominal incision. The sutures made to avoid excessive bleeding after ovariectomy were removed by just excising the uterus at the base of the said sutures. Fat and mesentery were removed from the uteri, then the organ was put in pre-weighed micro-tubes to get the wet mass of each uterus. Uterine dry weight was obtained after 21 days of tissue storage in calcium sulphate crystals.

Results and discussion:

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 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.

Conclusion on developmental toxicity:

There is **no clear evidence of an adverse effect on development**.

7.9.8 Hazard assessment of physico-chemical properties

Not assessed.

7.9.9 Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not assessed.

7.9.10 Conclusions of the human health hazard assessment and related classification and labelling

No classification relevant effects were observed for human health hazards.

7.10 Assessment of endocrine disrupting (ED) properties

7.10.1 Data on endocrine activity

OECD CF level 1: Existing data and non-test information

Table 76: OECD CF level 1 data

Non-test data			
Estrogen activity			
Method	**Result**	**Description of Result**	**References**
QSAR toolbox v.2.3	+	Moderate ER binder	/
Molecular Docking Analysis	+	Binding energy of PPB -6.04 kcal/mol for Erα-LBD and -5.82 kcal/mol for ERß-LBD, indicating considerable binding potency	Liang *et al*., 2023
Thyroid activity			
Molecular Docking Analysis	+	PPB fits in the active pockets of TRα-LBD and TRβ-LBD. PPB formed one hydrogen bond with amino acids Leu311 and Asn331. Binding energy: TRα-LBD: -6.15 kcal/mol and TRβ-LBD: -5.73 kcal/mol	Liang *et al*., 2022

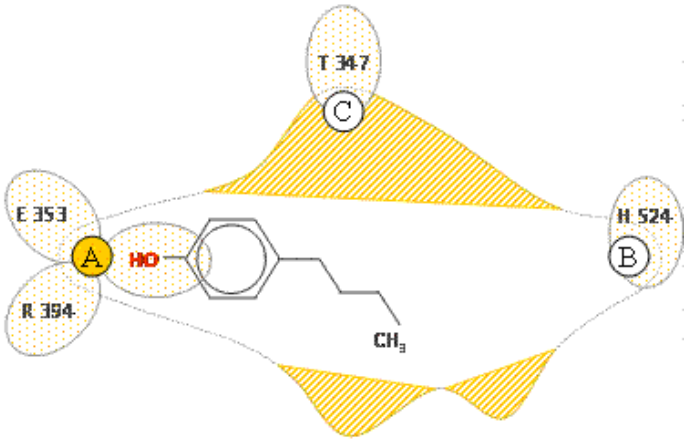
Estrogen receptor binding profiler (OECD (Q)SAR toolbox)

The ER-binding profiler classifies chemicals as non-binders or binders depending on molecular weight (MW) and structural characteristics of the chemicals i.e. a cyclic molecular structure with a single non-impaired hydroxyl- or amino group in the para- or meta-position on the ring. Substance with these features are ER binders. Binding potency is related to the size and shape of the molecule, which can be grossly measured by molecular weight. The ER profiler defines four categories of ER-binders:

1. Very strong binders: Chemicals with MW between 200 and 500 Da and two rings with a hydroxyl group connected to each of them.
2. Strong binders: Chemicals with at least one 5- or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 200 and 500 Da.
3. Moderate binders: Chemicals with at least one 5- or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 170 and 200 Da.
4. Weak binders: Chemicals with at least one 5- or 6-members carbon ring with an unhindered hydroxyl or amino group and MW less than 170 Da.

Propylparaben with one 6-carbon ring, an unhindered hydroxyl group and a MW of 180.2 Da falls in the category of moderate binders.

Figure 3: Scheme that reflects the ER binding of Propylparaben (ER-binding profiler, OECD (Q)SAR toolbox)



Liang *et al*. (2023)

In a molecular docking analysis the binding of 4 parabens (methylparaben, ethylparaben, propylparaben and butylparaben) to ERs was examined by using AutoDock Tools.

Parabens have considerable binding potencies to ERα and ERß:
Binding energies were resp. -5.81, -5.84, **-6.04** and -6.27 kcal/mol for Erα-LBD and -5.75, -5.69, **-5.82** and -5.96 kcal/mol for ERß-LBD for methyl-, ethyl-, **propyl**- and butylparaben.

Hydrogen bonds were found mainly with Leu327, Arg394 or Lys449 in ERα-LBD, and Arg346 or Lys401 in ERβ-LBD, respectively.

Liang *et al*. (2022)

In a molecular docking analysis the binding of 4 parabens (methylparaben, ethylparaben, propylparaben and butylparaben) to TRs was examined by using AutoDock Tools.

Bonds with

- TRα-LBD:
  Methylparaben formed 2 hydrogen bonds with the aminoacid Pro 393 and butylparaben with Ser277 and Arg228. While ethylparaben formed to hydrogen bonds with Leu311, propylparaben only formed one bond with this amino acid.

- TRβ-LBD:
  Methylparaben and ethylparaben formed one hydrogen bond with Leu365, while propylparaben and butylparaben formed one hydrogen bond with Asn331.

Binding energies:

Table 77: Binding energies with TRα-LBD and TRβ-LBD (From Liang *et al*., 2022)

Kcal/mol	MPB	EPB	PPB	BPB
TRα-LBD	-6.13	-6.01	-6.15	-6.36
TRβ-LBD	-5.45	-5.54	-5.73	-6.03

Conclusion: OECD CF level 1 data

* Estrogen activity: Propylparaben is predicted to have moderate ER binding potential.

* Androgen activity: /

* Thyroid activity: TR binding: stronger binding to TRα-LBD compared to TRβ-LBD.

* Stereoidogenesis: /

OECD CF Level 2: *In vitro* assays providing data about selected endocrine mechanisms/pathways

Table 78: OECD CF level 2 data

Estrogenic activity				
Method	**Short Method description**	**Result**	**Description of results**	**References**
US EPA Comp Tox Chemicals Dashboard	Estrogen receptor activity ToxCast: models	ER activity Estrogen	Positive in 14 ER assays out of 21 ToxCast pahway Model: ER Agonist: AUC= 0.205 (agonist: above the threshold of 0.1) ER Antagonist: AUC= 0.00 CERAPP Potency level (Consensus): Agonist: 1.00 (strong), antagonist:1.00 (strong) and binding: 1(strong) CERAPP potency level (From literature): weak agonist, strong antagonist, weak binding	US EPA Comp Tox Chemicals Dashboard
Standard in vitro assays				
US EPA OPPTS 890.1250	ER binding assay, rat uterine cytosol	ER binder	For PPB the Relative binding activity is 0.0006 %, IC50 is 1.5 x 10^{-4} M. In comparison with other parabens, ER binding affinities are BPP > PPB = EPB > MPB.	Blair *et al*., 2000 Reliability 2
OECD TG 455	Stable transfected hERα transactivation assay	Agonist ER activity (antagonism not evaluated in this study)	Relative transcriptional activation of estrogen receptor ERα by PPB, compared to 17β-estradiol is 69000 times less than E2, with PC50 (concentration inducting 50 % of the activity of E2) of 2.0 x 10^{-6} M, thus weak estrogen.	Kim *et al*., 2011 Reliability 1

			In comparison with other parabens, relative transcriptional activation of ER are BPB > IBPB > EPB > IPPB > PPB.	
Non-standard in vitro studies				
/	Recombinant hEr yeast assay with EPB, MPB, PPB, BPB and pHBA	Agonist estrogenic activity	PPB shows estrogenic response in yeast screen. Specificity of response based on the inhibition of PPB effect by anti-estrogen 4-hydroxy tamoxifen. All parabens showed estrogenic activity, except the metabolite pHBA. Potency for PPB is 30000-fold less than E2, with equivalent potency to nonylphenol. In comparison with other parabens, estrogenic activities are BPB > EPB > PPB > MPB.	Routledge *et al.*, 1998 Reliability 2
/	Yeast two-hybrid assay with ERα	Estrogen agonist of ERα	PPB shows estrogenic response in yeast two hybrid assay using a estrogen receptor ERα, with a REC10 of 1 x 10^{-5} M (conc. of PPB showing 10 % of the agonist activity of 10^{-7} M E2). In comparison with other parabens, estrogenic activities are BPB > PPB > EPB > MPB.	Nishihara *et al*., 2000 Reliability 3
/	Competitive binding for ERα and ERβ	ERα and ERβ binder	Competitive binding for both ER receptors in presence of DES (10^{-6} M -> 100 %), gave similar IC50 values for PPB, resp. 9.0 x 10^{-5} M for ERα and 6.0 x 10^{-5} M for ERβ. Relative binding affinity was resp. 0.033 % for ERα and 0.044 % for ERβ, compared to 100 for DES. The estrogenic activities of all parabens in this binding assay were IBPB > BPB > IPPB > PPB > EPB > MPB.	Okubo *et al*., 2001 Reliability 2

	MCF-7 proliferation, after 6 days treatment (E-screen)	Agonistic estrogenic activity	Maximal proliferation at 2 x 10^{-5} M PPB and an EC50 of 1.9 x 10^{-6} M PPB. Specific effect as PPB induced proliferation was suppressed by ICI 182,780. Relative proliferative potency compared to E2 is 1.5 x 10^{-6} M. The estrogenic activities of all parabens in this proliferation assay were IPPB > IBPB > BPB > PPB > EPB > MPB.	
/	Recombinant hERα yeast assay	Agonistic estrogen activity	Weak estrogen activity: 30000 less potent than 17β-estradiol. MPB, EPB and BPB was 3000000, 200000 and 8000 times less than 17β-estradiol resp.	Miller *et al*., 2001 Reliability 3
/	Competitive binding assay using cytosol of MCF-7 breast cancer cells - ERE-CAT transfected human breast cancer cells. - RNA analysis of pS2 gene in MCF-7 cells (northern blot) - Cell proliferation in MCF-7	ERα binder Agonistic ERα activity Agonistic ERα activity ERα mediated estrogenic activity	Competitive inhibition of E2 (16 x 10^{-10} M) by 26 % at 1.6 x 10^{-4} M, and by 77 % at 1.6 x 10^{-3} M. Comparison of potency to other parabens: Competitive binding assay: BPB > PPB > EPB > MPB. Increase of CAT gene expression (1.3-fold) at 10^{-5} M PPB after 24 h exposure, and 1.4-fold at 10^{-5} M PPB after 7 d exposure. Increase of pS2 gene expression, an endogenous oestrogen regulated gene, at 10^{-5} M PPB. Stimulation of MCF-7 cell proliferation at 10^{-5} M PPB up to level similar of that of E2 (range 10^{-10} to 10^{-7} M). Effect was specific ER-mediated, as suppressed by ICI 182,780. It is specific ERα-mediated as no significant proliferation is seen in MDA-MB-231 human	Byford *et al*., 2002 Reliability 2

			breast cancer cells (endogenous ERβ-receptor). MCF-7 proliferation: BPB > PPB > EPB > MPB.	
/	Proliferation assay with MCF-7 cells after 6 days treatment (= E-screen)	Agonistic estrogenic activity	Full concentration response curve cell proliferation with a max. effect similar to E2 (except MPB and metabolite). EC50 value for PPB EC50 was 1.5 x 10^{-6} M (tested conc.: from 10^{-9} M to 10^{-2} M). Specific effect, as PPB induced proliferation was fully blocked by ER antagonist ICI 182,780 at 10^{-8} M. No secondary effects: no cytotoxicity up to 10^{-3} M. Relative potency of PPB expressed as EEF (estrogen equivalent factor= ratio EC50 E2/EC50 PPB) was ± 10^{-6}, and similar for EPB, while about 10^{-5} for IBPB, BPB, BZPB and IPPB, and less for MPB and metabolite. Tested mixtures of E2 and parabens follow concentration addition model.	Van Meeuwen *et al*., 2008 Reliability 2
/	- Yeast two hybrid reporter assay with human ERα (hERα) and medaka ERα (medERα). - Competitive binding assay, using ligand screening system with ERα	Estrogenic agonist activity (via hERα and medERα) ERα binder	The EC10 of PPB (10-fold induction above baseline) was 4.1 x 10^{-6} M for hERα assay and 1 x 10^{-6} M for medERα (the latter thus more sensitive), and relative activity 3.2 x 10^{-5} for hERα and 4.4 x 10^{-4} medERα (tested conc.: 1.6 x 10^{-7} - 10^{-5} M). In the competitive binding assay the IC50 of PPB was 3.1 x 10^{-5} nM , and RBA of 3.3 x 10^{-4} (conc. tested: 3.8 x 10^{-9} M to 3.8 x 10^{-5} M) (DES as positive control).	Terasaki *et al*., 2009 Reliability 1

/	- Competitive binding assay for ERα and ERβ using a fluorescent estrogen	ERα and ERβ binder	Competitive binding for both ER receptors compared to Fluormone ES2 gave similar IC50 values for PPB, resp. 1.87 x 10^{-5} M for ERα and 1.65 x 10^{-5} M for ERβ. RBA compared to estradiol were 0.016 and 0.018 for ERα and ERβ, resp. The binding affinities of all parabens for both ER in this assay were IBPB > BPB > IPPB > PPB > EPB > MPB (not detectable).	Vo *et al*., 2010 Reliability 3
/	- GH3 cells exposure (24 h) to 4 parabens - ERE-transfected luciferase assay	Estrogenic activity mediated by ER Estrogenic activity mediated by ERα	Dose-dependent increase of CaBP-9k mRNA and protein, but only sign. at 10^{-4} M and 10^{-5} PPB (tested conc.: 10^{-7} to 10^{-4} M, compared to 10^{-9} M E2). Suppression by fulvestrant (anti-estrogen) indicates specific ERα-mediated pathway. Parabens have no pronounced effect on expression of ERα-receptor but strong increase in PR-receptor mRNA and protein expression as function of highest PPB concentration (also observed for E2). The latter is also specifically blocked by fulvestrant treatment. Induction of ERE luciferase activity by parabens similar to enhanced expression of CaBP-9K and PR, and specific inhibition after fulvestrant point to the fact that xenoestrogenic effects of parabens on CaBP-9K and PR are mediated through estrogen receptor signalling pathway. Observations on CaBP-9K by parabens are similar to those for alkylphenols, known to be weak estrogens in *in vitro* and *in vivo* assays.	Vo *et al*., 2011 Reliability 2

/	- MCF-12A 3D cell development assay	Estrogen-like effect	Disturbed acini formation similar to E2.	Marchese and Silva, 2012 Reliability 2
/	- Exposure (single or repeated dose) of MCF-7 human breast cancer cells or of MCF-10A human breast epithelial cells	Estrogenic agonist activity (ER-mediated proliferation)	(tested conc.: 2 x 10^{-10} to 2 x 10^{-6} M compared to 10^{-8} M E2). Proliferation after 48, 96, 144 and 196 h. **Proliferation** of MCF-7 cells sign. increased by E2, PPB and other parabens at single exposure of 2 x 10^{-10} to 2 x 10^{-7} M (high dose at 2 x 10^{-6} M is likely cytotoxic). Repeated exposure (96, 144 and 194 h) gave sign. increase of MCF-7 proliferation after PPB exposure, up to similar extent as E2. E2 and PPB have no significant effect on proliferation of MCF-10A cells, neither after single or repeated exposure. Only low doses of MPB and BPB (2 x 10^{-9} to 2 x 10^{-7} M) caused sign. increased proliferation of MCF-10A cells. Not secondary effects: clear low dose response (hormesis?). Mechanisms in both cell lines seem to be different, and effects of PPB more similar to those of E2, while MPB and BPB other response.	Wróbel and Gregoraszczuk, 2013 Reliability 2
/	MCF-10A cell proliferation assay	Estrogen-like effect	Proliferation of cells similar to E2.	Khanna and Darbre, 2013 Reliability 3
/	Human estrogen receptor α (hERα), hERβ and androgen receptor (hAR) models	Estrogen activity (agonist)	PPB was found to have agonistic estrogen activity. REC20 of 4.3 x 10^{-6} M and 1.7 x 10^{-7} M for Erα and Erβ resp.	Watanabe *et al*., 2013 Reliability 1

			Agonistic activity expressed as REC20 (20 % relative effective conc.), the conc. causing 20 % of the maximal response to E2 or DHT, antagonistic activity as RIC20 (20 % relative inhibitory conc.), the conc. causing 20 % inhibition. In comparison to EPB and BPB: MPB: REC20 ERα (agonist)= 4.3 $x10^{-6}$ M, REC20 Erβ (agonist)= 1.2 x 10^{-6} M BPB: REC20 ERα (agonist)= 2.9 x 10^{-7} M, REC20 Erβ (agonist)= 1.5 x 10^{-7} M	
/	Gene and protein expression in MCF-7 and MCF10A cell lines	Estrogenic	MCF-7: Sign. increased mRNA and protein expression of ERα, ERβ and PR. MCF-10A: mRNA expression: sign. increase of Erα (ESR1) only after 6 h, no alteration of (ESR2) and an increase of PR at 24 h. Protein expression: increased expression of protein levels of ESR1, ESR2 but not of PR.	Wróbel and Gregoraszczuk, 2014 Reliability 2
/	Yeast hERα assay (YES) and Effect-directed analysis (EDA)	Estrogenic	EC50= 3.3 x 10^{-6} mol/L Identified in fraction showing estrogenic activity.	Berger *et al*., 2015 Reliability 3
/	Action of GPR30 gene in MCF-7 and MCF-10A cells	Estrogenic effect on tumor proliferation	Sign. increase of GPR30 mRNA expression in MCF-7 (at 24 h) and MCF-10A cells (at 6 and 24 h). Sign. increase in GPR protein band density normalized to β-actin density in MCF-7 cells (at 48 and 72 h), but only sign. increased in MCF-10A cells at 48 h.	Wróbel and Gregoraszczuk, 2015 Reliability 2

/	ERα transactivation assay	Estrogenic	Estrogenicity= 1.1 ng/L EEQ	Scott *et al*., 2017 Reliability 3
/	Luciferase reporter assay and proliferation assay using T47D and MCF-7 tumor cells	Estrogenic	After 24 h PPB showed 4.7 % RTA (relative transactivation activity compared to 10 nM E2) at 0.5 µM and increased to 288 % at 50 µM (0.5**, 1******, 5******, 10******, 50****** and 100***** µM) in T47D-KBluc cells. ** p<0.05, ****** p<0.0001. PPB induced cell proliferation of T47D cells compared to 0.5 nM E2 (sign. at 10 µM, but not at 1 µM).	Majhi *et al*., 2020 Reliability 2
/	MCF-7 cell proliferation assay MVLN transactivation assay Erα and ERß -binding	Estrogenic	Sign. increase in MCF-7 cell proliferation after 48h exposure at ≥ 1 µM Dose-dependent increase of MVLN transactivation (steep curve) Erα: enthalpy change ΔH increased with the injection of Erα-ligand, indicating binding potency of PPB for ΔG: 8.13 kcal/mol for PPB, indicating that binding-proces with ERß-ligand is exothermic Corresponding KD: 0.68 µM for PPB ERß-binding EC50= 13.9 µM for PPB (compared to 9.4 nM for E2)	Liang *et al*., 2023 Reliability 1

Androgenic activity				
Method	**Short Method description**	**Result**	**Description of results**	**References**
Non-standard *in vitro* studies				
US EPA Comp Tox Chemicals Dashboard	High throughput ToxCast: models	AR activity androgen	Positive in 3 AR assays out of 15. ToxCast Pathway Model: Agonist: AUC= 0.00 Antagonist: AUC= 0.00 COMPARA (Consensus): Agonist: 0.00 Antagonist: 0.00 Binding: 0	US EPA Comp Tox Chemicals Dashboard
/	AR-reporter gene assay (AR-Eco screen) using stably transfected CHO-K1 cells and AR-binding assay	Anti-androgenic activity (weak) AR Binding	IC50= 8.6 x 10^{-5} M (reduction of 50 % of luciferase activity at 1.7 x 10^{-10} M DHT). IC50 >1.9 x 10^{-4} M (AR-binding): 40 % inhibition of testosterone binding at 1.9 x 10^{-4} M.	Satoh *et al*., 2005 Reliability 1
/	*In vitro* androgen receptor-mediated transcriptional activity assays, (stably transfected human embryonic kidney cells) (agonist & antagonist)	No androgenic activity Anti-androgenic activity	Parabens and metabolite showed no androgenic effects in range 10^{-9} M to 10^{-4} M. PPB inhibited sign. the transcriptional activity of testosterone (by 33 %) at 10^{-4} M PPB (anti-androgenic effect) while metabolite pHBA showed no anti-androgenic effects. No secondary effects: no cytotoxicity when tested at 10^{-5} M, combined with 1.25 x 10^{-10} M testosterone.	Chen *et al*., 2007 Reliability 2

			Comparison of inhibition between parabens: MPB (40 %) > PPB (33 %) > BPB (19 %).	
/	Recombinant rat AR binding assay	AR binder	Relative binding affinity to androgen receptor, compared to DHT is 0.0019 %, with EC50 of 9.7 x 10^{-4} M for PPB (similar to data by Fang *et al.,* 2001). Comparison of potency of PPB with 9 other compounds. In comparison with other parabens, AR binding affinities are IBPB > BPB > PPB.	Kim *et al*., 2010 Reliability 2
/	AR activation in reporter gene assay with AR-transfected CHO cells.	Equivocal as AR antagonist	Individual compounds and 4 different equimolar mixtures (fungicides, parabens...) have been tested in range 3 x 10^{-8} to 3 x 10^{-5} M, or 2.5 x 10^{-8} to 5 x 10^{-5} M. Paraben mixture included MPB, EPB, PPB, BPB and IBPB. Activity assessed with and without 10^{-11} M AR agonist R1881. Only IBPB showed an antagonistic effect on AR activation. PPB and BPB individually inhibited the R1881 induced anti-androgenic response, but this might be a secondary effect as it only occurred at cytotoxic concentrations. Paraben mixture showed antagonist effect, which however could be fully attributed to IBPB.	Kjærstad *et al*., 2010 Reliability 2
/	Human estrogen receptor α (hERα), hERβ and androgen receptor (hAR) models	No androgen activity	PPB showed no AR agonistic nor antagonistic activity.	Watanabe *et al*., 2013 Reliability 1
/	Yeast hAR assay (YAS)	Anti-androgenic	IC50= 3.9 x 10^{-5} M	Ma *et al*., 2014 Reliability 2

/	Human breast carcinoma MDA-kb2 reporter gene assay	No androgen activity (agonist and antagonist)	No agonistic nor antagonistic activity was observed after exposure to 25 µM PPB.	Kolšek *et al*., 2015 Reliability 2
/	Yeast anti-hAR assay (YAAS) Effect-directed analysis (EDA)	Anti-androgenic	IC50= 6.5 x 10^{-6} M Identified in fraction showing anti-androgenic activity.	Berger *et al*., 2015 Reliability 3
/	Recombinant Yeast Two-hybrid Assay	Androgenic activity	No anti-androgenic activity between 0.005 nM and 0.5 µM. A rapid increase of the anti-androgenic effect was shown at 0.5 µM and higher. Compared to other parabens, AR disrupting activity decreases as follow: BZPB > PNPB > MPB > EPB > PPB > BPB > pHBA. This confirms the findings of Chen *et al*., 2007. Hydrolysis by porcine liver esterase decreased anti-androgenic activity.	Ding *et al*., 2017 Reliability 2

Steroidogenesis				
Method	**Short Method description**	**Result**	**Description of results**	**References**
Non-standard *in vitro* studies				
/	Homogenized liver SULT inhibition assay using human liver and skin cytosol	Change in hormone balance: increase of estrogen level	50 % liver estradiol sulfon transferase inhibition at 30 ± 5 µM. 50 % skin estradiol sulfon transferase inhibition at 390 ± 140 µM.	Prusakiewicz *et al*., 2007 Reliability 2
/	Aromatase activity in microsomes from human placenta, and catalytic inhibiting capacity	Inhibition of aromatase (-> anti-estrogenic effect as it inhibits the conversion of androgens to estrogens)	Conc.-dependent and sign. inhibition (up to 50 %) of aromatase: At 10^{-5} M PPB (idem BPB and IPPB), (at 10^{-4} M EPB, MPB and BZPB). (PPB tested in range 10^{-7} M to 10^{-4} (compared to 10^{-6} M 4-OH-androstenedione= 4OH-A)	Van Meeuwen *et al*., 2008 Reliability 2

			Aromatase inhibitory potencies for the diverse parabens: PPB > EPB > MPB > IPPB > BZPB > BPB.	
/	Gene and protein expression of aromatase (Cyp19a1): by real time-PCR and western blot in MCF-7 and MCF-10A	Change in hormone balance: E2 secretion due to increase in CYP19a gene expression	Cyp19a1 mRNA expression detected in both cell lines, but only sign. increased in MCF-7 cells due to PPB and MPB, while sign. decrease in MCF-10A due to all parabens. **Effect on E2 secretion (ELISA) in both cell lines:** Stimulation (2-fold) of E2 secretion in MCF-7 cells due to PPB, sign. at 10^{-10} to 2 x 10^{-7} M compared to E2, (while less increased for MPB (only 2 x 10^{-10} to 2 x 10^{-8} M significant) and BPB (only 2 x 10^{-10} M significant)). In MCF-10A cells, sign. decreased E2 secretion for PPB at 2 x 10^{-7} and 2 x 10^{-6} M.	Wróbel and Gregoraszczuk, 2013 Reliability 2
/	Growth of isolated mice antral follicles	Disrupted steroidogenesis	Reduced antral follicle growth. Changed expression of genes involved in cell cycle, apoptosis, and steroidogenesis. Decreased DHEA, increased testosterone, and estradiol level.	Gal *et al*., 2019 Reliability 3
/	Secretion of E2 and T by H295R cells mRna expression of steroidogenic genes	Disrupted steroidogenesis	Hormone secretion: E2: negligible effect T: sign. decreased at 100**** µM (p<0.01) mRNA expression: Sign. increase of StAR at 100** µM Sign. increase of CYP11B2 at 100** µM	Liang *et al*., 2023 Reliability 1

			Sign. decrease of CYP19 at 1**** and 10**** £M Sign. decrease of 17ß-HSD at 1**** and 10** µM	

Thyroid activity				
Method	**Short Method description**	**Result**	**Description of results**	**References**
T-screen assay	GH3-cells (rat pituitary tumor cell line)	TR binding and activity	After 96 h: PPB induced cell proliferation in a conc.-dependent manner, sign. at 100 µM. => Demonstrating TR binding capacity.	Liang *et al.*, 2022 Reliability 2

Other MoA				
Method	**Short Method description**	**Result**	**Description of results**	**References**
Non-standard *in vitro* studies				
Prostaglandins (PG)	SC5 mouse juvenile Sertoli cells		IC50= 2.85 x 10^{-6}	Kristensen *et al*., 2011 Reliability 3
Glucocorticoid PPARγ	*Murine 3T3-L1 fibroblasts during 7 days of differentiation (with synthetic glucocorticoid mixture) *Activation of glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor (PPARγ) in reporter assays	GR mediated cells differentiation (agonistic activity) Activation of GR and PPARγ (both agonistic activity)	Exposure of parabens (MPB, EPB, PPB, BPB, BZPB) and metabolite 4-hydroxybenzoic acid (pHBA) and related benzoic acid (BA) during adipocyte differentiation phase. **Evaluation of morphology, lipid accumulation and mRNA expression of adipocyte marker genes:** Parabens promote in conc.-dependent way the 3T3-L1 differentiation, and potency is dependent on length of linear alkyl chain: MPB < EPB < PPB < BPB. Potency increased	Hu *et al*., 2013 Reliability 2

	*Competitive binding to GR (PolarScreen GR assay) *Adipose conversion in Human adipose-derived multipotent stromal cells (hADSC)	No GR binding GR mediated cells differentiation (agonistic activity)	when additional aromatic ring (BZPB). The paraben effect is confirmed by mRNA expression of adipocyte gene markers. After 18 h of exposure, GR-responsive luciferase reporter assay is activated by parabens, with potency similar to differentiation, but this seems not by direct binding to the ligand binding domain of GR as results of parabens in competitive binding assay did show no inhibition. Parabens transactivated PPARϒ, and potency was related to length of alkyl chain. Use of antagonists of GR and PPARγ decreased the response induced by parabens, confirming the specific activation of both receptors by parabens. Only for BPB (the most potent), the specificity was demonstrated by antagonism assay which showed suppression of activation. Adipose conversion of hADSC (differentiation during 7 days with 5 x 10^{-5} M parabens) and maintained for another 7 days in adipocyte status with parabens. No cytotoxicity of parabens up to 10^{-4} M.	
PPARγ	3T3-L1 lipid accumulation test PPARγ CALUX assay	Obesogenic	Strong PPARγ-dependent lipid accumulation (DLAmax= 1.91). Sign. increased expression of adipocyte marker gene aP2. 1.75-fold higher expression of PPARγ (10 µM).	Pereira-Fernandes *et al*., 2013 Reliability 1

Glucocorticoid	MDA-kb2 reporter cell line assay	Glucocorticoid-like activity	EC50= 1.3 x 10^{-5} M	Klopcic *et al*., 2015 Reliability 2
Glucocorticoid	Reporter gene assay, using the carcinoma MDA-Kb2 cells	Glucocorticoid activity	Exposure to 25 µM PPB induced an increase in >50 % of luciferase activity showing agonistic glucocorticoid activity. No antagonistic glucocorticoid activity was identified for PPB.	Kolšek *et al*., 2015 Reliability 2
PXR CAR PPARα	Reporter gene assay: PXR, CAR and PPARα: rat liver microsomes PPARα: human COS-7 simian kidney cells		Activity of hPXR dose-dependently increased, but not significantly at any conc. (0.3, 1, 3, 10 and 30 µM); REC20= 28 ± 17 µM. However, a sign. Increase of rPXR activity was seen at 30** µM; REC20= 4.9 ± 1.8 µM. Activity of rCAR dose-dependently increased but not sign.; REC20= 11 ± 5.3 µM. rPPARα activity was sign. Reduced at 3** µM and sign. Increased at 30** µM; REC20= 27 ± 2.9 µM.	Fujino *et al*., 2019 Reliability 2
PPARγ	3T3-L1 differentiation and lipolysis assay	Obesogenic	Modified lipid differentiation, with higher lipid content. Increased expression of PPARγ. Decreased level of glycerol released in basal conditions.	Elmore *et al*., 2020 Reliability 1

(Anti-)-estrogenic activity

US EPA CompTox dashboard

Estrogen activity was positive in 12 out of 18 high-throuput *in vitro* estrogen receptor assays included in the ToxCast ER bioactivity model:

NVS-NR-hER, NVS_NR_mERa, OT_ER_EraERa_0480, OT_ER_EraERa_1440, OT_ER_EraERb_0480, OT_ER_EraERb_1440, OT_ER_ErbERb_0480, OT_ER_ErbERb_1440, OT_Era_EREGFP_0480, ATG_Era_TRANS_up, ATG_ERE_CIS_up, TOX21_Era_BLA_Agonist_ratio.

Summary ToxCast model prediction:

> ToxCast pahway Model:
> ER Agonist: AUC= 0.205
> ER Antagonist: AUC= 0.00
>
> CERAP Potency level (Consensus):
> Agonist: 1.00 (strong), antagonist: 1.00 (strong) and binding: 1
>
> CERAPP potency level (From literature): weak agonist, strong antagonist, weak binding

***In vitro standard guideline* studies**

Blair *et al*. (2000)

A validated (standardized) estrogen receptor (ER) competitive binding assay using Rat Uterine Cytosol (OPPTS 890.1250) was used to determine the ER affinity for a large, structurally diverse group of chemicals. Relative binding affinity (RBA) values were determined as well as the IC50 values, i.e., the concentrations at which the maximal binding of the reference chemical [^{3}H]-estradiol was reduced for 50 %. Overall, 188 chemicals were assayed. In general, the results indicate that chemicals with 2 ring structures separated by 2 carbon atoms have higher RBAs compared to chemicals with a single ring structure or 2 rings separated by 1 carbon atom.

Propylparaben exhibited a weak binding to ER with a RBA of 0.0006 % (log RBA= -3.22). The mean IC50 value (50 % inhibition of [^{3}H]-17β-Estradiol binding) was estimated to be $1.5 \times 10^{-4} \pm 0.1$ M.

Kim *et al*. (2011)

In this study, the estrogenic activity of 13 chemicals has been determined using the validated Stably Transfected Transcriptional Activation Assay (STTA), known as OECD TG 455. The aim of this assay is to evaluate the ability of chemicals to function as an estrogen receptor alpha (Erα) ligand and activate an Erα agonistic response. The ability of the chemical to activate Erα receptors has been determined by detection of luciferase activity. PC50, i.e. the concentration of a test chemical at which the response is 50 % of the response induced by the positive control (17β-estradiol 1 nM), and the relative transcriptional activation (RTA) in comparison to estradiol have been determined.

Propylparaben show a weak estrogenic activity, with a PC50 of 2.0×10^{-6} M, and a log RTA of -2.84. This activity is about 69000-fold lower than 17β-estradiol.

In vitro non-standard guideline studies

Routledge *et al*. (1998)

In this study, authors evaluated the estrogenic activity of 4 parabens and para-hydroxy benzic acid (pHBA), which is the main metabolite of parabens, in a recombinant yeast estrogen screen. The DNA sequence of the human estrogen receptor (hER) was integrated into the yeast genome, which also contained expression plasmids carrying estrogen-responsive genes encoding for the β-galactosidase. In presence of estrogens, the enzyme is synthesized, and its activity can be measured spectroscopically. An inhibitor (4-hydroxy-tamoxifen) has been used to check the binding of the test substances to hER. Additionally, a competitive binding assay using rat uterine cytosol as ER source has been performed with butylparaben, as well as rat uterotrophic assay with methyl- and butylparaben.

Propylparaben showed a weak estrogenic activity *in vitro*, with an estrogenic response approximately 30000-fold less potent than 17β-estradiol. The activity is inhibited in a dose-dependent manner in presence of 4-hydroxy-tamoxifen.

Nishihara *et al*. (2000)

In this study, authors tested the estrogenic activities of 517 chemicals with a Yeast Two-Hybrid assay based on ERα. Two plasmids expressing ERα and the coactivator TIF2 have been introduced into yeast cells carrying a β-galactosidase reporter gene. After incubation for 4 hours with the test substance, the estrogenic activity has been determined by measuring the absorbance of the cells. The results were evaluated by relative activity, expressed as REC10, i.e. the concentration of the test chemical showing 10% of the agonist activity of 10^{-7} M E2.

Propylparaben showed a weak agonist estrogenic activity, with a REC10 of 1×10^{-5} M.

Okubo *et al*. (2001)

The estrogenic activity of 6 parabens has been investigated by assaying estrogen-receptor (ER)-dependent proliferation of human breast cancer MCF-7 cells. The proliferation of MCF-7 cells was determined after treatment with the test substances and 17β-estradiol for 6 days. Both 17β-estradiol and diethylstilbestrol (DES) have been used as positive control. An estrogen antagonist (ICI 182,780), which is known for interacting with ER, has been used as inhibitor of cell proliferation. Additionally, the binding affinities of the test substance to human ERα and ERβ were examined using the Ligand Screening –Estrogen Receptor (α) and (β) – System from Toyobo Co., Japan. The concentration of the test substance giving 50 % inhibition of ligand binding (IC50) in comparison with DES was determined *in vitro.* Finally, the effects of butyl- and isobutylparaben on expression of ERα receptor and progesterone receptor have been investigated by RT-PCR, as well as by Western Blot (for ERα only).

For the proliferation assay, the concentration giving half-maximal activation (EC50) of propylparaben was 1.9×10^{-6} M. In comparison, an EC50 value of 1.6×10^{-12} M was determined for 17β-estradiol. However, the maximal proliferation rate was similar to 17β-estradiol. When various concentrations of ICI 182,780 were added, cell proliferative effects were suppressed dose-dependently and completely. Propylparaben is suggested to be estrogenic, because its effects were completely inhibited by the pure estrogen antagonist.

Regarding the binding affinity, IC50 values of DES for ERα and ERβ were 3×10^{-8} M and 2.6×10^{-8} M, respectively. The IC50 values of propylparaben were 9×10^{-5} M and 6×10^{-5} M for ERα and ERβ, respectively. Relative binding affinities of propylparaben for ERα and ERβ were estimated to be 0.033 % and 0.044 % of that of DES. The estrogenic activities of all parabens in this binding assay were IBPB > BPB > IPPB > PPB > EPB > MPB.

Miller *et al*. (2001)

Estrogenic activity of 73 substances was assessed in a yeast assay where yeast cells were transfected with hERα together with expression plasmids containing the estrogen responsive elements and the *lac*-Z-reporter gene encoding the enzyme β-galactosidase.

Propylparaben showed weak estrogen activity: 30000 less potent than 17β-estradiol. In comparison, estrogenic activity of methylparaben, ethylparaben and butylparaben was 3000000, 200000 and 8000 times less than 17β-estradiol respectively.

The major criteria for activity appear to be the presence of an unhindered phenolic OH-group in a para position and a molecular weight of 140 – 250 Da.

Byford *et al*. (2002)

Estrogenic activity of 4 parabens has been assessed using estrogen sensitive MCF-7 human breast cancer cells. The study included ligand ability to (1) bind to ERα from MCF7 cell lysates in a competitive binding assay, (2) regulate an estrogen-responsive reporter gene (ERE-CAT) stably transfected into MCF-7 cells, (3) regulate expression of the endogenous *pS2* gene which is estrogen-regulated in MCF-7 cells, and (4) regulate the estrogen-dependent proliferation of MCF-7 cells.

During the competitive binding assay to ERα, about 26 % and 77 % inhibition of [^{3}H]-Estradiol binding was detected at 0.16 and 1.6 mM of propylparaben, respectively.

Regarding the assay of stably transfected ERE-CAT Reporter Gene in MCF7 Cells, the incubation of MCF7 cells with a propylparaben concentration of 10^{-5} M for 24 h led to a 1.3-fold increase in CAT gene expression. After an incubation period of 7 day, an 1.4-fold increase in CAT gene expression was noted at 10^{-5} M. In comparison, after 24 h and 7 d incubation periods with 10^{-8} M 17β-Estradiol, 2.6- and 2.9-fold increases of CAT gene expression were observed.

In the northern blot of pS2 mRNA from Propylparaben treated cells, an increase in expression of pS2 mRNA was observed at 10 µM.

During cell proliferation assay, the test substance gave a weak stimulation of cell proliferation at 10^{-6} M and a stronger stimulation at 5 x 10^{-6} M and 10^{-5} M. At 5 x 10^{-5} M, propylparaben stimulated the cell proliferation to the same extent as 17β-Estradiol at 10^{-7} to 10^{-10} M. The proliferation increase by 10 µM of the test substance was inhibited by the pure anti-estrogen ICI 182,780, suggesting that effects were ER-mediated.

Control experiments were performed using the MDA-MB-231 human breast cancer cell line, which lacks ERα and is unresponsive to 17β-Estradiol for proliferation. Proliferation of these cells was also unaffected by presence of up to 10^{-4} M propylparaben.

Van Meeuwen *et al*. (2008)

In this study, the estrogenic activity of several parabens has been assessed in estrogen-receptor (ER)-dependent proliferation assay of human breast cancer MCF-7 cells. Specificity of the effect has been checked by addition of ICI 182,780, an ER antagonist. Moreover, binary mixtures of ethylparaben and propylparaben, as well as mix of diverse parabens with 17β-estradiol (E2), have been tested. Cytotoxicity has been assessed in all experiments.

Additionally, the authors investigated if the substances were able to inhibit the enzyme aromatase, responsible of the conversion of androgens into estrogens. For this assay, one human placenta was used as source of microsomes. The catalytic aromatase inhibiting capacities of the compounds were tested by incubating the microsomes with radioactive-labelled androstenedion (^{3}H-androstenedion), which is a precursor of estrogens in steroidogenesis pathway. When activated, the aromatase provokes the release of tritiated

water (^3H), which can be then measured in a scintillator. Inhibition of aromatase activity led to a reduction of H^3 release.

Propylparaben did not show cytotoxicity. It stimulated the cell proliferation to the same extent as E2, with an EC50 value of 1.5 x 10^{-6} M. This effect was specific, as it could be fully blocked by the ER antagonist ICI 182,780. The estrogenic potency factor relative to E2 was around 10^{-6} for propylparaben. Assessments of paraben mixtures, as well as combination of one paraben with E2, showed additivity of the effects.

Terasaki *et al*. (2009)

Estrogen agonist activities of 7 parabens (methyl-, ethyl-, *n*-propyl-, *i*-propyl-, *n*-butyl-, *i*-butyl- and benzylparaben) and their relative mono- and di-chlorinated derivates have been investigated using a yeast two-hybrid assay based on either the human or medaka estrogen receptor α (hERα or medERα, respectively). The expression plasmids for the ligand-binding domain of each hormone receptor and coactivator have been introduced in yeast cell lines that carried the β-galactosidase reporter gene. The estrogen agonistic activity of each compound has been determined by measuring the concentration able to produce a chemiluminescent signal 10-fold stronger than the blank control (EC10) in the range 1.6 x 10^{-7} to 1 x 10^{-5} M. Based on these values, the relative activity (RA) to 17β-estradiol (E2) has been determined. Additionally, the estrogenic binding activity to hERα has been further assessed with a competitive enzyme-linked immunoassay (ER-ELISA) commercially available. In this assay, the tested substance has been put in competition with E2, and DES has been used as positive control. IC50 and relative binding affinity (RBA) to DES have been determined.

In yeast two-hybrid assay with the hERα, *n*- and *i*-propylparaben showed an EC10 of 4.1 x 10^{-6} M and 2.2 x 10^{-6} M, respectively, leading to a RA_{E2} of 3.2 x 10^{-5} and 5.9 x 10^{-5}. The medERα had a higher sensitivity (about 3-fold) for the parabens. Indeed, *n*- and *i*-propylparaben resulted in an EC10 of 1 x 10^{-6} M and 6 x 10^{-7} M, respectively, giving RA_{E2} of 4.4 x 10^{-4} and 7.7 x 10^{-4}. Mono-chlorinated derivatives showed activity almost in the same range, whereas di-chlorinated derivatives didn't show any estrogenic activity.

The ER-ELISA almost confirmed the estrogenic activity observed in the yeast two-hybrid assay. In competition with E2, *n*- and *i*-propylparaben showed an IC50 of 3.1 x 10^{-5} M and 4.6 x 10^{-6} M, respectively, leading to a RBA_{DES} of 3.3 x 10^{-4} and 2.2 x 10^{-3}.

Regarding the estrogenic activities of all parabens and their chlorinated derivates, the study concluded that parabens with a bulk substituent (i.e. butyl and benzyl groups) have higher potential than those with a sterically smaller group, and that chlorinated derivates are less estrogenic than their parent compounds.

Vo *et al*. (2010)

This study includes a receptor-binding assay to determine the relative binding affinity (RBA) of the substances to estrogen receptors in comparison with 17β-Estradiol. Competition of various parabens for binding receptors was determined with and ERα and ERβ screening system using a fluorescent estrogen, Fluormone ES2. The inhibition rate was calculated from the absorbance.

Propylparaben exhibited a weak binding affinity to both ERα and ERβ with an RBA of 0.016 % and 0.018 % respectively. The mean IC50 value (50 % inhibition of Fluormone ES2 to ER) was estimated to be 1.87 x 10^{-5} M for ERα, and 1.65 x 10^{-5} M for ERβ.

Vo *et al*. (2011)

In this study, the authors investigated the effect of parabens on GH3-cells, a rat pituitary cell line sensitive to estrogenic stimulation and estradiol-dependent for proliferation. In these cells, the Calbindin-D_{9k} (CaBP-9k) is present at high levels. CaBP-9k is a high affinity calcium binding protein regulated by 17β-estradiol (E2), and other steroids hormones, such as glucocorticoids and progesterone. An estrogen responsive element (ERE) and a

progesterone responsive element (PRE) are present in the CaBP-9k promoter and are known to mediate the transcriptional regulation of this gene. The expression level of CaBP-9k is thus a sensitive estrogenic biomarker. Several parabens have been tested at concentrations between 10^{-7} M and 10^{-4} M in the study: methyl-, ethyl-, propyl-, isopropyl-, butyl- and isobutylparaben.

An increase of CaBP-9k mRNA and protein level has been observed after 24 h exposure, depending on the dose and the length of the alkyl chain of the paraben. Propylparaben showed a significant increase at 10^{-4} M. Addition of fulvestrant, an anti-estrogen, clearly suppressed the paraben-induced increase in CaBP-9k expression.

The authors also tested the effect of parabens on mRNA and protein levels of estrogen receptor (ERα) and progesterone receptor (PR). Whereas the level of ERα was not significantly affected after exposure to parabens, there was a strong increase in PR mRNA and protein expression, in a pattern almost similar to the CaBP-9k expression one. This effect on PR expression was also suppressed by addition of fulvestrant.

To investigate further the role of ERE as enhancer, GH3 cells were transfected with luciferase reporting plasmids containing three copies of ERE sequence. In this case again, the induction of luciferase activity occurred similarly to the enhanced expression of CaBP-9k and PR following paraben exposure. And fulvestrant strongly inhibited the luciferase activity. This confirms that parabens act on CaBP-9k and PR via estrogen receptor signaling pathway.

Marchese and Silva (2012)

The authors developed an *in vitro* 3D model for breast glandular structure development using MCF-12A cells. These cells express ERα, ERβ and GPER (G-protein coupled to estrogen receptor 1). Under normal conditions, MCF-12A cells formed well-organized acini. These structures combine a basal deposition of basement membrane components and a hollow lumen, implicating cell polarity, controlled cell proliferation and apoptosis. Exposure to 10 pM to 10 nM E2 disrupted the acini formation, showing larger and misshapen structure with filled lumen (i.e., no apoptosis).

Exposure to 10 µM propylparaben showed similar effect on acini morphogenesis, resulting in structures significantly larger than controls, no more spherical, and filled lumen. Whereas these effects were partially reverted when MCF-12A cells were co-exposed to E2 or BPA and ICI 182,780 (an ER antagonist) and/or G-15 (GPER inhibitor), the effect was only partial in cells exposed to propylparaben, showing only a reduction of acini size, but not on filled lumen.

Wróbel and Gregoraszczuk (2013)

This study evaluated the effects of methyl-, butyl- and propylparaben on two different cell lines: MCF-7 human breast cancer cells (ER-sensitive) and MCF-10A human breast epithelial cells. In both cell lines, the effect on cell proliferation after single or repeated exposure to several doses of parabens (2 x 10-10 M to 2 x 10-6 M) has been assessed.

The level of estradiol secretion after exposure to the parabens has been determined using an ELISA assay. The gene and protein expression of aromatase (*CYP19A1*) have also been assessed by real-time PCR and Western Blot, respectively.

A single exposure to propylparaben (2 x 10^{-10} M to 2 x 10^{-7} M) provoked a statistically significant increase in the proliferation of MCF-7 cells of about 40 % compared to the control after 6 days. The repeated exposure to propylparaben led to increased proliferation of MCF-7 to all doses (25 – 30 %), already after 3 days. None of the parabens showed an effect on MCF-10A.

This study demonstrated that different cell lines react differently after exposure to parabens. Moreover, they showed that, regarding the expression of *CYP19A1*, the

estrogenic action of parabens is not only a consequence of the estradiol receptor binding ability.

Khanna and Darbre (2013)

This study assessed the ability of parabens (methyl-, propyl- and butylparaben) to increase cell proliferation of the MCF-10A cells. These cells are ERα but not ERβ competent and proliferate only after addition of 17β-estradiol (70 nM) under non-adherent conditions.

Addition of propylparaben significantly increases the number and the size of colonies. A dose-dependent increase was observed from 10^{-7} to 10^{-5} M.

Watanabe *et al*. (2013)

Estrogen activity of 17 parabens was examined in a reporter gene assay using Chinese hamster ovary cells transfected with plasmids using FuGENE 6 transfection Reagent.

Agonistic activity was determined as REC20 (20 % relative effective concentration), the concentration causing 20 % of the maximal response to 10^{-9} M E2 while antagonistic activity as RIC20 (20 % relative inhibitory concentration), the concentration causing 20 % inhibition.

Propylparaben was found to have agonistic estrogen activity with a REC20 of 4.3 x 10^{-6} M and 1.7 x 10^{-7} M for ERα and ERβ respectively. Methylparaben did not show agonistic activity towards ERβ (ERα not tested). REC20 ERα (agonist) was 4.3 x 10^{-6} M and 2.9 x 10^{-7} M for, REC20 ERβ (agonist) 1.2 x 10^{-6} M and 1.5 x 10^{-7} M respectively for ethylparaben and butylparaben

The relative potencies of the ERα agonistic activities decreased in the following order: benzyl-, isobutyl-, isopentyl- > phenyl- > heptyl-, hexyl- > octyl-, pentyl- > butyl-, isopropyl- > nonyl- > propyl- > ethylparaben.

The relative potencies of their ERß agonistic activities decreased as follows: benzyl- > phenyl- > isobutyl- > isopentyl- > pentyl- > butyl-, hexyl-, isopropyl-, propyl- > heptyl- > octyl- > nonyl- > ethyl- > decylparaben.

Furthermore, it was reported that the metabolism of parabens by carboxylesterases in rat liver microsomes markedly reduced the estrogenic activity.

Wróbel and Gregoraszczuk (2014)

The authors evaluated the effects of parabens (methyl-, propyl- and butylparaben) on mRNA and protein expression of estrogen receptors (ESR1 and ESR2 coding for ERα and ERβ, respectively) and progesterone receptor (PR). Two different cell lines, i.e. the MCF-7 breast cancer and MCF-10A non-cancerous cell lines, were exposed to 20 nM propylparaben for 6 and 24 h for mRNA expression or 48 and 72 h for protein expression.

MCF-7 cells are most sensible to propylparaben, as exposure significantly increases mRNA and protein levels of ESR1, ESR2 and PR. Moreover, co-exposure with 100 nM ICI 182,780 inhibits the propylparaben-induced protein level increase.

In non-cancerous MCF-10A cells, the results were more contrasted. Whereas for ESR1 and PR, both mRNA and protein level were higher after propylparaben exposure, only the protein expression of ESR2 increased. Moreover, the addition of ICI 182,780 was not able to prevent the effects of propylparaben on proteins expression, suggesting that these increases depend on different pathways.

Berger *et al*. (2015)

The aim of this study was to identify endocrine disruptors in plastic baby teethers (toys used to soothe baby's teething pain). The authors performed effect-directed analysis (EDA)

using yeast hERα (YES) and yeast anti-hAR assay (YAAS). Results of YAAS are discussed below.

Only 1 of the 10 teethers analyzed showed estrogenic (and anti-androgenic) activity. This sample consisted of Ethylene-vinyl acetate (EVA) and was the only one filled with cooling gel. GC-MS analysis of the estrogenic fraction allowed the identification of propylparaben. The estrogenic activity of propylparaben was then confirmed *in vitro* (YES) using the pure substance. Propylparaben showed an EC50 of 3.3 x 10^{-6} mol/L.

Wróbel and Gregoraszczuk (2015)

Authors investigated the estrogen effect of 17β-estradiol and methyl-, propyl-, and butylparaben tumor cell proliferation by measuring GPR30 gene and protein expression, cAMP levels, phosphor- and total ERK1/2 and Akt protein expression in MCF-7 breast cancer cells and MCF-10A breast epithelial cells.

It is known that estrogen-GPER signaling (GPR30) initiates rapid response through ERK1/2 -activation and cAMP generation.

Cells were exposed to 20 nM and 0.2 % ethanol. 10 μM Forskolin was used as a positive control to test the effects of propylparaben on cAMP.

In MCF-70, GPR30 gene expression was significantly increased after 24 h ($p<0.001$) and GPR30 protein expression significantly increased after 48 and 72 h of exposure to propylparaben. In the MCF-10A cells enhanced GPR30 gene expression significantly at 6 and 24 h while GPR30 protein level was only significantly increased at 48 h and not at 72 h.

Propylparaben exposure only increased cAMP levels significantly in MCF-10A cells ($p<0.001$).

Propylparaben significantly decreased phosphorylation of ERK1/2 in MCF-10A after 80 to 120 min, while in MCF-7 cells a significant increase was observed from 5 to 120 min after treatment. No effect was observed in Akt activation in MCF-10A cells, but significant effect of Akt activation in MCF-7 cells after exposure for 30 to 120 min.

Results suggest that propylparaben is able to modulate tumour cell proliferation by activating ERK1/2 and has the potential to inhibit apoptosis by acting on Akt phosphorylation.

Scott *et al*. (2017)

The authors assessed the estrogenic effect of organic contaminants found in Australian rivers including propylparaben on two Australian fish species (see details below). Additionally, they performed an *in vitro* transactivation assay (Erα-GeneBLAzer assay) to check the estrogenic potency of the contaminants.

The transactivation assay showed also a very weak estrogenic activity just above the detection limit of 1 ng/L (1.1 ± 0.1 ng/L EEQ).

Majhi *et al.* (2019)

The aim of this study was to assess the potential DNA damaging effect of propylparaben exposure in breast epithelial cells.

To check if this effect was ERα mediated, the authors also performed luciferase reporter assay, using T47DKBluc cells and compared the luciferase activity with 10 nM E_2 to determine the relative transactivation activity (RTA). Propylparaben showed a clear significant increase of luciferase activity at all doses (0.5**, 1******, 5******, 10******, 50****** and 100****** μM), showing a 4.7 % RTA at 0.5 μM, but up to 288 % at 50 μM.

A proliferation assay has also been performed to confirm the estrogenic activity. Propylparaben stimulated significantly the proliferation of T47D cells at 10 µM, but not at 1 µM.

Liang *et al*. (2023)

Parabens (methyl-, ethyl-, propyl- and butylparaben) were examined for their estrogen receptor binding and activation potential.

In a MCF-7 proliferation assay, cells were exposed to 0.2 – 100 µM of propylparaben for 6 days. Propylparaben induced proliferation significantly after exposure to 1 – 100 µM.

In a MVLN transactivation assay, cells were exposed to 2 – 100 µM of propylparaben for 48 h. Luminescence increased dose-dependently (steep curve).

To test the binding affinity to ERα in a isothermal titration calorimetry (ITC) assay, hERα recombinant protein was exposed to 100 µM of propylparaben. ITC profiles of propylparaben interactions with ERα-LBD was monitored at 298K. The dissociation constant (KD) and Gibbs free energy (ΔG) were calculated to be resp. 0.68 µM and 8.42 kcal/mol. The enthalpy change (ΔH) increased with injection of Erα-LBD into the propylparaben solution, indicating binding potency for ERα.
The binding affinity to ERß was tested in a Hit Hunter assay. Authors exposed hERß to 10^{-4} – 10^{2} µM of propylparaben. EC50 of propylparaben was 13.9 µM (9.4 nM for E2).

(Anti-)-androgenic activity

US EPA CompTox dashboard

Androgen activity was positive in 3 out of 11 Tox21/toxCast *in vitro* assays used in AR pathway model (Kleinstreuer *et al*., 2017).

- NVS_NR_cAR
- TOX21_AR_BLA_Antagonist_ratio
- TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881

Summary ToxCast Model predictions:

ToxCast Pathway Model:
Agonist: AUC= 0.00
Antagonist: AUC= 0.00

COMPARA (Consensus) :
Agonist: 0.00
Antagonist: 0.00
Binding: 0

In vitro non-standard guideline studies

Satoh *et al*. (2005)

Androgen and anti-androgenic effects of parabens were assessed in a reporter gene assay using stable transfected CHO-K1 cell lines (AR-Ecoscreen system). The binding affinity was determined in a competitive AR-binding assay.

The binding affinity of propylparaben to AR (IC50) was > 1.9 x 10^{-4} M, with an inhibition of 40 % of testosterone binding at 1.9 x 10^{-4} M.

No agonistic androgen activity was observed for propylparaben at 1.0 x 10^{-4} M, neither for the other tested parabens. Antagonistic activity was shown with an IC50 of 8.6 x 10^{-5} M.

In comparison, methylparaben and ethylparaben did not show antagonistic activity at 1.0 x 10^{-4} M.

Chen *et al*. (2007)

The study evaluated the androgenic and anti-androgenic effects of 3 parabens, the main paraben metabolite pHBA and 2 other antimicrobials. To identify the androgenic potency of the substances, an *in vitro* androgen receptor-mediated transcriptional activity assay was used. The assay is based on a stably transfected cell line that lacks critical steroid metabolizing enzymes (human embryonic kidney (HEK) 293 cells) that are stably transfected with an hAR gene (PCDNA6-hAR) and a luciferase reporting gene. The cells are highly responsive to endogenous steroids as well as synthetic compounds. The assay has been performed in absence and in presence of testosterone, to detect androgenic and anti-androgenic effects, respectively. Additionally, a cytotoxicity MTT assay for cell proliferation or cytotoxicity has been performed.

During the hAR transcriptional activity assay, neither the parabens nor the metabolite pHBA showed androgenic effects in range 10^{-9} M to 10^{-4} M. In presence of testosterone, propylparaben reduced by 33 % the transcriptional activity of the male hormone at 10^{-4} M (anti-androgenic effect).

No cytotoxicity or cell proliferation has been observed with propylparaben, alone or in combination with testosterone.

Kim *et al*. (2010)

In this study, the binding affinities of 9 substances for androgen receptor (AR) have been determined. The ability of test substance to displace a radiolabeled androgen (R1881) with high affinity for the AR is used as a measure of binding affinity to the AR. The assay is based on a commercially available rat recombinant fusion protein containing both the hinge region and ligand binding domain of the AR and Dihydrotestosterone (DHT) as receptor ligand.

Propylparaben showed a weak binding to AR with a RBA of 0.0019 % in comparison with DHT. The mean IC50 value (50 % inhibition of [^{3}H]-R1881) was estimated to be 9.7 x 10^{-4} M.

It should be noted that di(n-butyl) phtalate (DBP) and di(2-ethylhexyl) phtalate (DEHP), known anti-androgenic chemicals, did not show any significant AR binding activity.

Kjaerstad *et al*. (2010)

The aim of this study was to investigate the additive endocrine disrupting effects of 4 equimolar mixtures and to see if the observed effect was predictable using the concentration addition model. The androgenic and anti-androgenic effects of the single compounds and the mixtures have been tested in an androgen receptor (AR) reporter gene assay. The mixtures contained 1) substances known to be antagonists of the AR, 2) a mix of substances with and without activity on AR, 3) parabens, which are known to have weak to moderate endocrine disrupting effects including anti-androgenic effects, i.e. methylparaben, ethylparaben, propylparaben, butylparaben and isobutylparaben, and 4) azole fungicides, known to have endocrine disrupting properties.

The ability of the test compounds and the mixtures to activate the AR and to inhibit androgen-induced activation of the AR was tested in a reporter gene assay based on AR-transfected Chinese Hamster Ovary (CHO) cells transfected with a vector expressing the AR and a luciferase reporter plasmid. The substances were tested in absence or in presence of R1881, an androgen showing high affinity for AR. Hydroxyflutamide (HF), a strong AR antagonist, was included in every experiment as a positive control. Additionally, cytotoxicity of each substance and mixture has been assessed.

None of the tested mixtures or single compounds showed agonistic effects on AR. Regarding parabens, only isobutylparaben inhibited the R1881 induced activation of AR at 2.5 x 10^{-5} M and higher concentrations. Butylparaben and propylparaben showed anti-agonistic effects only at cytotoxic concentrations. The mixture of parabens showed higher response than expected, antagonizing the AR at concentrations of 2 x 10^{-6} M and above.

Watanabe *et al*. (2013)

Androgen activity of 17 parabens was examined in a reporter gene assay using Chinese hamster ovary cells transfected with plasmids using FuGENE 6 transfection Reagent.

Agonistic activity was determined as REC20 (20 % relative effective concentration), the concentration causing 20 % of the maximal response to 10^{-10} M DHT while antagonistic activity as RIC20 (20 % relative inhibitory concentration).

Propylparaben showed no antagonistic nor agonistic androgen activity in this study, neither did the other tested parabens.

Ma *et al*. (2014)

The authors assessed the androgenic and anti-androgenic activities of diverse parabens and parabens binary mixtures using a validated yeast hAR assay (YAS assay).

Propylparaben did not show any agonist activity but decreased significantly the β-galactosidase activity when co-exposed with 10^{-8} mol/L DHT (positive control). With an IC50 of 3.9 x 10^{-5} ± 3.9 x 10^{-6} M, propylparaben is an hAR antagonist.

Kolšek *et al*. (2015)

In a reporter gene assay, using the carcinoma MDA-Kb2 cells, 15 substances were tested for their androgen and glucocorticoid activity. The MDA-kb2 cell line is a stably transformed MDA-MB-453 cell line with the murine mammalian tumor virus (MMTV) luciferase neo reporter gene construct. Androgen and glucocorticoid receptor can both act on the MMTV luciferase reporter.

After a 30 minutes exposure to 25 µM propylparaben followed by the addition of 0.5 nM DHT and a 24 h incubation, the luciferase activity was assessed. No antagonistic androgenic activity was observed. Furthermore, propylparaben did not show agonistic androgen activity in the presence of 100 nM mifepristone (RU486), a known glucocorticoid antagonist.

Berger *et al*. (2015)

The aim of this study was to identify endocrine disruptors in plastic baby teethers (toys used to soothe baby's teething pain). The authors performed effect-directed analysis (EDA) using yeast hERα (YES) and yeast anti-hAR assay (YAAS). Results of YES are discussed above.

Only 2 of the 10 teethers analyzed showed anti-androgenic activity. One sample consisted of Ethylene-vinyl acetate (EVA) and GC-MS analysis of the anti-androgenic fractions allowed the identification of propylparaben and methylparaben. The other sample contained 6 unidentified androgenic substances. The anti-androgenic activity of propylparaben was then confirmed *in vitro* (YAAS) using the pure substance. Propylparaben showed an IC50 of 6.5 x 10^{-6} mol/L.

Ding *et al*. (2017)

Androgenic activity of 7 parabens was examined in a recombinant yeast two-hybrid assay, where yeast cells were transformed with hAR (pGBT9) plasmid, coactive plasmid and the reporter gene expressing β-galactosidase. The impact of hydrolysis of the tested parabens with different sizes of alkyl and aromatic side chains was investigated *in vitro* via esterase-

mediated hydrolysis. Furthermore, authors examined the contribution of the different side chains to the interaction with the AR ligand (AR LBD) by molecular docking.

Yeast cells were exposed to 0.005 nM to 50 µM of parabens. None of the tested parabens, including propylparaben, showed androgenic activity in this concentration range.

Propylparaben did not show anti-androgenic activity up to 0.5 µM, but the anti-androgenic effect increased rapidly at concentrations higher than 0.5 µM.

AR disrupting activity decreased as follows: BZPB > PNPB > MPB > EPB > PPB > BPB > HBA. Side chains with an aromantic moiety showed a stronger potency to AR disruption than alkyl side chains. This is in line with the findings of Chen *et al.* (2007) that methylparaben, propylparaben and butylparaben show anti-androgenic activity at 10 µM (resp. 40 %, 30 % and 19 %).

The molecular docking resulted in an order of binding potency to the AR LBD: BZPB > PNPB > MPB > EPB > PPB > BPB > HBA > HPB > OPB. Furthermore, a significant linear correlation was found between Glide docking energy and the maximum anti-androgenic activity (R2= 0.84, p= 0.01). Parabens with smaller docking score have stronger anti-androgenic activities.

It was shown that most of the tested parabens (except DPB, OPB and HBA) formed hydrogen bonds with AR LBD through the two amino acids Thr877 and Asn705, as is the case for DHT. These results suggest that propylparabens capable of competing with DHT induce anti-androgenic activity through those 2 enzymes.

Hydrolysis by porcine liver esterase was examined at concentration of 5, 25 and 50 µM of MPB, EPB, PPB, BPB, PNPB and BZPB. Anti-androgenic activity of those parabens were all significantly reduced after 2 h, indicating that hydrolysiation deactivated the anti-androgenic activity. Hydrolysation of propylparaben was very fast: V_{max}= 0.13 ± 002 mM/min (maximum reaction rate), K_m= 0.27 ± 0.04 mM (Michaelis constant). K_m values of tested parabens decreased with increasing alkyl side chain indicating that parabens with a longer alkyl side chain have lower hydrolysis rate. Furthermore, higher K_m values of parabens with an aromatic side chain show that these are less susceptive to hydrolysis by porcine hydrolysis. However, the hydrolysis rate in porcine liver esterase may differ from that in human plasma likely due to the different enzymatic concentration and enzyme specificity.

Furthermore, it should be noted that parabens can also metabolise via other pathways, like photochemical transformation. Furthermore, it is known that parabens can conjugate (glucuronidation/sulfonation) *in vivo*, which decrease their activity. Other metabolization pathways of parabens should thus be further explored.

Steroidogenesis

Prusakiewicz *et al*. (2007)

Parabens were examined for their ability to inhibit estrogen sulfo transferases (SULTs) in human skin cytosolic fractions and normal human epidermal keratinocytes (NHEK). Skin expresses the enzymes needed to convert circulating androgens and estrogen sulfates into estradiol. Propylparaben achieved a 50 % inhibition of skin cytosolic estradiol sulfation at 390 ± 140 µM, while in liver cytosol 50 % inhibition was achieved at 21 ± 5 µM. It was concluded that the potency of SULT inhibition increased as the paraben ester chain length increased. The hydolysis product p-hydroxybenzoic acid (pHBA) did not show an inhibition of the estradiol sulfation.

Van Meeuwen *et al*. (2008)

In this study, the authors investigated if the substances were able to inhibit the enzyme aromatase, responsible of the conversion of androgens into estrogens. For this assay, one human placenta was used as source of microsomes. The catalytic aromatase inhibiting capacities of the compounds were tested by incubating the microsomes with radioactive-labeled androstenedion (^{3}H-androstenedion), which is a precursor of estrogens in steroidogenesis pathway. When activated, the aromatase provokes the release of tritiated water (^{3}H), which can be then measured in a scintillator. Inhibition of aromatase activity lead to a reduction of H^3 release.

Additionally, the estrogenic activity of several parabens has been assessed in estrogen-receptor (ER)-dependent proliferation assay of human breast cancer MCF-7 cells (see above).

Propylparaben showed a statistically significant inhibition at 10^{-6} M of aromatase activity, resulting in a reduction to up to 55 % of the maximum aromatase activity. IC50 value for aromatase activity has been estimated to be 3.5 x 10^{-6} M.

Interestingly, at similar concentrations, the authors observed MCF-7 proliferation and aromatase inhibition. As aromatase is the enzyme responsible for conversion of androgens to estrogens, its inhibition could be considered as an opposite, thus anti-estrogenic effect, to the cell proliferation. However, some phytoestrogen also presents similar dualistic mode of action.

Wróbel and Gregoraszczuk (2013)

This study evaluated the effects of methyl-, butyl- and propylparaben on two different cell lines: MCF-7 human breast cancer cells and MCF-10A human breast epithelial cells. In both cell lines, the level of estradiol secretion after exposure to the parabens has been determined using an ELISA assay. The gene and protein expression of aromatase (*CYP19A1*) have also been assessed by real-time PCR and Western Blot, respectively. Additionally, the study evaluated the effect on cell proliferation after single or repeated exposure to several doses of parabens to assess the estrogenic potential of propylparaben (see above).

Exposure for 24 h to 2 x 10^{-8} M propylparaben significantly increased the expression of *CYP19A1* mRNA in MCF-7 cells, while a significant decrease was shown in MCF-10A cells. A similar pattern has been observed on protein expression. This observation was correlated with the estradiol secretion. Indeed, after 72 h exposure to propylparaben, a 2-fold increase was detected at doses between 2 x 10^{-10} M to 2 x 10^{-7} M, whereas a significant decrease of estradiol secretion was observed at highest doses (2 x 10^{-7} M and 2 x 10^{-6} M).

This study demonstrated that different cell lines react differently after exposure to parabens. Moreover, they showed that, regarding the expression of *CYP19A1*, the estrogenic action of parabens is not only a consequence of the estradiol receptor binding ability.

Gal *et al*. (2019)

This study investigated the effect of propylparaben on mouse antral follicles. After extraction, the follicles were cultured and exposed to 0, 0.01, 0.1, 1, 10 and 100 µg/mL (corresponding to 0.05 to 555 µM) for up to 72 h (n ≥ 8 / group).

Exposure to 100 µg/mL propylparaben inhibited strongly follicle growth. No effect on growth were observed at lower doses. Further analysis showed that propylparaben increased the expression of genes involved in cell-cycle regulation (cdk4 and cdkn1α), in apoptosis (bax), and in steroidogenesis (star). The authors finally investigated the level of hormones in culture media. Whereas no effect was observed on progesterone, propylparaben increased the production of estradiol and testosterone. A decrease in DHEA was observed transiently at 10 µg/mL. The gene expressions and hormone levels were

generally not consistent over time and/or not dose dependent. However, the authors concluded that propylparaben is able to disrupts antral follicle growth and steroidogenic function.

Liang *et al*. (2023)

In this study the effects of parabens (methyl-, ethyl-, propyl- and butylparaben) on steroidogenesis were examined using H295 R cells. Cells were exposed for 48h to 1, 10 and 100 µM of propylparaben.

Propylparaben disturbed the hormone balance: effects on secretion of E2 was negligible, while T was statistically significantly decreased at 100**** µM. Furthermore, steroidogenic mRNA expression was examined in H295R cells. The gene expression of StAR and CYP11B2 increased statistical sign. at 100** µM while CYP19 and 17ß-HSD statistically significantly decreased at 1**** and 10** µM.

Thyroid activity

Liang *et al.* (2022)

In a T-screen assay, GH3 (rat pituitary tumor cells) were exposed for 96 h to 4 parabens with following concentrations: 0, 50, 100, 200, 500, and 1000 µM for methyl- and ethylparaben, while 0, 5, 10, 20, 50, and 100 µM for propyl- and butylparaben. Results were compared to a solvent control (DMSO 0.1 %).

All parabens induced cell proliferation confirming TR binding and TR activity. Proliferation was only significant at 100**** µM for propylparaben, while at 500**** and 1000**** µM for methyl- and butylparaben and at 200****, 500**** and 1000**** µM for ethylparaben.

Other MoA

Kristensen *et al*. (2011)

This study investigated the effects of several known endocrine disruptors on the production of prostaglandins.

In a prostaglandin screen assay performed in SC5 mouse juvenile Sertoli cells, propylparaben showed an IC50 of 2.85×10^{-6}. The production of prostaglandin D2 was also reduced to less than 50 % in human mature mast cells. A co-exposure study with PPARγ agonists or antagonists suggest that the reduction of prostaglandin production is unlikely to be mediated by PPAR, even if propylparaben slightly increased PPAR activity.

Hu *et al*. (2013)

In this study, the effects of 5 parabens (methyl-, ethyl-, propyl-, butyl- and benzylparaben) on adipogenesis have been investigated. Murine 3T3-L1 cells are a well-known *in vitro* model to observe the conversion of fibroblast-like preadipocytes into mature, spherical, and lipid-filled adipocytes. The cells have been exposed to parabens for 7 days. Then the differentiation in adipocytes has been investigated by observation of morphology. To confirm the observations, an Oil Red O (ORO)-staining used to quantify the lipid accumulation in the cells, and the mRNA expression of adipocyte-specific markers, i.e. peroxisome proliferator-activated receptor (PPAR) γ, CCAAT-enhancer-binding protein (C/EBP), FABP4 and FAS, that are genes involved in lipid metabolism, adiponectin and leptin mRNA. Propylparaben enhanced clearly 3T3-L1 adipocyte differentiation, showing significantly positive results in all these tests (only the increase of leptin mRNA was not significant). The potency to induced adipogenesis was correlated with the length of the alkyl chain, showing the following ranking: benzyl- > butyl- > propyl- > ethyl- > methylparaben. Adipocyte differentiation has been also observed in human cells (hADSC).

Further investigation using only butylparaben revealed that this substance promotes the adipogenesis by modulating the early events of the differentiation process.

As activation of the glucocorticoid receptor (GR) is known to induce adipocyte differentiation, the ability of parabens to activate the GR has been evaluated using a GR-responsive luciferase reporter assay (MMTV-Luc). Propylparaben was able to increase the activation of GR in this assay, but not significantly. Regarding the potency, the same ranking as in previous experiment has been shown, with only butyl- and benzylparaben showing significant increase of GR activity. Activation of GR has been confirmed for butylparaben by performing a GR-activation assay in COS-7 cells, known to have no or little endogenous GR expression, and by showing the increase of lipin 1 mRNA, which is a biomarker of early event during adipocyte differentiation. Additionally, a competitive binding assay (PolarScreen GR competitor assay) has been performed to determine whether parabens can bind to the ligand binding domain of human GR in competition with a tracer glucocorticoid (Fluormone GS1). Interestingly, no parabens were able to bind the GR.

Activation of PPARγ has also been checked using a PPARγ-responsive luciferase assay. Propylparaben significantly increased the PPARγ activity. As previously, the potency of parabens increased with the length of the alkyl chain, but benzylparaben did not activate PPARγ. Activation of PPARγ by butylparaben has been confirmed by increased expression of mRNA of known target genes of PPARγ peripilin and FABP4.

In presence of GR or PPARγ antagonist, the adipocyte differentiation and increased mRNA expression of target genes induced by butylparaben was significantly suppressed, confirming the adipogenic effects of parabens through activation of these receptors.

Pereira-Fernandes *et al*. (2013)

The aim of this study was to develop a screening system for obesogenic compounds and to assess the potential of diverse chemicals, including parabens. The authors evaluated a lipid accumulation test in 3T3-L1 mouse pre-adipocyte cells and developed a reproducible standardised protocol in which the intracellular lipid content was quantified using Nile red stain. The degree of lipid accumulation (DLA) was calculated as the relative fluorescent units (RFU) of the test compound relative to the RFU of the solvent control.

Propylparaben show a strong dose-dependent obesogen potential, inducing a DLAmax of 1.91, and a LOEC of 100 µM. The obesogenic effect of propylparaben was further confirmed based on the gene expression level of the adipocyte marker gene aP2. When co-exposed with insulin, propylparaben also strongly increased the insulin-mediated adipogenic effect on 3T3-L1 cells (DLAImax= 2.33).

The authors then demonstrated the role of PPARγ for this obesogenic effect. First, they repeated the lipid accumulation assay in presence of T0070907, a known PPARγ antagonist, and the propylparaben-induced obesogenic effect was totally suppressed. Finally, they evaluated the potential PPARγ transactivation of the substance in a PPARγ CALUX assay. Propylparaben showed a strong activation of PPARγ, with a relative luminescence 1.75 higher than the control.

Klopcic *et al*. (2015)

The authors assessed the glucocorticoid-like activity of 4 substances present in all-days products propylparaben, butylparaben, DEHP and tetramethrin, individually and in mixture. They performed a reporter gene assay using the MDA-kb2 cell line, which expresses the endogenous glucocorticoid receptor and a luciferase reporter gene. All compounds were exposed to 10 nM and 1 µM.

Individually, propylparaben showed glucocorticoid-like activity at 1 µM, which was 1.57-fold higher than the solvent control, (EC50= 1.3×10^{-5} M).

Kolšek *et al*. (2015)

In a reporter gene assay, using the carcinoma MDA-Kb2 cells, 15 substances were tested for their androgen and glucocorticoid activity. The MDA-kb2 cell line is a stably transformed MDA-MB-453 cell line with the murine mammalian tumor virus (MMTV) luciferase neo reporter gene construct. Androgen and glucocorticoid receptor can both act on the MMTV luciferase reporter.

To assess agonistic glucocorticoid activity, cells were pretreated for 30 min with 0.5 µM of flutamide, a known AR antagonist. Propylparaben exhibited cytotoxicity at concentrations higher than 25 µM. After exposure to the single concentration of 25 µM propylparaben, an increase in >50 % of luciferase activity was seen.

Antagonistic glucocorticoid activity was assessed by treating the cells for 30 min with propylparaben or 100 nM RU486 (positive control) prior to addition of 500 nM hydrocortisone. After 24 h the luciferase activity was determined. No antagonistic glucocorticoid activity was identified for propylparaben.

Fujino et al. (2019)

The authors assessed the potential of 17 parabens to activate and/or inhibit PXR (human and rat), CAR (rat), and PPARα (rat), using reporter gene assays. Indeed, these receptors are involved in hormone metabolism and could therefore play a role in endocrine disruption.

Propylparaben (concentrations: between 0.3 and 30 µM) increased the activity of hPXR in a dose-dependent manner up to about 2x, but the increase was not significant. However, the dose-dependent increased activation of rPXR reached statistical significance at 30µM. The authors calculated a REC20 (20% relative effective concentration to the positive control rifampicin or PCN) of 28 ± 17 µM for hPXR and 4.9 ± 1.8 µM for rPXR. No inhibition was observed.

Propylparaben (concentrations: 1, 3, 10 and 30 µM) also increased dose-dependently, but not significantly, the activity of rCAR (about 1.5-fold). REC20 was reached at 11 ± 5.3 µM (positive control: artemisinin). No inhibition was observed.

Regarding rPPARα, propylparaben (concentrations: 1, 3, 10 and 30 µM) reduced the receptor activity at the 3 lowest doses (significant at 3 µM) but increased significantly the activity at 30 µM. REC20 was reached at 27 ± 2.9 µM (positive control: BZF, increased luciferase with 2.9 fold compared to control at 30 µM, REC20 = 5.5 ±0.52).

Elmore *et al*. (2020)

This study investigated the effect of methyl- and propylparaben on adipogenesis in 3T3-L1 white adipocytes and brown adipocytes.

In 3T3-L1 cells, propylparaben significantly enhanced the formation of lipid droplets during the maturation, with a greater neutral lipid content in a concentration-dependent manner (significant from 0.01 to 1 µM). Propylparaben also decreased the level of glycerol released in basal lipolytic conditions. A significantly higher mRNA expression of PPARγ and other genes involved in adipogenic differentiation and lipid metabolism (Cebpa, Lpl, Mgll, Plin1) was observed during early differentiation.

On the opposite in brown adipocytes, propylparaben exposure decreased the lipid content during the differentiation. No effect was observed on glycerol release during lypolisis.

CONCLUSION ON ENDOCRINE ACTIVITY

The *in vitro* studies show strong evidence for an estrogenic mode of action of propylparaben. Different types of *in vitro* assays with different approaches for endpoint measurements have been used (i.e., ER binding in Competitive binding assays, estrogen

activity in transactivation assays using recombinant yeast cells, stably transfected cell lines, cell proliferation assays using human MCF-7 breast cancer cell line). Both specificity and lack of interference by secondary effects (e.g., cytotoxicity) are shown. Propylparaben shows weak estrogenic activity, 5 to 6 ranges lower than 17β-estradiol.

Anti-androgenic activity of propylparaben is shown by five out of eight *in vitro* studies while in one study such activity could only be seen at cytotoxic concentrations.

Interference with aromatase in steroid pathway has also been investigated, but the results are not sufficient to conclude on this point, as different cellular models show different responses.

Four studies suggest that propylparaben interferes with the glucocorticoid and another showed Glucocorticoid-like activity. Three studies showed an induction of PPARγ activity.

Conclusion: OECD CF level 2 data

* Estrogen activity: Propylparaben shows agonistic ER activity.

* Androgen activity: Propylparaben shows anti-androgenic activity.

* Thyroid activity: No info

* Steroidogenesis: Interference with aromatase is suggested, but different cellular models show different responses. Propylparaben impacted steroidogenic gene transcription as well as secretion of T in H295R cells.

* Glucocorticoid and PPARγ: Glucocorticoid-like activity observed in different studies. Interference with the PPARγ receptors is suggested.

7.10.2 Endocrine disruption – Environmental data

As it is expected from the rodent data that endocrine disruption could be driven by propylparaben itself, available information about the toxicokinetic of the substance in fish is presented here:

In the study of Bjerregaard *et al*. (2003), Juvenile rainbow trout were exposed to a single dose of propylparaben (150 mg/kg). Approximately 8 % of the administered dose was retained in the liver and muscle after a few hours. It was possible to retrieve propylparaben from muscle until 24 h and from liver until approximately 48 h after feeding.

Following oral exposure, in fish, a chemical is absorbed from the gastrointestinal tract and reached the liver (which is the target organ for vitellogenin induction) immediately without prior dilution in the body (Alslev *et al.*, 2005). Knowledge on esterase activity in fish is very scarce, but in a study on 5 different fish species, nonspecific esterase activity was found in intestine, liver, and bile. Rainbow trout was found to have the lowest activity of the five species tested (Li and Fan, 1997).

Note: Reliability is based on the currently provided data in the (literature) studies.

OECD CF Level 3: *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s)

Table 79: OECD CF level 3 assays: environmental data

Method (guideline)	Short description of Method	Result	Description of Result	References	Reliability
Non-standard assay	Exposure: Juvenile rainbow trout Intraperitoneal injection: 100 & 300 mg/kg (as 1 mL/kg fish) at day 0 & 6. Effect measurement: Plasma vitellogenin, in samples at day<0, day 6 and day 12	Increased VTG	**Time-dependent sign. Increase of VTG** at 100** mg/kg (15-fold) and 300** mg/kg (1000-fold), though fish toxicity at highest dose. **Not a secondary effect** due to toxicity or starvation. Potency PPB comparable to BPB, 60-fold more potent than EPB (related to length of alkyl side chain).	Pedersen *et al*., 2000	3 (not a relevant environmental route used, high mortality at each dose)

Non-standard assay	Exposure: Sexually immature rainbow trout Oral: 7 to 1830 mg/kg/2 days, 5 times during 10 days period Aquatic: 50 – 250 µg/L, during 12 days period (no feeding) Effect measurement: Plasma vitellogenin PPB accumulation in liver and muscle	Increased VTG	**Time-dependent increase of VTG**, no difference between sexes. **Food exposure**: ED50 VTG increase was 35, 31, 22 mg/kg/2 days at day 3, 6 and 11. **Aquatic exposure**: only significant VTG increase at 225/250 µg/L ($p<0.05$), resulting into 29 mg/kg/d uptake, and VTG effect similar to the 33 mg/kg/2-day food uptake. Food exposure slightly more potent than aquatic exposure, higher level in liver. Though target is hypothalamus-pituitary-gonad gland. Accumulation: < 0.1 mg PPB end of experiment, likely metabolisation, half-life in liver is 8.6 h, and in muscle 1.5 h. Comparison to 4-tert-OP: for food exposure PPB is slightly more potent (22 mg/kg/2 days vs 35 mg/kg/2 days for 4-tert-OP), while for aquatic exposure 4t-OP more potent with LOEC 4.8 µg/L compared to PPB with LOEC 250 µg/L.	Bjerregard *et al*., 2003	2
Non-standard assay	Exposure: Adult male medaka, Oryizias latipes, exposed for 7 days to 0.055, 0.55, 5.5 and 55 mM (or 9.9, 99.1, 991 & 9911 mg/L) propylparaben compared to E2, and other UV screens 4-MBC (4-methyl-benzylidene camphor) and OMC	Increased VTG	**Dose-dependent increase of VTG by PPB exposure**, up to levels similar to those induced by 10^{-5} times E2) (tested in range 3.7 – 185 nM). **Dose-dependent, significant increase of VTG-1, VTG-2, CHG-L, CHG-H and Erα ($p<0.01$)**. No effect on ERβ and AR, except for the highest PPB concentration with significant increased ERβ expression.	Inui *et al*., 2003	3 (important data not reported: e.g. test conditions, analytical data, mortality, …)

	(octyl-methoxycinnamate). <u>Effect measurement:</u> VTG in plasma by ELISA VTG-1, VTG-2 (subtypes), CHG-L, CHG-H (subtypes of choriogenin, liver derived precursor protein for fish egg envelope), Erα, ERβ and AR in livers by real-time RT-PCR				
Non-standard assay	<u>Exposure:</u> 20 days exposure of juvenile zebrafish to PPB (0.55, 2.2, 4.99 µM or 0.1, 0.4 and 0.9 mg/L) compared to E2, positive control (0.055 nM= 100 ng/L). Each condition 30 fish, solutions refreshed 3/week <u>Effect measurement:</u> VTG concentration in whole body homogenates of zebrafish, determined by ELISA	Decreased VTG	Sign. Lower VTG ($p<0.001$) in PPB treated fish (mean 240, 218, 270 ng/mL for respectively 0.55, 2.2 and 4.99 µM PPB) compared to control (mean 400 ng/mL, range 395 – 540), while about 100 times increase by E2 treatment (35553 ng/mL, mean value). (Authors suggest anti-estrogenic effect, though to be consider with cautious, differences are minor with control group).	Mikula *et al*., 2006	2

Non-guideline individual-separated exposure system	*Xenopus tropicalus* (NF stage 51) Test conc: 0.05, 0.5 and 5 mg/L Exposure period: 14 d Parameters examined: Body weight, total body length, snout - vent length (SVL), hind limb length (HLL) and reached developmental stage, thyroid gene expression		No effect was seen on growth and development, but *dio3* an *ttr* were downregulated.	Pohl J, 2015	3 (e.g. no thyroid histopathology performed, spacing factor of 10 between concentrations, validity criteria only partially fulfilled, analytical monitoring performed but not reported)
Non-guideline study	*Drosophila melanogaster* (fruit fly) Test conc: 200, 500, 1000 and 2000 mg/L in food	Fecundity Growth Lifespan Climbing activity SOD	Stat. sign. decrease of fecundity ($p<0.01$) Stat. sign. delayed growth at 1000**** and 2000*** mg/L Stat. sign. effect on mean lifespan: F: at 1000** and 2000** mg/L, M: at 1000**** and 2000** mg/L Decreased with age in all treatments, but with higher activity in the 1000 mg/L group compared to control Stat. sign. increase at 1000**** mg/L, while decreased at 2000** mg/L	Li *et al*., 2015	2
According to OECD TG 236 (Fish Embryo Acute Toxicity)	8 h and 80 hpf exposure of zebrafish embryos (*Danio rerio*)	Mortality	Mortality: 100 % mortality at 10 mg/L. Sign. decrease (with 75** %) of epiboly stage at 8 hpf. Increased abnormalities (yolk-sac and	Torres *et al*., 2016	2

	Sea urchin (*Paracentrotus lividus*), larvae Mortality, length, abnormalities	Embryonic development length Abnormalities	tail) and decreased heart rate at 8 hpf; increased eye, head, pericardial edema, tail, yolk-sac abnormalities, and decreased heart rate at 80 hpf. Decreased hatching rate (sign. At 6000**, 8500** and 10000** µg/L). Sign. decrease of larval length at 400**, 1000** and 10000** µg/L. Sign. increase of abnormal larvae at 400**, 1000** and 10000** µg/L.		
Non-standard assay	7 days exposure of rainbowfish and mosquitofish to propylparaben	Increased VTG	Weak increase (3-fold) of vitellogenin protein in rainbowfish. No effect observed in mosquitofish.	Scott *et al*., 2017	2
Modified OECD TG 231 (AMA)	Amphibian Metamorphosis assay Acute toxicity: 5.0, 12.5 and 50 mg/L Exposure: 14 d exposure of Silurana (Xenopus) tropicalis tadpoles to 0.05, 0.5, 5mg/L Effect measurement: Body weight and length, snout-belly and hind limb	Acute toxicity No thyroid effect	Acute toxicity at 12.5 mg/L (100 % mortality) No delay in metamorphosis, no effect on body length nor any developmental marker. No change in thyroid epithelial cell height.	Carlsson *et al*., 2019	2

	lengths, thyroid epithelial cell height				
Daphnia magna Reproduction Test (OECD TG 211)	Exposure Nom. conc.: 0.10, 0.32, 1.00, 3.16 and 10.0 mg/L (TWM: 0.07, 0.25, 0.80, 3.10 and 10.8 mg/L (66.3 – 108 % of nom.). Effect measurement: Male offspring	No males observed	No indication of hormonal activity.	REACH registration dossier, Unpublished study report, 2019	1
Non-standard assay	*Drosophila melanogaster* (72 h old) Exposure duration: 6 h Exposure conc.: 50, 100 and 200 mM	Pupation Maturation Offspring number and fecundity	No sign. effect was seen on the pupation percentage after exposure to PPB (99 ± 0.01, 100 ± 0.00 and 100 ± 0.00 compared to control 100 ± 0.00). However, the mean pupation time (h) increased sign. (70.6** ± 0.87, 71.6** ± 0.85 and 71.4** ± 0.86 compared to the control 69.2 ± 0.87) PPB exposure showed no effect on maturation percentage (93 ± 0.03, 94 ± 0.03, 97 ± 0.02 compared to control 99 ± 0.01), while maturation time (h) increased sign. (72.7** ± 0.89, 72.5* ± 0.87, 72.2* ± 0.87 compared to control 70.5 ± 0.86). No effect was seen on mean offspring number (12.91 ± 0.74, 13.26 ± 0.74, 13.82 ± 0.67 compared to control 13.16 ± 0.73).	Atli E., 2022	2

			Daily mean fecundity was sign. decreased at all concentrations: 4.97* ± 0.42, 4.87 **± 0.42, 4.84** ± 0.40 compared to control (7.09 ± 0.56). PPB caused a delay in development and decreased fecundity and mean offspring number		
Non-standard assay	dengue mosquito (*Aedes aegypti L.*): eggs and larval stage Exposure conc.: Eclosion (at 12 and 24h): 0.02, 250, 500, 1000 mg/L larval mortality (at 12, 24 and 36 h): 0.02, 25, 50, 100, 250, 500 and 1000 mg/L Pupation (4d exposure): 0.02, 25, 50, 100, 250, 500, 1000 mg/ L Emergence, fecundity and F1: 0.02, 25, 100 mg/L	Eclosion (number of hatched eggs), larval mortality, pupation, emergence, fecundity and F1	Eclosion: Sign. reduction in hatched eggs after 12 and 24h of exposure at all concentrations: after 24 h in the control was 85.7 ± 3.6 %, 23.4**** ± 25.2 % at 0.02 mg/L, 0****** % egg at 250, 500 and 1000 mg/L. Larval mortality: Time and dose-dependent increase in mortality: at 36h: 0 % in control and 0.02 mg/L, 8.7 ± 14.0 %, 12.0 ± 7.0 %, 11.7 ± 9.2 %, 71.7 ± 10.5 %, 99.0 ± 1.1 %, and 100 % was observed for resp. 25, 50, 100, 250, 500, and 1000 mg/L => LC50= 182.6 mg/L Pupation: dose-dependent reduced pupation: 78.3 ± 9.5 % pupation in control, 45.3 ± 3.0 %, 28.7 ± 14.9 %, 21.7 ± 18.4 %, 20.3 ± 8.9 %, and 3.3 ± 3.5 % in 0.02, 25, 50, 100, and 250 mg/L. Emerging adults from acute test: sign. reduced in a dose dependent manner: 68.1 ± 7.9 % in control, 44.9**** ± 16.2 %, 34.9****** ± 15.6 %, 24.6****** ± 13.2 % and 13.1****** ± 7.4 % at 0.02, 25, 50, and 100 mg/L, 0****** % at 250, 500 and 100 mg/L	Calma and Medina, 2020	2

			Fecundity (mean number of eggs): non sign. difference with control (84.05 ± 19.51): 53 ± 1.41, 77.25 ± 20.53 and 74.5 ± 0.71 at 0.02, 25, and 100 mg/L. F1 eclosion: dose-dependent reduction, hatched eggs in control 90.2 ± 4.8 %, eggs from treated parents (0.02, 25, and 100 mg/L) put in untreated water: resp. 96.7 ± 1.0 %, 100 ± 0 %, and 87.1 ± 0 % Eggs from treated parents further exposed to 0.02, 25, and 100 mg/L: resp. 89.6 ± 9.6 %, 93.1 ± 2.7 % and (sign. P<0.05) 58.7 ± 0 %.		
Non-standard assay	Zebrafish embryos (2 hpf) Exposure conc.: 2,5 and 10 µM), 0.1 % DMSO (v/v) Exposure duration: 120 hpf	Gene Transcription (HPG axis) Steroid hormone levels and VTG	Sign. downregulation of gnrh (gnrh2, gnrh3 and gnrh1), fshß and their receptors (gnrhr1, gnrhr2 and gnrhr4) U-shaped response for fshr, lhß and sex hormone receptors (er2b and ar) and steroidogenic related genes (i.e. cyp11a, cyp17, cyp19a, cyp19b, 3β-hsd, hmgra, hmgrb, star and 17β-hsd) Sign. increased VTG levels at 2**** µM Sign. increased T levels at 2 **and 10**** µM	Liang *et al*., 2023	1
Non-standard assay	Adult male mosquitofish (*Gambusia affinis*) Exposure conc.: 0, 0.15, 6.00 and 240 µg/L Exposure duration: 4 and 32 days	Gene transcription of HPG and liver axis Histopathology of testis	<u>After 4d exposure</u>: No sign. difference in expression of genes in the brain (erα, erß, arα, arß, gnrh, gnrhr and cyp19a1b) compared to the control Upregulation of erα, erß, arα, arß, vtgß, vtgC and cyp19a in liver at all conc. Upregulation of star at 6 and 240 µg/L. <u>After 32d exposure</u>: Increased gene transcription in brain at 0.15** µg/L and sign. decrease at 240** µg/L of all	Ma *et al*., 2023	1

			genes (era, erb, ara, arb, gnrh, gnrhr and cyp19a1b) Only expression of erß and cyp 19a sign. increased at 6** and 240** µg/L. No effect on proportion of germ cells, sign. decrease in ratio of mature sperms at 240 µg/L after 32 d exposure, while % of primary spermatocyte was higher at 240** µg/L at 32 d compared to 4 d exposure: delayed spermatogenesis		

Pedersen *et al*. (2000)

Guidelines : non-guideline

GLP : non-GLP

Material and methods:

Test substances: n-propylparaben
Test species: Rainbow trout (Oncorhynchus mykiss)
Type of test: flow through (fresh water)
Species life stage: sexually immature juveniles (80 – 120 g), mixed sex population
Acclimation period: 1 week
Route of administration: intraperitoneal injection
Test conditions:
Photoperiod: 12 h light/12 h dark
Feeding fish were starved to avoid possible hormonal exposure via the food
Test design:
Applied concentration: 0 (solvent control), 100 and 300 mg/kg
Positive control: 17β-oestradiol
Vehicle: 48 % DMSO
Replicates: 10 fish in 80 L steel tanks
Exposure period: injection at day 0 and 6
Test duration: 12 days
Parameters examined: Vitellogenin plasma levels
Data analysis and Statistics:
VTG plasma levels were determined with a direct sandwich ELISA
Kruskall-Wallis to test differences between control and exposed groups, calculated only on day 12 due to testing of repeated measures which are invalid for non-parametric approaches. This test was followed by Dunns test for multiple comparisons with unequal sample sizes.
Values expressed as mean ± S.E.M. and $p<0.05$
Statistical analysis using Systat 7.0

Results and discussion:
90 % of the fish survived in the control DMSO, 50 % at 100 mg propylparaben/kg and only 10 % at 300 mg propylparaben/kg.

A time- and concentration-dependent increase of vitellogenin, a biomarker for oestrogen activity, was observed after intraperitoneal injection in sexual immature juvenile rainbow trout.

Plasma VTG was measured at day 0, 6 and 12. Significant differences compared to the control group were seen at dosage of 100** mg/kg (increase of 15 times) and 300** mg/kg at day 12 of the exposure period. At 300 mg/kg already a pronounced estrogenic response could be observed after 6 days with a 1000 times increase of the mean basal VTG-level to 288 µg/mL. At the highest dose only a single fish (10 %) survived the second injection due to the high toxicity of propylparaben, but no further increase of VTG was observed in this specimen.

VTG declined slightly in time in the control group. This might be attributed to the starvation of the fish during the whole experiment. However, if toxicity of propylparaben or starvation influenced the result of this study, it would have led to an underestimation of the actual oestrogenicity through a general suppression of protein synthesis, including vitellogenesis.

For compounds with non-detectable oestrogenic effect, toxicity, starvation and large interindividual variability might contribute to a decrease in sensitivity which was not the case for propylparaben in this study. Egg yolk synthesis in oviparous animals can only be evoked by oestrogen (Korsgaard and Petersen, 1976) or chemical mimicking oestrogen (Sumpter and Jobling, 1995 and Ankley *et al.*, 1998) and not by general toxicity of a compound.

Bjerregaard *et al*. (2003)

Guidelines: non-guideline

GLP: non-GLP (confirmed by author)

Material and methods:

Test substances: propylparaben (97 % -Sigma)
Test species: Rainbow trout (Oncorhynchus mykiss) from commercial Danish fish farms (Lihme Fishery and Højbjerg Fiskeri, Jutland)
Type of test: flow through
Species life stage: sexually immature juveniles (47 – 133 g), mixed sex population
Acclimation period: - 3 – 14 days,
- without feeding
- flow through
- fish placed in 1000 L tanks and transferred to 60 L stainless steel Tanks
- Temperature: raised from 5 to 12 °C over 8 days
- Photoperiod: 12 h light/12 h dark

Route of administration: - food: gavage
- water

Test conditions:
Photoperiod: 12 h light/12 h dark
Temperature: 12 °C
Feeding:
Feeding experiment 1:
Aqualife 19 from BioMar A/S, Brande, Denmark
Propylparaben or 17β-estradiol were dissolved in acetone an added to the food homogenate (crushed food mixed with water)
Feeding experiment 2:
Gelatine powder (Merck) was mixed into the food homogenate. Propylparaben was added to the homogenate, given to the fish in the form of cubes. The content of propylparaben in the food cubes was chemical analysed.
Water exposure: fish (101 ± 20 g at start) were not fed during the exposure period of 12 d, 112.5 L/tank.
Additional info given by the author:
- pH water: pH: 7.55 (from the analysis of the water supply company)
- oxygen content: oxygen concentration was not determined, but with two air stones heavily bubbling it is assumed that oxygen tension to be close to 100%.

Test design:
Applied concentration:
Chemical analysis: Water samples were analysed for propylparaben during both exposure routes.
Feeding:
Experiment 1:
Tanks were daily cleaned of faecal material and the water was filled up again to the original volume.

Table 80: Nominal and measured dose in experiment 1

Nom. Dose (mg/kg/2d)	Meas. Dose (mg/kg/2d) (adjusted for fish size)
Solvent control	
50	84
250	359
500	758
1000	1830

Experiment 2:

Table 81: Nominal and measured dose in experiment 2

Nom. Dose (mg/kg/2d)	Meas. Dose (mg/kg/2d) (adjusted for fish size and measured food conc.)
Solvent control	
10	7.2
25	33
35	36
50	39

Propylparaben was detected in the water of both experiments, but in experiment 2 it was below the concentration to induce vitellogenin synthesis via water (0.9 µg/L ± 1.9).

Water exposure: 50 and 250 µg/L/2d (meas. conc. 225 µg/L/2d). Before fish were added, propylparaben concentrations were maintained at 48 ± 2 and 221 ± 4 µg/L resp. Once fish were added, concentration propylparaben dropped to 37 ± 1 and 176 ± 3 µg/L after 6 days.

Positive control: 17β-oestradiol (98 %-Sigma):
Food: 50 and 400 µg/kg/2d, corresponding to 7 and 29 mg/kg/d uptake (considering that all the disappearing concentration of propylparaben in the water is taken up into the fish)
Water: 1 µg/L

Vehicle: acetone (food experiment);
Approximately 0.02 % ethanol (water experiment) (60 mL of 96 % ethanol added to 325 - 340 L water, added every 24 h)

Replicates: feeding experiments: 5 fish in 112.5 L steel tanks with a water flow of 316 - 348 L every 24 h
Experiment 1: 30 fish: 3 fish excluded due to illness, remaining fish: 13 males/14 females
Experiment 2: 55 fish: 25 males/26 females/4 fish died

Water experiment: 5 fish escaped from the aquarium, remaining fish: 14 males/11 females

Kinetics experiment: 10 fish were fed with 150 mg/kg

Exposure period: feed: 10 days
Water: 12 days

Parameters examined: - Vitellogenin plasma levels (VTG) to derive dose-responsive relationships and NOELs for estrogenicity
kinetics: Concentrations of propylparaben in liver and muscle at the end of the test
Sexes were determined by microscopical examination of the gonads (feeding experiment)

Data analysis and Statistics:
Sandwich ELISA method to determine vitellogenin levels in plasma
Lillifors test for normality and Barlettt's test for variance homogeneity

Normality distributed data sets were compared by ANOVA
Nonparametric statistics (Kruskal-Wallis test followed by Dunn's test for multiple comparisons of unequal group sizes) were used in the comparison of remaining data sets
SYSTAT 8.0
Values expressed as means ± S.E.M., $p<0.05$

Results and discussion:
3 out of 30 fish were excluded due to illness in food experiment 1; 4 of the 55 fish died in experiment 2 and 5 fish of 30 escaped from the aquarium in the water experiment.

Vitellogenin
"Vitellogenin response" was considered when a plasma vitellogenin level more than twice the highest measured vitellogenin concentration from any fish at day 0 and any control fish during the exposure period was observed. The calculation was performed for each sex separately.
Food:
Experiment 1: a time and dose dependant, increase of VTG was seen. No differences were observed between the sexes.
Experiment 2: Deviations in fish size and the measured concentration in the food reduced the doses to which fish were exposed between 25 and 50 mg/kg. A dose and time dependant increase of VTG was observed between 33 and 39 mg/kg/2d. No differences were observed between the sexes.
Estrogen activity: ED50 values for increase in vitellogenin synthesis were 35, 31 and 22 mg kg/2d at day 3, 6 and 11, respectively.
Water:
Only significant VTG increase was observed at 250** µg/L (meas. conc.: 225 µg/L). No differences in response between sexes.

Kinetics
In the food experiment only the liver concentrations of fish (37 ± 21 µg/g ww) exposed to 1830 mg/kg/2 days could be determined, while for water exposure only concentrations in liver (7 ± 2 mg/g ww) and muscle (0.87 ± 0.05 mg/g ww) of the fish exposed to 250 µg/L could be determined.

After oral exposure to a dose of 1830 mg/kg/2d less than 1 ‰ of the total amount of propylparaben was retained in muscle and liver 24 h after the end of the experiment (10 d).

After 12 d exposure via the water to 225 mg propylparaben concentrations of 6700 and 870 mg propylparaben/kg were measured in the liver and muscle, respectively.

Half-life of propylparaben in liver is 8.6 h, in muscle 1.5 h (when first-order kinetics is supposed).

After oral exposure, chemicals absorbed from the gastrointestinal tract reach the target organ for vitellogenin induction (liver) immediately prior to dilution in the fish body (Alslev *et al*., 2005), while in rodents chemicals passes first the liver (metabolism) and are than distributed in the body before reaching the target organ.

At the end of the experiment less propylparaben remained in the fish. Therefore, it can be concluded that after oral administration, propylparaben is quickly metabolized or poorly absorbed. The latter is considered more unlikely as propylparaben in mammals is completely absorbed in the gastrointestinal tract and quickly hydrolysed to p-hydroxybenzoic acid and propanol by esterases in the organs.

In this experiment some unmetabolised propylparaben escapes first pass liver metabolisation and is distributed to other organs which may indicate that esterase activity

is lower in fish than in mammals. Furthermore, rainbow trout showed the lowest esterase activity in an experiment with 5 different fish species (nonspecific esterase activity was found in intestine, liver, and bile) (Li and Fan, 1997).

Food exposure is slightly more potent than water exposure: no estrogenic activity via water after uptake of 7 mg/kg/d (50 µg/L exposure), but via the food the most sensitive fish responded to 7 mg/kg/2d; similar response between exposure via the water and food after uptake of 29 mg/kg/d (225 µg/L exposure) and exposure to 33 mg/kg/2d resp. This observation that food exposure is slightly more potent than water exposure can be explained by the fact that the dose that reaches the liver is higher when the chemical enters via the portal vein than via the general circulation after uptake via the gills.

As the liver is not the target organ for reproduction, potentially more effects on reproduction can be observed if fish are exposed via the water because propylparaben may reach the hypothalamus-pituitary-gonad axis before metabolisation.

Propylparaben shows estrogenic activity in fish when exposed orally or via water.

Inui *et al*. (2003)

Guidelines: non-guideline

GLP: non-GLP

Material and methods:

Test substances: Propylparaben (n-propyl-p-hydroxy-benzoate; PPB) from Wako Pure Chemical Industries (Osaka, Japan) and other UV-screens
Test species: medaka (Oryzias latipes) from local commercial company
Type of test: renewal every other day
Species life stage: adult males, with body length between 2.5 and 3.5 cm
Acclimation period: 7 days in 2 L of pure water, fed daily with tropical fish flake food (TetraFin, Tetra GmbH, Germany)

Route of administration: via water

Test conditions: no data reported

Test design:
Applied concentration: 0.055, 0.55, 5.5 and 55 mM or 9.9, 99.5, 995 and 9915 mg/L
Positive control: E2: 3.7, 37 and 185 nM
Solvent control: 0.1 % ethanol
Replicates: 5 fish/treatment
Exposure period: 7 days
Parameters examined:
Vitelogenin plasma concentration
mRNA expression of egg proteins vitelogenin (VTG), choriogenin (CHG) and sex hormone receptors

Data analysis and Statistics:
ELISA to determine VTG levels
Real time analysis RT-PCR to estimate mRNA expression
Statistical evaluation using ANOVA
Values expressed as means ± S.E.M., $p<0.01$

Results and discussion:

Vitellogenin levels

A dose-dependent increase of VTG was seen after exposure to propylparaben up to levels similar to those induced by exposure to 10^{-5} times E2.

The highest plasma vitellogenin concentration was around 100 µg/mL in the exposed fish according to figure 1 of the article (please note that the authors sent in a corrigendum stating that the units to figure 1 are µg/mL and not ng/mL as indicated in the paper).

mRNA expression of vitelogenin (VTG), choriogenin (CHG) and sex hormone receptors:
A dose-dependent significant increase of expression of VTG-1, VTG-2 and CHG-L was observed at all concentrations ($p<0.01$), while CHG-H-expression increased significantly at 5.5 and 55 mM ($p<0.01$). ERα expression was significantly increased from 99.5 mg/L , while expression of ERβ dose-dependently increased but only significantly after exposure to the highest concentration (9915 mg/L of propylparaben).

Expression of AR was not significantly different from control.

Mikula *et al*. (2006)

Guidelines: non-guideline

GLP: non GLP

Material and methods:

Test substances: Propylparaben from Sigma Aldrich, St Louis, USA
Purity: >99 %
Test species: zebrafish (Danio rerio) from local breeder, member of aquaristic association Cyperus Brno-HomogenousTreatment of fish in accordance with legislation of Czech Republic: Protection of Animals Act 246/92 as amended and Decree of 207/04; and in accordance with test project 7/2005 of the University of Veterinary and Pharmaceutical Sciences Brno
Type of test: semi-static (replacement of water 3 times/week)
Species life stage: 20 d post hatch
Acclimation period: 3 d, fish were fed with shelled eggs of brine shrimp (Artemia salina)

Route of administration: water

Test conditions:
- *photoperiod:* 12 h light, 12 h dark
- *water temperature:* 25 ± 1.5 °C
- *pH:* 7.5 – 7.8
- *dissolved oxygen*: did not fall below 60 % in any of the tanks
- feeding: shelled eggs of brine shrimp (Artemia salina)

Test design:
Exposure duration: 20 d
Applied concentration:
0, 0.1, 0.4 and 0.9 mg/L
Positive control: ≤0.1 mL/L of 17β-estradiol (min. 98 %, Sigma-Aldrich; St. Louis, USA)
Solvent: <0.1 % ethanol
Replicates: 300 fish divided in ten 15 L glass tanks (10 L of water according to ISO7346-2)
2 replicates/conc.

Parameters examined:
VTG: 12 fish, randomly selected on day 20 (6 fish from each tank)

Data analysis and Statistics:

ELISA kit: determination of VTG in whole body homogenates
Standard descriptive statistics: geom. Mean and CI
Kruskal-Wallis followed by a multiple comparison of the mean ranks

Results and discussion:

Significant ($p<0.001$) decrease in vitellogenin was seen in all concentrations compared to control indicating an anti-oestrogenic effect (see Table 82).

Table 82: VTG concentrations

PPB	VTG conc. (geom. Mean, IC95): ng/mL
Control (0 mg/L)	400
0.1 mg/L	240*****
0.4 mg/L	218*****
0.9 mg/L	270*****
100 ng/L 17β-estradiol	35533*****

A different effect is seen than in other studies which may be due to the fact that other organisms and other exposure periods were used.

Pohl J. (2015)

To investigate the thyroid disruption of propylparaben, *Xenopus tropicalis* tadpoles (NF stage 51) were exposed to 0.05, 0.5 and 5 mg/L propylparaben for 14 days in a semi-static individual exposure system (12 tadpoles/concentration). Body weight, total body length, snout -vent length (SVL), hind limb length (HLL) reached developmental stage and thyroid gene expression were examined. Thyroid histopathology was not examined.

No effect was seen on mortality, growth, and development, but dio3 an ttr were downregulated. The downregulation of dio3 could possibly be associated with a reduced serum content of thyroid hormone, while ttr might be connected to a previously described xenoestrogenic effect of propylparaben *in vitro* and in fish.

Li et al. (2015)

Authors investigated the effects of fecundity and lifespan by exposing *Drosophila melanogaster* to food containing 200, 500, 1000 and 2000 mg/L of propylparaben. 1% ethanol was used as solvent. Eggs were counted for 5 days. Then, every 12h, numbers of individuals were recorded from egg to pupae and from pupae to adults and the transition periods. To measure the lifespan, newly eclosion males and females were exposed and survival was looked at over a period of 80 days. Furthermore, a climbing assay was conducted to see if fruit flies were able to climb up a glass vial (8 cm or above of vertical distance) after exposure to propylparaben. SOD activity and lipid peroxidation was also measured.

Fecundity decreased statistically significantly ($p<0.01$) at day 3, 4 and 5. Daily mean egg production in the solvent control at day 5 was ±116, while resp. ±100, ±97, ±68, ±4 at 200, 500, 1000 and 2000 mg/L.

A delay in growth period was observed from 10.5 days for the solvent control to resp. 10.8 days and 14.6**** days at 1000 and 2000 mg/L. However, at lower exposure concentrations the development time decreased (resp. 9.3** days and 10.4 days) compared to the control (10.5 days).

Table 83: effect of PPB exposure to growth period of *Drosophila melanogaster* (from Li *et al.*, 2015)

PPB (mg/L)	Egg to prepupa (days)	Prepupa to fly (days)	Growth period (days)
Solvent control	6.0	4.2	10.5
200	4.7**	4.4	9.3**
500	5.9	4.4	10.4
1000	6.4	4.4	10.8
2000	9.7****	4.6	14.6****

**p<0.05, ****p<0.001

The mean lifespan of female and male fruit flies decreased statistically significantly at 2000 mg/L, but statistically significantly increased at 1000 mg/L.

Table 84: Effects on lifespan of *Drosophila melanogaster* after exposure to propylparaben (from Li *et al*., 2015)

PPB (mg/L)		Mean lifespan (day)	Maximum lifespan[a] (day)	90 % survival time (day)
Solvent control	F	67.8	76.7	72.4
	M	65.7	75.3	70.3
200	F	65.5	73.5	68.5
	M	63.5	72.1	67.2
500	F	68.2	74.5	70.4
	M	68	74	69.9
1000	F	71.9**	81.7****	76.5****
	M	71.3****	80.6**	75.3****
2000	F	63.4**	72.6**	67.3****
	M	61.4**	70.5**	65.1****

**p<0.05, ****p<0.001
[a]The maximum life spans in this study were calculated as the average life span of the 10% longest surviving flies

Climbing activity decreased with age in all exposed groups. While a higher climbing activity was reported in the 1000 mg/L exposure group compared to the control, lower activity was seen in the 200 mg/L group.

A significant increase in enzyme activity of SOD activities and reduction in MDA level was seen at 1000**** mg/L while the opposite effect was seen at 2000** mg/L.

***Torres et al.* (2016)**

Guidelines: Based on OECD TG 236 (Fish Embryo Acute Toxicity Test)

GLP: non GLP

Material and methods:

Test substances: Propylparaben from Sigma Aldrich, St Louis, USA
Purity: >99 %
Test species: zebrafish (*Danio rerio*) from stock Biotério de Organismos Aquáticos
Sea urchin (*Paracentrotus lividus*) collected in a clean rocky shore of Portugal (Vila Nova de Gaia (N 41°2'26.18", W 8°39'2.24")
Type of test: semi-static (daily renewal)
Species life stage: zebrafish: freshly fertilised eggs
Sea urchin: 30 min post fertilisation

Route of administration: water

Test conditions:

- *photoperiod:* Zebrafish: 14 h light, 10 h dark
  Sea urchin: 48 h in the dark
- *water temperature:* Zebrafish*:* 26.5 °C
  Sea urchin: 20 °C
- *pH:* na
- *dissolved oxygen*: daily medium renewal to keep oxygen concentrations constant
- feeding: stock adult female and male zebrafish: Tetramin (Tetra, Melle, Germany) supplemented with live brine shrimp (Artemia spp.)

Test design:

Exposure duration: 8, 32 and 80 hpf (zebrafish)
48 h (sea urchin)

Applied concentration:

Zebrafish: solvent control, 0, 10, 100, 1000, 3500, 6000, 8500, 10 000 µg/L
Sea urchin: solvent control, 0, 10, 64, 100, 160, 400, 1000, 10000 µg/L
Water control and solvent control were grouped when no significant difference between them

Solvent: 0.01 % DMSO

Replicates: Zebrafish*:* 10 fertilised eggs/replicate in 24-well plates
8 replicates/conc.
Sea urchin: 20 eggs/mL per well

Parameters examined:

Zebrafish: Mortality rate (at 8, 32 and 80 hpf), 75 % of epiboly stage (at 8 hpf), abnormal cell growth (at 8 hpf), abnormalities (head, tail, eyes, yolk-sac (at 32 and 80 hpf), pericardial edema (at 32 and 80 hpf), heart rate (at 32 and 80 hpf)), hatching rate (at 80 hpf), muscular involuntary contractions (at 80 hpf)

Sea urchin: abnormal larvae, length

Data analysis and Statistics:

Analysis of data using SPSS v. 21.0
Levene and Kolomogorov-Smirnov test: homogeneity and normality
ANOVA followed by Newman-Keuls multiple comparison test if homogeneity and normality were met
Kruskal-Wallis test followed by Games-Howel multiple comparison rank test if homogeneity and normality not met

Results and discussion:

Zebrafish:

Table 85: Effects of propylparaben (n= 32 for control, n= 8 for exposure groups) (from Torres *et al*., 2016)

Conc. µg/L	Mortality rate	Hatching rate	Abnormal cellular growth	75% epiboly stage	Tot. abnormalities	Pericardial edema	Heart rate	Abnormal muscular contractions
controls	3.13	92.19	1.56	97.81	3.13	2.08	143.0	-
10	2.5	87.5	1.25	96.25	2.08	2.08	144	-
100	1.25	90.0	1.25	98.75	2.08	2.08	146.0	-
1000	4.37	85.68	1.25	94.49	4.17	2.08	139.75	-
3500	3.75	66.25	1.25	96.25	100**	91.67**	124.5**	-
6000	1.25	18.75**	1.25	91.25	100**	100**	67.5**	-
8500	21.25	2.5**	1.25	87.5**	100**	100**	70.29**	-
10000	100**	0.0**	0	87.5**	-	-	-	-

**p<0.05

Authors observed a sign. Increase in head-, yolk-sac-, eye-, tail abnormalities and pericardial edema and a sign. Decrease in heart rate at ≥ 3.5 mg/L. No abnormal muscular contractions were seen.

Sea urchin:
Sign. effect on total abnormalities (increase) and larval length (decrease) at 0.4**, 1** and 10** mg/L.

Scott *et al*. (2017)

Guidelines: non-guideline

GLP: non GLP

Material and methods:

Test substances: Propylparaben from Sigma Aldrich (New South Wales, Australia)
Purity: ≥99 %
Test species: rainbowfish (Melanotaenia fluviatilis): purchased from acommercial aquarium fish wholesaler (Aquarium Industries, Vic-toria, Australia)
Mosquitofish (Gambusia holbrooki): caught in New south wales national park
Type of test: semi-static, renewal every 48 h
Species life stage: adult male fish
Acclimation period: 24 h, 16 – 24 adult fish/treatment in duplicate tanks

Route of administration: water

Test conditions:
- *photoperiod:* rainbowfish and mosquitofish: 16 h light, 8 h dark
- *water temperature:* rainbowfish*:* 24 ± 1 °C
Mosquitofish: 22 ± 1 °C
- *pH:* na
- *dissolved oxygen*: na
- feeding: frozen brine shrimp (rain-bowfish; Hikari Bio Pure, Kyorin Co. LTD., Japan) and Nutrafin MaxColour Enhancing Flakes (mosquitofish; Hagen, Massachusetts,USA)

Test design:
Exposure duration: 7 days
Applied concentration:
solvent control, positive and negative control, concentration range from 2- to 115-fold higher than the highest environmental concentration reported previously (in case of propylparaben 218 ng/L) to incorporate a margin of safety. Measured concentration in rainbowfish: 8820 ng/L; mosquitofish: 12000 ng/L
Solvent: 0.001 % ethanol
Positive control: EE2
Replicates: 2

Parameters examined:
Estrogenic activity (see section on OECD CF level 2)
Mass, length and Gonopodium length (mosquitofish only)
Plasma VTG (rainbowfish), VTG homogenate concentration and gene expression (excl. liver) (mosquitofish)

Data analysis and Statistics:
Mann-Whitney U non-parametric test ($p<0.05$)

Results and discussion:

Table 86: Weight, length and gonopodium length in male fish (from Scott *et al*., 2017)

Male fish	Rainbowfish		Mosquitofish	
	Solvent control (n=23)	PPB (n=16)	Solvent control (n=20)	PPB (n=20)
Weight (mg)	2056	2331	185	187
Length (standard, mm)	52	55	22	22
Gonopodium length (mm)	-	-	7.0	7.3

VTG:

A male negative control (not sign. different from male solvent control, $p<0.05$) was used for statistical analysis. Whole body VTG was expressed as fold Vtg expression compared to the male negative control.

Propylparaben caused a small but statistically significant 3-fold increase of VTG protein in rainbowfish at a concentration of 8820 ± 295 ng/L ($p= 0.035$). No effect has been shown on *Vtg* mRNA nor protein in mosquitofish after exposure to up to 12000 ± 150 ng/L propylparaben.

The *in vitro* transactivation assay (Erα-GeneBLAzer assay) showed also a very weak estrogenic activity just above the detection limit of 1 ng/L (1.1 ± 0.1 ng/L EEQ).

Because the effective concentration is much higher (40 – 55-fold) than the highest concentration reported in Australian rivers (218 ng/L) and only weak estrogenic activity is detected *in vitro*, authors concluded that propylparaben shows a low, but not negligible, risk of estrogenic effects in wild fish.

Carlsson *et al. (*2019)

The aim of this study was to assess the thyroid disruption properties of 3 indoor dust chemicals including propylparaben in a modified Amphibian Metamorphosis Assay (AMA, OECD TG 231) in *Silurana (Xenopus) tropicalis* tadpoles. Main deviations are the species used for the assay (to reduce the time required for the assay) and the growth in individual beaker. The authors exposed *S. tropicalis* tadpoles (n= 12/group, 12 replicates of 1 tadpole/treatment) for 2 weeks to 0.05, 0.5 and 5mg/L propylparaben. Stability was tested in an additional set up. It was found that propylparaben decreased quickly in beakers containing tadpoles: measured concentrations were respectively 0.026 mg/L, 0.50 mg/L and 4.2 mg/L in new water and 0.00075 mg/L, 0.013mg/L and <LOD (=0.001 mg/L) in old water. Therefore, a flow through system was chosen for the main study. For the acute toxicity, tadpoles were exposed to 5.0, 12.5 and 50 mg/L.

Propylparaben showed an acute toxicity, with 100 % mortality from 12.5 mg/L. At lower concentration, no delay in metamorphosis, no effect on body length nor any developmental marker were observed. Histopathological investigation did not reveal any change in thyroid epithelial cell height. This study showed no evident indications of specific thyroid-disrupting effect caused by propylparaben.

Daphnia magna Reproduction Test (REACH registration, Unpublished study report, 2019)

Following the Revised OECD Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption an *in vivo* assay with *Daphnia magna*, primarily not designed to detect endocrine-active substances, can be responsive to juvenile hormone (JH) agonists leading to the production of male offspring. However, this endpoint

is responsive to, but not diagnostic of, ED modality because it can also be in response of natural factors (short photoperiod, temperature fluctuations, decreased food density and increased F0 population density).

In this study, performed according to OECD TG 211, no male daphnids were observed, thus indicating that no hormone related activity occurred in this species.

Atli *E. (*2022)

Development and fecundity were investigated by exposing 72 h old instar larvae of the fruit fly *Drosophila melanogaster* for 6 h to 50, 100 and 200 mM propylparaben. Larvae were exposed in tubes containing drying papers absorbed with the resp. concentrations, meaning that exposure was via oral (feed) and dermal route (skin absorption). Concentrations were chosen below the LD50 (300 mM). Results were compared to the solvent control (0.1 % ethanol).

Table 87: Effect of propylparaben on pupation, pupation time, maturation, maturation time, number of offspring and fecundity (from Atli *et al*., 2022)

	Pupation % (result ± St. Dev.)	Pupation time (h, mean – result ± SE)	Maturation % (result ± St. Dev)	Maturation time (h, mean- result ± SE)	Nb of offspring (mean- result ± SE)	Fecundity (result ± SE)
Solvent control	100 ± 0.00	69.2 ± 0.87	99 ± 0.032	70.5 ± 0.86	13.16 ± 0.73	7.09 ± 0.56
50 mM	99 ± 0.032	70.6** ± 0.87	93 ± 0.103	72.7** ± 0.89	12.91 ± 0.74	4.97* ± 0.42
100 mM	100 ± 0.00	71.6** ± 0.85	94 ± 0.084	72.5** ± 0.87	13.26 ± 0.74	4.87* ± 0.42
200 mM	100 ± 0.00	71.4** ± 0.86	97 ± 0.048	72.2** ± 0.87	13.82 ± 0.67	4.84** ± 0.40

**p<0.05

It can be concluded that propylparaben statistically significantly impacted development by delaying pupation and maturation time in larvae of *Drosophila* and caused a statistically significant decrease in fecundity. Most changes in insect development and reproduction are due to changes in the endocrine system, more particularly during pupation the concentration of ecdysteroids increases while juvenile hormone decreases. Furthermore, propylparaben increases development time from egg to pupa at 100 and 2000 mg/L (Li *et al.*, 2015).

Calma and Medina (2020)

Acute and chronic exposure to propylparaben was assessed in the dengue mosquito *Aedes aegypti L.* In the acute assay eggs were exposed to 0.02, 250, 500, 1000 mg/L and egg hatching (eclosion) was counted after 12 and 24 h. To assess larval mortality a new set of eggs was exposed to 0.02, 25, 50, 100, 250, 500, 1000 mg/L and survival was counted after 12, 24 and 36 h. The chronic assay started with second instar larvae. Following concentrations were tested: pupation and emergency: 0.02, 25, 50, 100, 250, 500, 1000 mg/L. Adults resulting from the emergency assay were used to examine fecundity and F1 hatching. Resulting F1 eggs were divided in an untreated and treated water group.

Table 88: Acute and chronic effects in dengue mosquito *Aedes aegypti L*.

mg/L	0	0.02	25	50	100	250	500	1000
% Hatching after 24 h	85.7 ± 3.6 %	23.4*** ± 25.2	-	-	-	0******	0******	0******
% mortality after 36 h	0	0	8.7 ± 14.0	12.0 ± 7	11.7 ± 9.2	71.7***** ± 10.5	99.0***** ± 1.1	100*****
% Pupation (4 d exposure)	78.3 ± 9.5	45.3*** ± 3.0	28.7**** ± 14.9	21.7**** ± 18.4	20.3***** ± 8.9	3.3***** ± 3.5	0*****	0*****
% emerging adults from acute test	68.1 ± 7.9	44.9*** ± 16.2	34.9****** ± 15.6	24.6***** ± 13.2	13.1***** ± 7.4	0******	0******	0******
% Fecundity (mean number of eggs)	84.05 ± 19.51	53 ± 1.41	77.25 ± 20.53	-	74.5 ± 0.71	-	-	-

Liang *et al.*, 2023

Guidelines: non-guideline

GLP: non-GLP

Material and methods:

Test substances: propylparaben (CAS: 94-13-3)
Purity: > 99.0 %
Test species: zebrafish (wild type, AB strain)
Type of test: semi-static (daily renewal)
Species life stage: 2 hpf embryos collected from adult zebrafish
Route of administration: water
Test conditions:
Adult zebrafish
Photoperiod: 14 h light/10 h dark
T°: 28 ±0.5 °C
pH: 6.9 - 7.2
Hardness: 200 mg/L ($CaCo_3$)
Dissolved oxygen: 5 - 7 mg/L
Feeding fish were starved to avoid possible hormonal exposure via the food
Test design:
Applied concentration: 0 (solvent control), 2, 5 and 100 µM
Vehicle: 0.1 % DMSO (v/v)
Replicates: 3 replicates
Exposure period: 120 h
Parameters examined:
Vitellogenin plasma levels
Hormone production (E2 and T)
Gene expression

Data analysis and Statistics:

The difference between the treatment groups and the solvent control was evaluated by one-way analysis of variance (ANOVA) with the Dunnett's test.

Results and discussion:

From a previous study (Liang *et al*., 2022) it can be concluded that the solvent control did not show significant difference from the blank control, therefore results were compared to the solvent control.

Characteristic signals, which were detected in fish by using MALDI-TOF-MS, show that parabens could enter into the fish bodies.

After 120 h of exposure VTG-levels significantly increased at 2**** µM, T-levels significantly decreased at 2** and 10**** µM, while E2 levels were not significantly affected.

It was shown that propylparaben disturbed the HPG-axis in zebrafish larvae by sign. Downregulating of gnrh (gnrh2, gnrh3 and gnrh1), fshß and their receptors (gnrhr1, gnrhr2 and gnrhr4) and giving a U-shaped response for fshr, lhß and sex hormone receptors (er2b and ar) and steroidogenic related genes (i.e. cyp11a, cyp17, cyp19a, cyp19b, 3β-hsd, hmgra, hmgrb, star and 17β-hsd).

Table 89: gene transcription of the HPG-axis in zebrafish larvae

Genes	2 µM PPB	5 µM PPB	10 µM PPB
gnrh2	↙ ****	↙ ****	↙ ****
gnrh3	↙ ****	↙**	↙ ****
gnrhr1	↙ ****	↙	↙**
gnrhr2	/	/	/
gnrhr4	↙ ****	↙**	↙ ****
fshß	↙ ****	↙**	↙ ****
fshr	↗****	↙	↗****
lhß	/	↗****	↗****
Cyp11a	↗****	↙	↗****
Cyp 17	↗****	↙	/
Cyp19a	↗ ****	↙	↗****
Cyp19b	↙	↙	↙
Er2b	↙	/	↙
ar	/	↙	/
hmgra	↗****	↙	/
hmgrb	↗****	↙*	↗****
star	↙	/	/
3ß-hsd	↗****	↙	↗****

17ß-hsd	↗****	↙	↗****

Ma *et al*. (2023)

Guidelines: non-guideline

GLP: non-GLP

Material and methods:

Test substances: propylparaben (CAS: 94-13-3)
Purity: > 99.0 %
Test species: mosquitofish (*Gambusia affinis*)
Type of test: semi-static (half of exposure solution changed every day)
Species life stage: adult male mosquitofish (average length 2.07 ± 0.28 cm, weight 0.12 ± 0.05)
Route of administration: water
Test conditions:
Photoperiod: 14 h light/10 h dark
T°: 25 ± 1 °C
pH: 7.1 - 7.4
Hardness:
Dissolved oxygen: ≥ 5 mg/L
Feeding: twice/day with commercial fodder and brine shrimp, faeces, and residual food was removed from the tanks
Test design:
Applied concentration: 0 (solvent control), 0.15, 6 and 240 µg/L)
Vehicle: 0.05 % DMSO (v/v)
Replicates: 384 fish, 4 replicates/conc., 12 fish/replicate in 2 L exposure solution (2 L tanks)
Exposure period: 4 and 32 days
Parameters examined:
Mortality at 32 d
Gene expression HPGL-axis
Histopathology of the brain, testis, and liver
Data analysis and Statistics:
t-test and one-way ANOVA (Tukey's multiple comparison) to analyze difference in mRNA expression between control and treatment groups

Results and discussion:
No mortality was seen after 32 days of exposure.

Propylparaben affected brain structure in a dose-dependent manner. Cell cavities, cytomorphosis and blurred cell boundaries were observed at 240 µg/L in the 4 d and in all 32 days treatment groups. Additionally, at 32 days hepatic sinus dilatation and cytoplasmic vacuolation was demonstrated in all treatment groups.

Gene transcription in the brain was not affected after 4 d exposure. However, after 32 d genes expression was changed in a parabolic manner with a sign. increase at 0.15** µg/L and a sign. decrease at 240** µg/L of all genes (erα, erß, arα, arß, gnrh, gnrhr and cyp19a1b).

Propylparaben affected liver hepatocytes in a time and dose-dependent manner. After 4 d exposure mild and moderate liver injuries (hepatic sinus dilatation and cytoplasmic vacuolation) were observed in all treatment groups and increased after 32 days exposure (hepatic sinus dilatation or hyperemia, cytoplasmic vacuoles, nuclear aggregation, cytolysis, and partial cell necrosis).

Propylparaben significantly up-regulated gene expression (erα, erß, arα, arß, vtgB, vtgC cyp19a and star) in the liver after 4 d exposure at all concentrations tested ($p<0.01$). After 32 d exposure, only erß and cyp19a were still sign. upregulated at 6** and 240** µg/L.

Propylparaben did not affect testis tissue after 4 d of exposure. However, after 32 d of exposure, spermatogenic cell lesion, decreased mature seminal vesicle, sperm cells gathering, seminiferous tubules disorder and dilated intercellular space were seen. No sign. Difference in proportion of different sperm cells, but a delayed spermatogenesis was observed after 32 d exposure characterised by a sign. decrease in mature sperms (72.3 % at 240 µg/L after 4 d, 60.9** % at 240 µg/L at 32 d) and a sign. increase of primary spermatocytes (4.92 % at 240 µg/L after 4 d, 9.1** % at 240 µg/L at 32 d).

After 4 d of exposure, propylparaben did not affect gene transcription in the testis. However after 32 days, erß (at 240 µg/L), vtgC (at 0.15 µg/L), star (at all conc.), hsd17b3 (at 6 and 240 µg/L), hsd20b (at 6 µg/L) and shh(at 6 and 240 µg/L) were sign. up-regulated ($p<0.05$). The transcription of hsd3b was sign. downregulated at 0.15** but sign. upregulated at 240** µg/L. The increased vtg and decreased gnrh and gnrhr expression may be associated with the change in maturity ratio of the sperm cells.

Authors suggest that the effects on brain, liver and testis tissue might be due to mechanisms like disruption of ER- and AR-mediated pathways, oxidative stress, lipid metabolism disorder, DNA double-strand breaks, and apoptosis and that gene expression of the HPGL-axis might be a compensatory and feedback regulation for the damage caused.

It can be concluded that propylparaben had an estrogenic effect (consistent sign. Upregulation of erα and erß in testis and liver, sign. Upregulation of vtgB, vtgC, Erα and Cyp19a in liver and sign. Upregulation of vtgC in testis, an anti-androgenic effect (upregulation of arα and arß in liver) and impacted steroidogenesis (sign. Upregulation of star, hsd3b, hsd17b3 and hsd20b).

Conclusion: OECD CF level 3 data

Oestrogen:
- Intraperitoneal injection: increase of VTG from 100 mg/kg/d in juvenile rainbow trout (Oncorhynchus mykiss) (Pedersen *et al.*, 2000).

- Feed: clear dose and time dependant **increase** of VTG between 33 and 39 mg/kg/2d in immature juvenile rainbow trout, 10 days of exposure (Bjerregaard *et al.*, 2003), but sign. Decrease in immature Danio rerio (although minor difference with control (Mikula *et al*., 2006).

- Water: - Rainbow trout (Oncorhynchus mykiss):
  increase of VTG in sexually immature juveniles from 0.225 mg/L, exposure duration of 12 days (Bjerregaard *et al.*, 2003).
  - Rainbowfish:
  sign**. increase** in adults at 8820 ng/L (exposure duration of 7 days) (Scott *et al*., 2017).
  - Medaka (Oryzias latipes):
  increase of VTG from 99.5 mg/L in adult males after an exposure period of 7 days (Inui *et al.*, 2003).
  - Zebrafish (Danio rerio):
  decrease of VTG after 20 days exposure to 0.1 mg/L in juvenile (Mikula *et al.*, 2006), but although significant differences are minor compared to control.

Propylparaben disrupts gene transcription of HPG-axis after embryonic exposure of zebrafish (Liang et al., 2023). This was also seen in adult mosquitofish whereby the mRNA expression was affected in brain, testis and liver showing estrogenic and androgenic effect of propylparaben (Ma *et al*., 2023).

OEDC CF level 3/4: *In Vivo* Assays Providing Data about Selected Endocrine Mechanism(s) /Providing Data on Adverse Effects on Endocrine-Relevant Endpoints

Table 90: OECD CF level 3/4: fish data

Method (guideline)	Short description of Method	Result	Description of Result	References	Reliability
Non-standard assay	Exposure: Golden medaka, *Oryzias latipes*, exposed from 3-4 hpf for 316 hpf (= 13 dpf), then fed and further exposed up to 28 and 43 dpf, nominal PPB exposure conc. 40, 400, 1000 and 4000 µg/L Effect measurement: - **Morphological & developmental features** (cardiovascular, spinal, swim bladder inflation, swimming activity) till 316 hpf (= 13 dpf) - **Survival and growth (body length)** at 13, 28 and 43 dpf - **EROD activity *in vivo*** at 196 hpf - **Galbladder morphometric observations** from 124 – 244 hpf - **Histology** at 13, 28 and 43 dpf - **Sex ratio** at 28 dpf and 43 dpf	No histological effects in gonads No differences in sex ratio	No effect on developmental score or hatching time of PPB compared to control, except for highest concentration 4000 µg/L with higher mortality, swimming weakness and higher % of non-inflated swimbladder during 13 dpf. Sign. reduced length at 13 & 28 dpf for 400** – 4000** µg/L, was recovered at 43 dpf, while survival was significantly reduced as function of time and exposure concentration with only 20 % survival at 43 dpf at 4000** µg/L. Histological damage at 4000 µg/L at several organs at 13 dpf, but structure of gonads was unaltered. After 28 and 43 dpf, no histological effects in surviving organisms at 4000 µg/L. No differences in sexual ratio, seen at 28 dpf, compared to control for propylparaben treatment (up to 4000 µg/L) and up to 43 dpf.	Gonzalez-Doncel *et al*., 2014	3 (Exposure duration not long enough, intersex, and undifferentiated fish not taken into account in sex ratio)

Non-standard assay	Exposure Zebrafish embryos (n= 30/dose, 4 replicates, i.e. 120 embryos in tot) exposed from 2 to 120 hpf. Conc.: 1, 10, 25, 50, 100 and 200 µM PPB (0.1 % DMSO) Effects measurements: • Hatching delay, mortality, and developmental features • Gene expression	Developmental toxicity and mortality Altered gene expression	100 % mortality above 10**** µM at 72 hpf. Hatching sign. delayed at 72** and 120**** hpf, strong developmental abnormalities (100 % pericardial edema and 17.5 % spinal defects). Affected expression of genes involved in stress response, cell cycle, DNA damage, inflammation, fatty acid metabolism and estrogenic pathways (ER, AR and TR).	Bereketoglu and Pradhan, 2019	3
OECD TG 236 (Fish Embryo Acute Toxicity)	Exposure: Zebrafish embryos (n= 20/conc., 3 replicates) exposed from 1 to 96 hpf. Conc.: 0, 1, 2, 4, 6 and 8 mg/L PPB (0.1 % DMSO)	Acute toxicity Sublethal alterations Modified lipid distribution and metabolism	LC50= 3.98 mg/L Reduction in hatching rate observed at 72 h at all doses ($p<0.05$). Dose-dependent enlargement of yolk sac, but also increased occurrence of pericardial edema, deformed tail, and reduced blood circulation. Dose-dependent reduction of embryo length (also head and swim bladder) while increase of yolk sac. Increased level of neutral lipids and triglycerides in yolk sac while decreased level in body. Decreased phospholipase A2 activity.	Perugini *et al*., 2020	2
Non-standard assay	Exposure: zebrafish embryos (n= 40/well) exposed from 2 hpf 120 hpf Conc.: 0, 1, 2, 5, 10, 20 and 50 µM PPB (0.1 % DMSO)	Survival affected Hatching affected	PPB decreased survival sign. At the highest dose of 50**** µM, 5d-LC50= 34.45 µM (~6,21 mg/L). PPB sign. decreased the hatching rate after 72 h of exposure to 10** and 20** µM.	Liang *et al*., 2022	2

	(survival), 0, 5, 10 and 20 µM (other parameters) Effects measured: Hatching rate, survival, malformations, body length, heart beat, TH levels No sign. Difference between water and solvent control	Malformations observed Body length decreased Affected heart rate Disturbed TH levels	PPB induced malformations after 120h at 10**** and 20**** µM. Sign. decrease of body length after 120 h exposure at all conc. tested. PPB decreased the heart rate sign. After 120 h of exposure to 10**** and 20**** µM. PPB exposure evoked a decrease in T3 and T4 at 120 hpf at 5**, 10**** and 20**** µM.		

Gonzalez-Doncel *et al*. (2014)

Guidelines: non-guideline

GLP: non-GLP

Material and methods:

Test substances: Propylparaben from Sigma-Aldrich (Sigma Chemical and Co., St Louis, MO)

Test species: Golden medaka (*Oryzias latipes*) from stock culture of labo

Type of test: daily renewal
Species life stage: synchronized-aged 3 – 4 hpf embryo's (7-8 early morula stage) collected 2 – 4 h after start of daylight cycle

Route of administration: water

Test conditions:
- *photoperiod: 16 h light, 8 h dark*
- *water temperature:* 25 ± 1 °C
- *feeding*: no feeding during exposure period

Test design:
Exposure duration: 10 d, hereafter embryos were transferred to embryo rearing medium
Applied concentration: nom. conc.: 0, 40, 400, 1000 and 4000 µg/L
Meas. conc.: remained within 80 % of nom. conc.
EROD activity:
- positive control: a group of 5 animals was exposed to 0.5 µg/L β-naphtoflavone (aryl hydrocarbon receptor (AHR) agonist)
- β-naphtoflavone in addition of 400 or 1000 µg propylparaben/L

Solvent: 10 mg propylparaben solved in 0.55 mL of 0.1N sodium hydroxide (stock solution): calculated via extrapolation from reported data: 0.22 %
Replicates: Embryonic development:
5 replicates, 5 embryos/replicate, randomly distributed in 600 µL test solution; 4 independent experiments (n= 100)
EROD activity: 10 replicates, 5 embryos/replicate (n= 50)
Gallbladder area dynamics: 15 embryos/concentration, 4 independent experiments (n= 60)

Parameters examined:

Embryonic assessment:
Morphological (gallbladder) and developmental features and sublethal endpoints such as spinal (i.e., myoskeletal deformities), cardiovascular (i.e., pericardial edema and regression of heart compartmentalization), swimbladder (partial or total lack of inflation process) and swimming weakness (i.e., displaying uncoordinated movements or incapacity to respond to prodding) were monitored daily, from start of hatch to 72 h (i.e., 244, 268, 292 and 316 hpf).

Scoring system of Shi and Faustman (1989) with slight modifications: assessment at 76, 124, 196, 244 and 316 hpf using 13 developmental features at each time point.

Post hatching growth: Body length was examined immediately after embryonic assessment (i.e. 13 dpf); eleutheromebryos were than transferred to clean wells with embryo rearing medium (flow through, 8 L tanks) and length and survivorship was examined again at 28 and 43 dpf post-anesthesia.
EROD activity in 196 hpf embryos.
Gallbladder area: monitored daily from 124 to 244 hpf.
Histological analysis on surviving 13 dpf embryos from 0, 40, 400, 1000 and 4000 µg/L and 28 and 43 dpf larvae from 4000 µg/L.
Histological determination of gender in 28 dpf and 43 dpf exposed to 0 and 4000 µg/L.

Data analysis and Statistics:

Arc –sin transformation of proportional data for normalisation of distribution and stabilisation of variances; continuous data were log-transformed
Kolmogorov-smirnov "D" test for analysis of normality
Barlett's test for homogeneity of variance
Kruskal-Wallis test
Mann-Whitney U test with Bonferroni correction to identify any significant differences between any of the treatments and control ($p<0.05$)
Chi-square test: examination of differences in gender proportions between Propylparaben concentrations and control groups ($p<0.05$)
Statistical analysis by Statistical Package for the Social Sciences (SPSS)

Results and discussion:

Embryonic development:
At a concentration of 4000 µg/L, the mean development score started to decrease in comparison with the control. However due to the reduced number of alive animals (significant monotonic increase of mortality from 268 hpf), the variability increased considerably.
Development in controls was normal and no mortality or abnormalities were observed. Hatching in control started at day 11 and 95 % was emerged at day 12, they swam normally with significant recovery in the swim bladder inflation, responded normally to stimuli and had no symptoms of weakness.
No significant mortality, hatching failures (i.e. 7 ± 2.6 % for 4000 µg/L), or delayed or precocious hatching were observed after exposure to propylparaben. However significant difference was observed after exposure to 4000 µg/L for the endpoints recovery of the swimbladder inflation, weakness and cardiovascular defects. The latter might explain the mortality observed at this concentration and would result, in turn, in decreased weakness. As embryos were not incubated individually, it could not be deduced if a particular individual was affected severely enough to result in mortality.

Post-hatching growth
Length at 13 dpf and 28 dpf was significantly reduced at ≥ 400** µg/L. At 43 dpf, surviving larvae recovered and had similar lengths than the control.
Significant reduction of survivorship was observed at ≥ 400** µg/L by 43 dpf.

EROD activity:
Propylparaben did not increase NOS EROD activity (EROD activity value for embryo excluding galbladder area) nor OS EROD activity (EROD activity value for gallbladder area). B-naphtoflavone alone or in co-exposure with 400 or 1000 µg/L significantly induced OS EROD activity.

Galbladder area dynamics:
By 124 dpf an increase in gallbladder enlargement was observed at ≥ 400 µg/L.

Using the gallbladder as a biomarker of exposure a dose response relationship is revealed, except for the highest propylparaben concentration (4000 µg/L), where area values were well below those the immediate lower concentration (1000 µg/L) at all the time-points, suggesting diminished bile flow due to liver congestion. Therefore, the individual does not efficiently eliminate the toxicant or its metabolites, which are retained to the extent where embryo development is severely affected, as observed among the post-hatched individuals.

Histological analysis:
Due to the low survival in the 43 dpf embryos of the 4000 µg/L exposure concentration, 2 additional independent treatments were carried out for this lifestage. Minor and severe effects were seen at 4000 µg/L at 13 dpf: gallbladder dilatation, lack of swim bladder inflation, reduced length or weakness, myoskeletal and cardiovascular lesions and lack of startle response, yolk sack frying with thining of its wall, enlarged peritoneal cavity, hepatic atrophy with concomitant increase in gallbladder size, collapse of the air bladder, renal affection containing glomeruli with a reduction of the urinary space with an apparent increase in the number of podocytes in the periphery of the capillary bed indicating a delay in glomerular maturation and signs of generalized brain damage with massive cellular degeneration, probably of apoptotic nature. After 28 and 43 dpf no histological effects were found in surviving organisms at 4000 µg/L.

No significant difference in sex ratio was seen at 28 dpf and 43 dpf after exposure to 4000 µg/L. In medaka, ovary differentiation is observed between 21 and 28 dpf (Saito and Tanaka, 2009), while testis differentiation is attended after 60 dpf (Kurokawa *et al.*, 2007). Authors indicate that it will be not probable that neither differentiated ovaries, nor undifferentiated gonads in this study will later develop into testis or ovaries resp. because spontaneous intersex or gonad sex reversal rarely occurs in differentiated gonochorist species.

Bereketoglu and Pradhan (2019)

Guidelines: non-guideline

GLP: non-GLP

Material and methods:

Test substances: Propylparaben from Sigma-Aldrich (Sigma Chemical and Co., St Louis, MO)
Purity: ≥98 %
Test species: Zebrafish (type not mentioned)
Type of test: semi-static (renewal every other day)
Species life stage: 2 hpf
Route of administration: water
Test conditions:
- *photoperiod:* Adult fish: 14 h light, 10 h dark (not mentioned for embryos)
- *water temperature:* Adult: 25 ± 1 °C, embryos: 24.0 ± 1 °C
- *feeding*: Adult: fed twice/day with Artemia salina (Ocean Nutrition) and commercial flake food (Tetra)

Test design:
2 h old zebrafish embryos
Exposure duration: 120 h
Applied concentration: nom. Conc.: solvent control, 1, 10, 25, 50, 100 and 200 µM
Corresponding to ~ 0.1802 mg/L, 1.8 mg/L, 4.5 mg/L, 9.01 mg/L, 18 mg/L and 36.04 mg/L
Gene expression analysis: 1 and 10 µM
Solvent: 0.1 % DMSO
Replicates: 4 replicates; 30 embryos/replicate, 120 embryos/tested concentration

Parameters examined:
Mortality
Hatching rate
Abnormalities
Gene expression

Data analysis and Statistics:
Stat. difference between treatment and control: one way ANOVA followed by Dunnett's post-test
Statistical analyses: using GraphPad Prism 7 software (GraphPad software)
Principal component analysis (PCA): using SIMCA software (Umetrics)

Results and discussion:
Mortality: 100 % mortality observed above 10 µM (~ 1, 8 mg/L).
Hatching was significantly delayed at 10 µM at 72** and 120**** hpf. At 25 µM only 5-10 % of the embryos hatched at 96 h and died immediately after hatching with severe malformations.
Abnormal morphology was seen at 50 and 100 µM at 72 hpf, followed by shrunken embryos at 96 h, finally resulting in 100 % dead embryos.
At 96 h and at 10 µM, pericardial edema was seen in 100 % of the larvae and spinal defects in 17.5 %.
At 25 µM and higher, embryos were lighter colored in a dose-dependent manner at 72 hpf.
Several gene expressions were examined after exposure to 1 and 10 µM of propylparaben: genes involved in physiological pathways like stress response, cell cycle, DNA damage and inflammation, fatty acid metabolism as well as genes involved in endocrine functions. Only the latter are reported here:
At 10 µM (~1.8 mg/L), propylparaben downregulated the androgen receptor gene (ar) and upregulated the estrogen receptor 2 alpha gene (esr2a), suggesting an anti-androgen and estrogen activity.
Furthermore, an upregulation of thyroid hormone receptor alpha a (thraa) and thyroid hormone receptor beta (thrb) was observed and was significantly different respectively at 10 µM and from 1 µM, suggesting effects on thyroid signalling.

In this study also expression of genes related to oxidative stress were examined:

Downregulation of *nuclear factor, erythroid 2-like 2a (nrf2), kelchlike ECH-associated protein 1 (keap1)* and *superoxide dismutase 1 (sod1)*
Downregulation of *microsomal glutathione S transferase (mgst) and glutathione S transferase (gst)*
No significant change in *catalase (cat)* and *superoxide dismutase 3 (sod3)*

It can be concluded that exposure to propylparaben resulted in alteration of several physiological pathways and that it might also affect the endocrine system at early life developmental stages of zebrafish. Propylparaben can cause toxicity by inducing oxidative stress. Furthermore, authors demonstrated that propylparaben shows a significantly higher toxicity compared to methylparaben.

Perugini *et al*. (2020)

Guidelines: According to OECD TG 236

GLP: not mentioned

Material and methods:

Test substances: Propylparaben from Sigma-Aldrich
Purity: not mentioned
Test species: Zebrafish (wild-type AB strain)
Type of test: semi-static (renewal every 24 h)
Species life stage: 2 hpf
Route of administration: water

Test conditions:
- *photoperiod:* Adult fish/embryos: 14 h light, 10 h dark
- *water temperature:* Adult: 28 °C, embryos: 26.0 ± 1 °C
- *feeding*: Adult: fed three/day with dry feed and Artemia salina

Test design:

1 – 3 hpf zebrafish embryos

Exposure duration: 96 h

Applied concentration: nom. conc.: solvent control, water control, 1, 2, 4, 6, 8 mg/L

Solvent: 0.1 % DMSO

Positive control: 4 % 3,4-dichloroaniline

Replicates: 3 replicates; 20 embryos/concentration

Parameters examined:

Mortality
Sublethal alterations
Abnormalities
Hatching rate

Data analysis and Statistics:

FET: Bayesian approach

Beta distribution was performed to evaluate differences in the percentage of anomalies among tested concentrations and time of exposure.
Significant level: **P < 0.05.
LC50 was calculated using ToxRat software version 3.3 (ToxRat Solutions GmbH).

Morphometric analysis and ORO quantification: t-test analysis

to determine the statistical difference between exposed and control groups. Statistically significant if P values were **P < 0.05; ****P < 0.01; *****P < 0.001. Statistical analyses were performed using the GraphPad Prism 7 software (GraphPad software).

Results and discussion:

In this study, performed according to OECD TG 236 (Fish Embryo Acute Toxicity Test) 1 hpf zebrafish embryos were exposed to 0, 1, 2, 4, 6 and 8 mg/L of propylparaben for 96 h. Propylparaben was dissolved in 0.1 % DMSO.

No significant difference was seen in mortality between the water and solvent control.

A 96 h LC50 of 3.98 mg/L was determined based on coagulation of fertilized eggs, lack of somites formation, lack of detachment of the tail-bud from the yolk sac and lack of heartbeat. The most common adverse effect seen in all test groups at 96 h was lack of heartbeat and coagulation of embryos. Although no malformations were seen at 24 hpf in all treatments, there was absence of spontaneous movement.

Furthermore, hatching rate was reduced in the control group (55.9 %) and significantly decreased in all concentrations at 72 hpf when compared to the water control (resp. 41.7** %, 45** %, 17** %, 0** % and 0** % at 1, 2, 4, 6 and 8 mg/L). None of the surviving embryos at 6 and 8 mg/L hatched at 72 hpf. It can be concluded that at 72 hpf there was a delay in hatching.

At 1 mg/L significant enlargement of yolk sack and reduction in body length and head size was seen at 96 hpf ($p<0.05$).

At 2 mg/L and higher enlarged yolk sack, blood stasis, reduction in blood circulation, pericardial edema, skeletal deformities and reduction of body length and head size were observed.

The increase in yolk sack was found to correspond to a significant reduction in swim bladder size at 2 mg/L. It was noted that the effects on blood stasis, blood circulation and

pericardial edema appeared before the reduced heartbeat at 2 mg/L suggesting vascular endothelial dysfunctions as the cause of circulatory alterations.

At 2 mg/L hyperexcitability observed at 1 and 2 mg/L led to immobility at higher concentrations. Hyperactivity could be linked to the hampered lipid metabolism.

Furthermore, after exposure to propylparaben the distance between the eyes became very small an the neurocranium was strongly deformed.

Authors measured a decrease in ORO staining (Oil Red O staining, a direct measure of the content of neutral lipids and triglycerides) in the body but an increase in the yolk of propylparaben exposed embryos.

Treatment with propylparaben also hampered phospholipid metabolism, notably a decrease in PLA_2 activity, specifically in the head and cartilage level and yolk sac. PLA_2 activity is essential in craniofacial skeleton formation.

It can be concluded that propylparaben induced neurological and skeletal abnormalities which might be due to the altered lipid utilization in zebrafish early life stages.

Liang *et al*. (2022)

For the *in silico* data and the *in vitro* data of this study see section 7.10.1.

Guidelines: non-guideline

GLP: non-GLP

Material and methods:

Test substances: Propylparaben from TCI (Japan), CAS: 94-13-3
Purity: >99.0 %
Test species: Zebrafish, wide type, AB strain
Type of test: daily renewal of the solution
Species life stage: 2 hpf embryos

Route of administration: water

Test conditions:
- *photoperiod: /*
- *water temperature: /*
- *feeding*: /

Test design:
Exposure duration: 120 hpf
Applied concentration: nom. Conc.:
Survival: SC, 1, 2, 5, 10, 20 and 50 µM
Other parameters*:* SC, 5, 10 and 20 µM
Meas. Conc.: did not remain within 80 % of nominal concentration

Table 91: Geometric mean measured concentration

µM	0 h	24 h	Geom. mean meas.
SC	ND	ND	ND
5	3.62 ± 0.10	2.50 ± 0.05	3.08 (~0.56 mg/L)
10	7.64 ± 0.05	4.70 ± 0.45	5.99 (~1.08 mg/L)
20	16.43 ± 1.09	9.70 ± 0.16	12.62 (~2.27 mg/L)

Solvent: 0.1 % DMSO
Replicates: 6-well plates, 40 embryos/well, 3 replicates

Parameters examined:

Survival and hatching rate were examined after 72 h exposure
Hatching rate, malformation rate, body length, heart rate and thyroid levels were examined at 120 hpf

Data analysis and Statistics:

Statistical significance was evaluated using One-way analysis of variance with the Dunnett test.

Results and discussion:

Solvent control did not significantly differ from blank control.

Survival only sign. Affected at 50** µM (decrease of 94.2 %).

Table 92: Effects of propylparaben (from Liang *et al*., 2022)

Nom. conc.	SC	5 µM	10 µM	20 µM
Hatching rate (%)	87.9 ± 4.0	80.0 ± 2.5	66. 7 ± 8.0****	53.3 ± 6.3****
Malformation rate (%)	2.9 ± 2.1	1.7 ± 1.4	57.5 ± 15.6****	75.8 ± 8.0****
Body length (mm)	4.1 ± 0.2	4.0 ± 0.2**	3.8 ± 0.2****	3.5 ± 0.2****
Heart rate (30 sec)	76.2 ± 4.4	74.7 ± 5.6	72.7 ± 6.0****	71.1 ± 5.3****

**: $p < 0.05$ and ****: $p < 0.01$

Propylparaben affected (decrease) both Total T3 and T4 significantly at 5**, 10**** and 20**** mg/L.

Conclusion: OECD CF level 3/4 data

No significant difference in **sex ratio** at 43 dpf in medaka after embryonic exposure to 4000 µg/L (Gonzalez-Doncel *et al.*, 2014), but severe adverse effects (macroscopical and histopathological) were observed in some individuals at 13 dpf. As no signs of histological damage were found in surviving 28 and 43 dpf larvae it can be concluded that those severe effects lead to subsequent mortality.

Except for Gonzales-Doncel *et al.* (2014), Bereketoglu and Pradhan (2019), Perugini *et al*. (2020) and Liang *et al*. (2022) observed a **delay in hatching** in Danio rerio respectively at 10 µM (~1.5 mg/L), 1 mg/L and 10 µM (geom. mean meas. ~1.08 mg/L). Furthermore, those authors saw a sign. **Decrease in hatchting** resp. from 25 µM, 1 mg/L and 10 µM.

At 10 µM (~1.08 mg/L (geom. mean)), propylparaben downregulated the androgen receptor gene (ar) and upregulated the estrogen receptor 2 alpha gene (esr2a), suggesting an **anti-androgen and estrogen activity** (Perugini *et al*., 2020). Furthermore, they observed effects on **thyroid signaling** based on an upregulation of thyroid hormone receptor alpha a (thraa) and thyroid hormone receptor beta (thrb) respectively at 10 µM and from 1 µM, while Liang *et al*. (2022) noted a sign. decrease of Total T3 and T4 at 5, 10 and 20 µM.

OECD CF Level 4: *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

Table 93: OECD CF Level 4 data

Method	Short description of Method	Result	Description of Result	References	Reliability
Non-standard assay	Exposure: Juvenile zebrafish (20 dph) were fed 500, 1000 or 2000 mg/kg PPB, compared to control (solvent only) and positive control (20 mg/kg E2) Effect measurement: - Vitellogenin measurement in whole body homogenates (ELISA) after 20 days exposure - Determination of sex ratio after 45 days exposure: weight & length, and sex on paraffin sections by light microscopy	No effects on VTG Effect on sex ratio	No significant effects on VTG for paraben treated compared to control fish, while E2 gave significantly higher VTG values. Sex ratio skewed significantly to female in 500 mg/kg PPB with 71 % female compared to 40 % in control group ($p<0.017$). Similar shift in other PPB treatments (60.4 % female) but not significant compared to control. **Secondary effects**: no significant effect on weight and length compared to control group, but **slight mortality** in control and paraben treated group (1.7 to 8.6 %). However, E2 treatment resulted in fish mortality (45 % after 45 days), and no sex determination could be performed as there were no visible gonads, though well-developed urogenital papillae.	Mikula *et al.*, 2009	2
Non-standard assay	Exposure on *Copecod Tigriopus japonicus* Acute toxicity test: exposure to 0, 100, 200, 300, 400 and 500 µg/L PPB for 96 h Development and fecundity: exposure to 0, 0.05, 0.5, 5 and 50 µg/L PPB	Acute Toxicity Effects on development and fertility	LC50 of 114 µg/L in male (NOEC: 100 µg/L), and 357 µg/L in female (NOEC: 200 µg/L). Metamorphosis sign. delayed and reproduction sign. decreased at 50 µg/L ($p<0.05$)	Kang *et al.*, 2019	2

	Sex ratio: exposure to 0 and 50 µg/L PPB	Effect on sex ratio	Female to male ratio sign. increased at 50 µg/L (feminization) ($p<0.05$).		
OECD TG 234 (FSDT)	Exposure: Fertilised eggs of *Danio rerio* (30 eggs/test vessel) were exposed to nom. conc PPB of 6.40, 20.2, 64.0, 202 and 640 µg/L (7.66, 25.4, 59.9, 165 and 518 µg/L mean meas.) until 70 dpf Effect measurement: - Hatching rate - Post hatch survival at 35 dpf and 70 dpf - Length at 35 dpf and 70 dpf - Weight at 70 dpf - VTG - Sex ratio Histopathology	No effect on mortality, hatching rate and VTG Effect on size (length and weight) Effect on sex ratio and maturation stage	Sign. increase of length and weight. Females: NOEC length/weight= 59.9 µg/L (mm) Males: NOEC length= 165 µg/L (mm) Weight ≥ 518 µg/L (mm) No sign. effects on VTG: NOEC ≥ 518 µg/L (mm) Sign. Increase of females and decrease of undifferentiated fish at 518 µg/L (mm), NOEC= 165 µg/L (mm) Females exposed to PPB showed higher maturation stages than compared to the control, with increasing lesions in the ovaries with increasing test conc.	REACH registration dossier, Unpublished study report, 2020	1

Mikula *et al*. (2009)

Guidelines: non-guideline

GLP: non GLP

Material and methods:

Test substances: Propylparaben
Test species: zebrafish (Danio rerio)
Type of test: semistatic (replacement of water 3 times/week)
Species life stage: 20 d post hatch
Acclimation period: 3 d

Route of administration: diet

Test conditions:
- *photoperiod:* 12 h light, 12 h dark
- *water temperature:* 24.5 ± 1.5 °C
- *pH:* 7.7 ± 0.2
- *dissolved oxygen*: did not fall below 60 % in any of the tanks (between 61 – 72.6 %)
- feeding: mix of Artemia salina eggs and propylparaben, 17β-estradiol or solvent, resp., 3 times/day

Test design:
Exposure duration: 20 d (VTG), 45 d (sex ratio)
Applied dose:
0 (solvent control), 500, 1000 and 2000 mg/kg
Positive control: 20 mg/kg 17β-estradiol (min. 98 %, Sigma-Aldrich; St. Louis, USA)
Solvent: diluted in ethanol, mixed with food followed by evaporation of the solvent
Replicates: 350 fish divided in 10-15 L glass tanks (10 L of water according to ISO7346-2) 35 fish/tank
2 replicates/conc.

Parameters examined:
VTG: after 20 days, 6 fish from each tank, randomly selected, were killed and VTG was measured in whole body homogenates.
Weight and length
Sex ratio: after 45 days all remaining fish were killed and sexed using light microscopy

Data analysis and Statistics:
ELISA kit
Kruskal-Wallis non-parametric test: to compare differences in vitellogenin concentrations amongst fish, followed by a multiple comparison when significant differences were found ($p<0.05$)
Fisher's exact test: to analyse the difference in sex ratio between control and the different propylparaben exposure concentrations ($p<0.017$)
Statistical software (StatSoft Inc, 2007)

Results and discussion:
During gonadal development of juvenile Zebrafish, regardless of sex, premature ovaries are developed at the beginning of the differentiation period.

Three stages of gonadal differentiation were observed in zebrafish by Maack and Segner (2004):
1) juvenile hermaphroditic stage at 15-42 dpf ~ ±13-40 dph (Kimmel *et al.,* 1995: hatching period between 2 and 3 dpf) distinguished by immature ovaries. About half of those premature ovaries are transformed to testis,
2) gonad transition stage at 43-71 dpf ~ 41-69 dph where gonads differentiate into testis or ovaries resp. and,
3) premature stage at 72-99 dpf ~ 70-97 dph of testicular or ovarian development.
Maack and Segner (2004) concluded that the most susceptible period to have effects on development that were persistent after estrogen exposure occur during the gonad transition period.

In this study, no significant differences were recorded for length and weight between control (resp. 25.3 mm and 277.9 mg mean) and propylparaben exposed fish at day 45. However, weight and length from fish exposed to17β-estradiol were significantly lower.

No significant difference in Vitellogenin was detected at all paraben doses. The 17β-estradiol exposure group was excluded from further analyses because of the significant difference.

Table 94: VTG concentrations (from Mikula *et al*., 2009)

	VTG conc. (median): ng/mL
Control (0 mg PPB /kg)	854
500 mg PPB/kg	799
1000 mg PPB/kg	830
5000 mg PPB/kg	811
20mg 17β-estradiol/kg	1177946

Sex ratio:
Exposure to 20 mg 17β-estradiol/kg lead to 41.4 % mortality at the end of the 45 days exposure period. No sex ratio could be determined for this group as gonads were not visibly detectable, in contrast to the urogenital papillae which were well developed.
In the other exposure groups mortality was negligible (1.7 – 8.6 %).
Only a significant difference in sex ratio (skew towards females) was observed in the 500 mg PPB/kg group with 71 % females compared to 40 % in the control ($p<0.017$). Similar shifts were seen in the other propylparaben exposure groups (60.4 % females) but were not significantly different from the control.

The shift in sex ratio appears to be not ER–mediated because no inhibition or induction of vitellogenesis was observed, but it may be explained by interference on the HPG-axis or other hormones influencing the sex differentiation process (e.g. thyroid hormones).

Kang *et al.* (2019)

The marine copepod *Tigriopus japonicus* was exposed to methyl-, ethyl- and propylparaben.

To investigate the acute toxicity, more in particular for propylparaben, 10 males and 10 females of the copepod were exposed to 0, 100, 200, 300, 400 and 500 µg/L. A 96hLC50 of 113 µg/L and 357 µg/L was determined for males and females respectively.

Adverse effects on development (10 newborn nauplii <24 h old) and fecundity (10 ovigerous females) were evaluated after exposure to concentrations of 0, 0.05 (0.277 nM), 0.5 (2.774 nM), 5 (27.74 nM), and 50 µg/L (0.277 mM) of propylparaben. Significant delay in metamorphosis from nauplius to adult was observed at all concentrations ($p<0.05$). Reproduction was significantly decreased at 50 µg/L ($p<0.05$). Sex ratio was examined only at 50 µg/L propylparaben. A clear feminisation (significant change in female to male ratio-$p<0.05$) was seen which might be attributed to the binding of propylparaben to the estrogen-related receptor (ERR).

In this study it was also shown that propylparaben exhibited the highest acute toxicity of the tested parabens.

Fish Sexual Development Test (OECD TG 234) (REACH registration dossier, Unpublished study report, 2020)

Guidelines: OECD TG 234 (Fish Sexual Development Test)
Deviation: study prolonged with 7 d until 70 dpf in order to perform proper sex determination and thus reduce undifferentiated fish.
GLP: GLP

Material and methods:

Test substances: Propylparaben, purity: 99.7 %
Test species: zebrafish (Danio rerio)
Type of test: flow through
Species life stage: fertilised eggs (< 12 hpf)

Route of administration: freshwater (purified drinking water), flow rate of 10 volumes/vessel

Test conditions:
- *photoperiod:* 12 h light, 12 h dark
  Light intensity: approx. 1000 Lux
- *water temperature:* 26.8 – 27.5 °C
- *pH:* 7.52 – 7.66 (min. 7.2 – max. 7.83)
- *dissolved oxygen*: between 89 – 98 %, min. 78 %
- feeding: from 6 dpf: with breeding food *ad libitum*, from 14 dpf with brine shrimp nauplii (Artemia salina) and from 21 dpf with ground flake food *ad libitum*

Test design:
Exposure duration: until 70 dpf
Applied dose:
Stock solution: 250 µL HCl (37 %)/L was added to increase the stability of the test item. Equal amount of HCl was added to acidify the control.
Nom.: control, 6.40, 20.2, 64.0, 202 and 640 µg/L
Mean meas.: 0, 7.66, 25.4, 59.9, 165 and 518 µg/L => 80.5 % (640 µg/L) - 127.4 % (20.2 µg/L) of nom.
Replicates: 4 replicates/test concentration and control, 30 eggs/replicate

Parameters examined:
Hatching success
Survival: at 21 dpf, 35 dpf and 70 dpf
Weight and length: at 35 dpf and 70 dpf
VTG: at 70 dpf
Sex ratio: at 70 dpf (macroscopic, histological, and histopathological analysis)

Data analysis and Statistics:
All statistics were calculated using ToxRat® Professional 3.3
NOEC/LOEC: ANOVA, followed by Williams test or respective non-parametric approaches (e.g. Jonckheere-Terpsta test)
VTG determination by homologous ELISA kit

Results and discussion

No effect was observed on hatching success. Larvae started to hatch at 3 dpf and hatch was completed at 5 dpf. Complete hatch was seen in all replicates.

No significant difference in post-hatch survival was seen at 35 dpf and 70 dpf between treatments and the control, resulting in a NOEC ≥ 518 µg/L (mm).

At 35 dpf the mean total length in the control was 2.17 cm. Length in treatment groups ranged between 2.20 cm (at 59.9 µg/L) and 2.35 cm (at 518 µg/L) and was statistically significantly increased at the highest concentration ($p<0.05$).
At the end of the test (70 dph), the mean total length of the control group was 3.3 cm. Length in treatment groups ranged between 3.3 and 3.6 and was statistically significantly increased in females at ≥ 165 µg/L and in males at the highest test concentration ($p<0.05$). Considering all fish, control fish weighed 0.381g at 70 dph and mean wet weight ranged in treatment groups ranged between 0.789 g (7.66 µg/L) and 0.503 g (518 µg/L). Significant difference with the control was seen at the two highest test concentrations ($p<0.05$).

According to the registrant, one explanation for an increased size is a bacterial growth, which was seen in the highest concentration and which could serve as additional feed for fish exposed to this test level.

Size data resulted in:
70 dpf $NOEC_{length}$ females= 59.9 µg/L (mm), 70 dpf $NOEC_{length}$ males ≥ 518 µg/L (mm).

70 dpf $NOEC_{weight}$ females= 59.9 µg/L (mm), 70 dpf $NOEC_{weight}$ males= 165 µg/L (mm).

A concentration-dependent shift in sex ratio towards females was observed. The increase in number of females and decrease in undifferentiated fish was statistically significant at the highest dose ($p<0.05$). When considering females, males and undifferentiated fish, the mean % females in controls was 45.9 % which increased to 81.3** % at the highest test concentration, while the mean % of undifferentiated fish decreased from 26.3 % in control to 3.6 %** at the highest test concentration. A decrease in number of males was seen, but this did not follow a linear trend and was not statistically significant compared to the control (27.8 % in control vs 15.1% at 518 µg/L).

An in-depth histological examination of the gonads revealed a treatment-dependent effect on the maturation of females. More mature gonads were seen with increasing test concentration. At the highest concentration ovaries were mostly in stage 3 or 4, while the majority of ovaries in the control were at stage 1 and 2. This earlier maturation was observed together with a dose-dependent increase in egg debris and granulomatous inflammation in the ovaries. Following the registrant this is most likely due to the incapability to lay eggs as a substrate to facility egg production was absent.

Although not statistically significantly impacted, vitellogenin in females was increased at the two highest test concentrations (slightly) and in males and undifferentiated fish at the highest test concentration compared to the control. Mean concentrations of VTG in females ranged between 314.1 ng/µg Total protein (404626.4 ng VTG/mL plasma) at 59.9 µg/L propylparaben to 569.9 ng/µg Total protein. (605374.6 ng VTG/mL plasma) at 518 µg/L propylparaben. In males, VTG concentrations ranged between 63.9 ng VTG/mL plasma (59.9 µg/L) and 3796.44 ng VTG/mL plasma (at 518 µg/L). However, no dose-dependent relationship was observed. VTG increased dose-dependently in undifferentiated fish and reached the highest level at 518 µg/L with 1463.74 ng VTG/mL plasma but was not statistically significantly different from the control (443.67 ng VTG/mL plasma).

Summary of Findings of the FSDT study (2020):

Sex ratio:

- According to OECD TG 234 method: concentration-dependent increase of females (from 25.4 µg/L), statistically significant at highest concentration (81.3 %), together with increasing lesions in females (egg debris and granulomatomous inflammation) and treatment-dependent effect on the maturation of females (induced maturation: mostly stage 3 and 4 compared to stage 1 and 2 in control).

- Decrease in males compared to control in all treatments (although not dose-dependent and not statistically significant): 46 % reduction at highest dose.

- Dose-dependent decrease in undifferentiated fish, which was statistically significant at the highest dose (518 µg/L).

VTG:

Although not considered significant,
- increase in VTG in females at 165 µg/L and 518 µg/L,
- dose-dependent increase in males from 59.9 µg/L (3796.44 ng/mL at highest concentration vs 31.70 ng/mL in plasma in control: 119.8-fold increase)

Conclusion:

VTG increase in males and females supports the sex shift towards females (statistically significant at the highest dose). This sex shift was accompanied with more maturated stages in the ovaries and a decrease in undifferentiated fish. The whole of these findings confirm the weak estrogen agonist MoA of propylparaben.

Conclusion OECD CF level 4

Feeding study:

Shift in sex ratio towards females in zebrafish at lowest dose (500 mg/kg) tested, no significant effect on VTG (Mikula *et al.*, 2009). MoA is not clear: ER-mediated MoA or by interference with HPG axis or other hormones influencing sex differentiation.

Waterborne study:

FSDT: A skew in sex ratio towards females in zebrafish (dose-dependent, sign. at highest conc. of 518 µg/L) together with an increase in VTG in males and females (although not stat. sign., dose-dependent in males from 59.9 µg/L; not dose-dependent in females) Concentration-dependent increase in oocyte maturation.

Supporting information:

Also in the marine copepod *Tigriopus japonicus* a clear feminisation was observed which might be attributed to the binding of propylparaben to the estrogen-related receptor (ERR) (Kang *et al*., 2019).

OECD CF Level 5: *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

No data available

Conclusion OECD CF level 5

/

Table 95: Neuronal behaviour

Method	Short description of Method	Result	Description of Result	References	Reliability
OECD TG 236 (FET)	2 hpf zebrafish embryos Exposure duration: 96 hpf, than exposure is stopped until 6 dpf Exposure conc.: 0, 0.1, 1 and 10 ppb, solvent: $1x10^{-9}$ % ethanol (v/v) 3 replicates: Mortality, hatching, non-lethal malformations : n= 40/group), heart rate (n= 10/group)	Decreased hatching rate at 48 hpf Increased mortality at highest conc. Decreased heart rate Increased malformations Anxiety-like behaviour (light-dark preference test-scototaxis)	Sign. decrease of hatching rate at 48 hpf at 0.1**** and 10**** ppb, effect no longer observed at 72 and 96 hpf. Mortality increased sign. at 72 hpf at the highest conc. Of 10***** ppb (± 25 %) compared to control (± 8 %). The heart rate decreased sign. at all concentrations (0.1****, 1***** and 10***** ppb). PPB increased the incidence of malformations (bent spine, yolk sac edema, pericardial edema, and red blood cell accumulation) at all tested conc. (0.1*****, 1***** and 10***** ppb) after 96 hpf. At 6 dpf, a reduction of time spent in the light compartment was only seen at 1***** and 10***** ppb as well as time spent in the dark compartment which increased sign. at 1***** and 10***** ppb. Number of transitions between light and dark region (exploratory behaviour) was sign. reduced at 1** and 10**** ppb.	Lite *et al*., 2022	2

		Reduction of SOD, CAT	PPB reduced SOD activity in head sign. at all conc. (resp. 1.4, 0.9 and 0.6 U/mg protein at 0.1***, 1**** and 10***** ppb). Sign. reduction of CAT activity at all conc. (0.1**, 1*****, 10***** ppb).		
		Reduction of antioxidant enzymes activity (GPx and GST), reduction of non-enzymatic antioxidant GSH	Sign. reduction of GPx and GST at 1**** and 10**** ppb, max reduction of GPx of 6.4 U/mg protein and GST of 0.33 U/mg protein at 10***** ppb. Sign. reduction of GSH activity at 0.1*****, 1***** and 10***** pbb.		
		Inhibition of AchE activity	Sign. reduction at all conc. (0.1*****, 1***** and 10***** ppb) with max. inhibition at 10 ppb: 3.4 nmol/mL.		
		Increased nitric oxide levels	Sign. increase at 1** ppb, with a max. at 10** ppb (28 µM).		
		Increase of ROS, lipid peroxidase and apotosis	Sign. increase of ROS and apoptosis at all conc. (0.1***, 1*** and 10*** ppb, with an increase of resp. 77 % and 78 % at 10 ppb) Lipid peroxidase increased at all conc (0.1*****, 1***** and 10***** ppb) with an increase of 58 % at 10 ppb.		

Lite *et al*. (2022)

In this study, authors examined several parameters (mortality, hatching, heart rate, malformations, neurobehaviour, enzyme activity, ROS, apoptosis, and lipid peroxidase) in zebrafish embryos. 2 hpf old wild type (AB strain) zebrafish were exposed to 0, 0.1, 1 and 10 ppb of propylparaben until 96 hpf. Anxiety-like behaviour was examined at 6 dpf. Ethanol was used as a solvent in concentrations of 1 x 10^{-9} % (vol/vol). Results were compared to the water control.

Mortality was measured at 24, 48 and 72 hpf and increased sign. At 72 hpf at the highest conc. of 10***** ppb.

The hatching rate sign. decreased at 48 hpf at 0.1**** and 10**** ppb. This effect was however no longer observed at 72 and 96 hpf.

A sign. decreased heart rate was shown at all concentrations (0.1****, 1***** and 10*****ppb).

Propylparaben increased the incidence of malformations (bent spine, yolk sac edema, pericardial edema, and red blood cell accumulation) at all tested conc. (0.1*****, 1***** and 10***** ppb) after 96 hpf.

To examine the anxiety-like behaviour of the zebrafish larvae a light-dark preference test (scotaxis) was performed. For this, exposure of fish was stopped after 96 hpf until 6 dpf. A reduction of time spent in the light compartment was only seen at 1***** and 10***** ppb as well as time spent in the dark compartment which increased sign. At 1***** and 10***** ppb. Number of transitions between light and dark region (exploratory behaviour) was sign. Reduced at 1** and 10**** ppb.

In an enzyme assay SOD, CAT, gluthatione (GSH), Glutathione peroxidase activity (GPx), Glutathione S-transferase (GST) activity, Acetylcholinesterase (AchE) and nitric oxide (NO) level were examined.
Propylparaben sign. reduced GPx and GST at 1**** and 10**** ppb, with a max. reduction of GPx of 6.4 U/mg protein and GST of 0.33 U/mg protein at 10***** ppb. GSH activity was sign. Reduced at all concentrations (0.1*****, 1***** and 10***** pbb).
Propylparaben inhibited AchE activity sign. At all conc. (0.1*****, 1***** and 10***** ppb) with max. inhibition at 10 ppb: 3.4 nmol/mL. Inhibition of AchE activity is a marker for neurotoxicity.
NO-levels sign. increase at 1**** ppb, with a max. at 10**** ppb (28 µM).

Furthermore, propylparaben sign. increased of ROS levels and apoptosis at all conc. (0.1*****, 1***** and 10***** ppb, with an increase of 77 % of ROS and 78 % of apoptosis at 10 ppb.

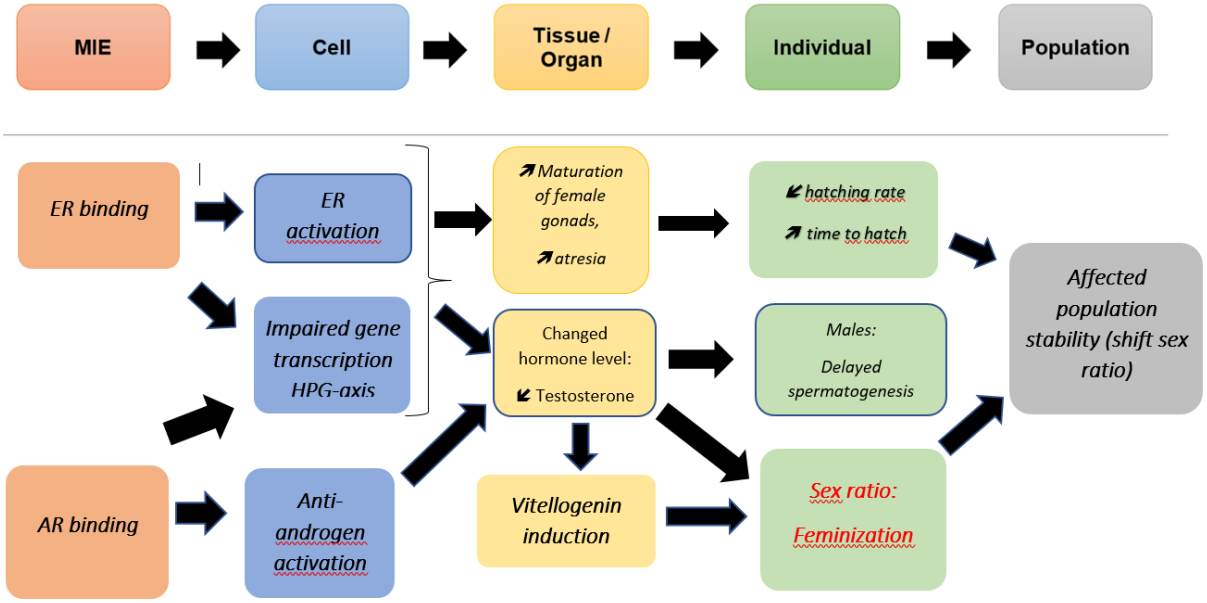
Lipid peroxidase increased at all conc. (0.1*****, 1***** and 10***** ppb) with an increase of 58 % at the highest conc. It is known that the disruption in the redox balance during early development results in malformations including growth parameters.

The reason for the observed malformations can be the collapse in redox homeostasis and increased apoptosis as it was demonstrated that propylparaben induced oxidative stress and reduced antioxidant enzyme activity.

Conclusion of ED properties environment:

Postulated Mode of Action in fish

Figure 4: scheme showing the postulated MoA(s)
The MoA presented here does not describe every detail of the biology but instead focuses on describing critical steps, acknowledging that other activities could also influence each of the key events described.



EATS-mediated adverse effect
Sensitive but not diagnostic of EATS

Several *in vitro* studies demonstrate a clear estrogenic agonistic activity of propylparaben via ER-binding, ER activation and cell proliferation. The estrogen agonistic activity was also confirmed *in vivo* in a reliable uterotrophic assay.

Although *in vitro* studies on anti-androgen activity show more diverging results, the majority of the studies show positive results. Moreover, anti-androgenicity was clearly demonstrated *in vivo* in rodents in a hershberger assay and in embryonic zebrafish, in which significant downregulation of the AR receptor was observed.

Furthermore in vitro data suggest also other ED modes of actions like glucocorticoid-like and PPARγ activity.

Several non-guideline studies demonstrate, a clear induction of vitellogenin in fish after exposure to propylparaben. Results of the Fish Sexual Development Test (OECD TG 234) also confirm this weak estrogen activity of propylparaben whereby a non-statistically significant increase in VTG in males and females was observed. It is however not surprisingly for a weak estrogen that this increase in VTG is not statistically significant. Furthermore, propylparaben downregulated the androgen receptor gene (ar), supporting an anti-androgen activity. In support, estrogenic and anti-androgen activity of propylparaben in rodents is confirmed resp. in 3 of 4 uterotrophic assays and in a hershberger assay.

Propylparaben disrupts gene transcription of the HPG-axis and those involved in steroidogenesis in embryonic zebrafish and in adult male mosquitofish and consequently changes steroid hormone levels (decrease of testosterone) in embryonic zebrafish demonstrating estrogenic and anti-androgenic effect as well as abnormal steroidogenesis after exposure to environmental relevant concentration of propylparaben. Additionally, several literature studies show that propylparaben also affect steroidogenic gene transcription and testosterone level in rodents, although not in a consistent matter.

A sex shift towards females (significant at the highest concentration) was observed in the FSDT. In a supportive feeding study with juvenile zebrafish, feminisation was seen at all concentrations but was only significant at the lowest concentration (71 % females

compared to 40% in control). This feminisation is further supported by the findings with the invertebrate marine copepod *Tigriopus japonicus* after exposure to a single concentration of 50 µg/L propylparaben.

Furthermore, propylparaben induced oocyte maturation in zebrafish accompanied by increasing ovarian lesions (egg debris and granulomatomous inflammation). An increase in oocyte atresia was also observed in rodents.

Moreover, a significant dose-dependent increase in mRNA expression of choriogenins CHG-L and CHG, an estrogen-responsive gene, was seen in adult male medaka *(Oryzias latipes)* in a supportive study.

Additionally, exposure of adult male mosquitofish leads to delayed spermatogenesis in presence of testis lesions (spermatogenic cell lesion, decreased mature seminal vesicle, sperm cells gathering, seminiferous tubules disorder and dilated intercellular space).

Although no effect on hatching success was seen in the FSDT, literature studies demonstrate that propylparaben can decrease the hatching rate and delay the time to hatch in fish as well as in invertebrates. Furthermore, propylparaben reduced fecundity in invertebrates (fruitfly *Drosophila melanogaster*, dengue mosquito *Aedes aegypti*, nematode *Caenorhabditis elegans* and the marine copedpod *Tigriopus japonicus*).

It should be noted that estrogen agonistic activity is a common endocrine modality amongst the members of the paraben family (SVHC identification as ED HH of butylparaben[1] and isobutylparaben[2]; available data demonstrating clear evidence for methylparaben). Furthermore, as indicated by (Darbre and Harvey, 2014) estrogen receptor binding activity of parabens may increase with increasing alkyl chain length and thus there might be a higher potential for estrogenic activity for long-chain length molecules (methyl<ethyl<propyl<butyl 4-hydroxybenzoate).

Furthermore, in fish, similar effects were seen for methylparaben in an FSDT (OECD TG 234), namely a significant increase in VTG and a trend towards feminisation.

Considering all relevant and reliable information in a Weight of Evidence Approach, it is concluded that there is scientific evidence that propylparaben can be identified as a endocrine disruptor for the environment according to the WHO/IPCS (2002) definition of an endocrine disruptor.

[1] Member State Committee support document for identification of butyl 4-hydroxybenzoate as a substance of very high concern because of its endocrine disrupting properties (article 57 (f) – human health properties): https://echa.europa.eu/registry-of-svhc-intentions/-/dislist/details/0b0236e18442b804

[2] Member State Committee support document for identification of isobutyl 4-hydroxybenzoate as a substance of very high concern because of its endocrine disrupting properties (article 57 (f) – human health properties): https://echa.europa.eu/registry-of-svhc-intentions/-/dislist/details/0b0236e1875e6bc9

7.10.3 Endocrine disruption – rodent data

OECD CF Level 3: *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s)

Table 96: OECD CF level 3: rodent data

Method	Short description of Method	Description of Result	References	Reliability
Non-standard assay	Uterotrophic assay Immature female mice and immature female Wistar rats SC (100 mg/kg/d) & oral (1, 10, 100 mg/kg/d)	**No sign. Difference** in uterine weight compared to control group (vehicle) for all parabens (BPB, MPB, EPB and PPB)	Hossaini, 2000	3 for the eMSCA (2 for the REG)
Non-standard guideline but similar to OECD TG 440	Uterotrophic assay Immature CD1 female mice (Im), adult ovariectomised CD1 female mice, immature Wistar (IW) female rats SC, once a day for 3 consecutive days 4 dose groups: 6.5, 20, 65 and 195 mg/kg	Immature CD1 mice: **sign. increase of uterine weight** compared to vehicle group ($p<0.05$) LOEL: 20 mg/kg NOEL: 6.5 mg/kg Ovariectomized CD1 mice: **sign. increase of uterine weight** compared to vehicle group ($p<0.05$) LOEL: 20 mg/kg NOEL: 6.5 mg/kg Immature Wistar rats: **sign. increase of uterine weight** compared to vehicle group ($p<0.05$) LOEL: 65 mg/kg NOEL: 20 mg/kg No sign. Changes in bw compared to vehicle group.	Lemini *et al*., 2003	3 for the eMSCA (2 for the REG)
Non-standard guideline	Uterotrophic assay Adult ovariectomised female CD1 mice (25-30 g) SC	**Sign. increase in uterine weight** at 65 mg/kg BW) and 195 mg/kg BW) compared to control group. The highest dose PPB nearly doubled uterine weight. Morphometrical study	Lemini *et al*., 2004	2

	65 and 195 mg/kg of PPB for 3 days	**Sign. increase of luminal epithelium heights, glandular epithelium height and myometrium.**		
OCSPP Guideline 890.1400	Hershberger assay 6-week-old immature castrated male Wistar albino rats Exposure by oral gavage for 10 d to 10, 250, and 750 mg/kg bw/d PPB + 0.4 mg/kg bw/d Testosterone propionate (TP) Controls: - vehicle control: 5 mg/kg day corn oil, - pos. control (3 mg/kg day flutamide (FLU) + 0.4 mg/kg day TP) – neg. control (0.4 mg/kg day TP, by SC injection)	**Sign. decrease in BWG** in all propylparaben doses **Sign. decrease in ventral prostate, seminal vesicles, LABC, Cowper's gland and gland penis** at 250 and 750 mg/kg bw/d compared to controls. **Decreased liver weights** at all doses compared to vehicle control**, no sign. effect on kidney weights.** <u>Hormone measurement</u> **Sign. increase in LH in serum** at all doses compared to controls. **Sign. decrease of FSH** at 10 and 250 mg/kg bw/d compared to vehicle and pos. control, but similar to controls at 750 mg/kg bw/d. <u>Histopathology</u> <u>Seminal vesicle:</u> hyalinization and anastomosis at 250 mg/kg bw/d, hyalinization, and a mass of cells in vesicle lumen at 750 mg/kg bw/d. <u>Ventral prostate:</u> At 250 mg/kg bw/d: tubular atrophy and minimal congestion, at 750 mg/kg bw/d: increase of alveolar volume, polyp structures and a mass of cells in prostate lumen. <u>Kidney:</u> congestion at 250 mg/kg bw/d and enlargement in the glomerular capsule and tubular degeneration at 750 mg/kg bw/d. <u>Liver:</u> Mononuclear inflammatory cells at 250 mg/kg bw/d, alterations like Kupffer cell proliferation among the	Özdemir *et al.*, 2018	2

		portal veins. LABC: massive ruptures of striated muscle fibers at 250 and 750 mg/kg bw/d. Cowper's gland: enlargement of nodules and hypertrophy on basal cells at 250 and 750 mg/kg bw/d.		
OECD TG 440	Uterotrophic assay in immature rats SD immature rats (PND 21) Exposure by oral gavage for 3 d to 10, 100, and 1000 mg/kg bw/d PPB Control: E2 (SC injection) + vehicles (oral + SC)	Abs and rela uterine weight slightly increased.	Sivaraman *et al*., 2018	2

Uterotrophic assays:

3 of the 4 available uterotrophic studies with propylparaben confirm the estrogen activity of the substance seen in the *in vitro* studies.

In the guideline study of Sivaraman *et al.* (2018), performed according to OECD TG 440, absolute and relative uterine weight increased slightly.

The Lemini *et al*. (2004) study (similar to OECD TG 440) is the most robust of the 3 non-standard tests. In this study subcutaneous injection in adult ovariectomised female mice resulted in a sign. Increase of uterine weight at the 2 doses tested (65 and 195 mg/kg bw/d). Furthermore, a significant increase of luminal epithelium heights, glandular epithelium height and myometrium was observed. The significant increase of uterine weight was confirmed in the study of Lemini *et al*. (2003) in mice (immature and ovariectomised females) and immature female Wistar rats. However, in the study similar to OECD TG 440 (Hossaini *et al*., 2000) no significant difference in uterine weight was seen in immature mice and rats. It should be noted that the OECD TG 440 is not validated for immature mice. Furthermore interpretation of the results of this study is difficult due to some of the deviations (body weight variation unknown, exceeded vehicle volume, …) and the fact that it is not known if the substance remained stable in the vehicle (peanut oil with 10 % ethanol).

Sivaraman *et al.* (2018)

On PND 21, immature female Sprague-Dawley rats (from 4 litters) were assigned to 6 dose groups (n= 6 rats/group) and exposed from PND 21 to PND 23 to 0, 10, 100 and 1000 mg/kg propylparaben, vehicle or 1 µg/kg/day 17α-ethinyl estradiol (E2). Substances were given by oral gavage for the 0, 10, 100 and 1000 mg/kg propylparaben, whereas the vehicle and E2 controls were injected subcutaneously (vehicles different for oral and subcutaneous exposure).

Exposure to propylparaben did not change the absolute or relative uterine weights at any dose tested.

Table 97: Body weight, absolute and relative uterine weight

Substance	Dose (mg/kg /day)	Route of exposure	BW (g)	Abs. uterine weight (g)	Rela. Uterine weight (%)
PPB	0	Oral gavage	64.1 ± 3.7	0.029 ± 0.004	0.045 ± 0.005
	10		64.3 ± 6.5	0.033 ± 0.006	0.052 ± 0.012
	100		63.3 ± 3.5	0.038 ± 0.005	0.040 ± 0.010
	1000		63.8 ± 4.1	0.037 ± 0.007	0.058 ± 0.014
E2	0	SC	63.8 ± 1.9	0.036 ± 0.007	0.056 ± 0.009
	1		64.8 ± 3.8	0.190**** ± 0.049	0.293**** ± 0.076

**** $p < 0.01$

A clear weak **agonist estrogenic activity** of propylparaben is demonstrated, based on its ability to induce uterine growth.

Hershberger assay:

In the hershberger assay (Özdemir *et al*., 2018), performed according to OCSPP Guideline 890.1400, propylparaben (TP-co-treatment) significantly decreased weights of testosterone stimulated ventral prostate, seminal vesicles, LABC, Cowper's glands and glans penis compared to the vehicle control (corn oil). It should be noted that the initial body weight in the treatment groups was lower than that of the control, however body weight gain decreased significantly in all treatment groups which is coherent with an anti-androgen effect.

Furthermore, exposure to propylparaben resulted in a significant increase in LH in all

treatment groups (10, 250 and 750 mg/kg/d) and in a significant decrease in FSH in serum at 10 and 250 mg/kg/d (compared to vehicle and positive control= testosterone propionate) and a similar level at 750 mg/kg/d compared to controls.

It can be concluded that propylparaben operates via **an anti-androgenic** modality.

Conclusion OECD CF Level 3

Estrogenicity: 3 of 4 uterotrophic assays confirm that propylparaben exhibits estrogenic activity.

Androgenicity: One hershberger assay performed in SD rats confirmed that propylparaben shows anti-androgenic activity.

OECD CF Level 4: *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

Table 98: OECD CF level 4: rodent data

Method	Short description of Method	Result	Description of Result	References	Reliability
OECD TG 421	Dose Range-Finding Study for Reproduction/ Developmental Toxicity Screening Test Rat (Wistar) (10/sex/dose (except for control group: 5/sex)) Duration of exposure: min. 35 d for M and during 21 d for premating, max 14 d 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 Oral: gavage Doses: 0, 500 and 1000 mg/kg bw/d	Reduced precoital interval Increased pre- and post-implantation loss	Female reproductive parameters: precoital interval decreased in tested groups, percentage of pre- and post-implantation loss increased. Other parameters unaffected Mean nb of pups at birth, mean nb of live pups and viability index unaffected	REACH registration dossier: Unpublished report, 2018	1 However, eMSCA disagrees and considers a reliability of 2 (low number of animals tested and not equivalent between treatment groups, only 2 doses tested, non-GLP)
OECD TG 422	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Rat (Wistar) (11/sex/dose) 4 weeks exposure for males and approx. 7 w for females (Day 1 of pre-pairing to post-partum day 3). Oral: feed Doses: 0, 1500, 4500 and 15000 ppm	Increased post-implantation loss Reduced nb of living pups	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 %). Mean nb of living pups lower at the low and high dose	REACH registration dossier: Unpublished study report, 2012	1

			groups, birth index decreased at the highest dose. Pup bw at PND 1 and 4 unaffected.		
Non-standard guideline	Wistar male rats 4 weeks exposure (From PND 19-21 to PND 46-48) Oral: feed Doses: 0, 0.01, 0.1 and 1 % corresp. To 0, 12.4, 125 and 1290 mg/kg bw/d	Reduced sperm production Reduced testosterone level	No effect on weight of reproductive organs. Dose-dependent decrease of the cauda epididymal sperm reserves and of the sperm concentration (sign. at the mid and high doses). Daily sperm production in testis sign. decreased in all treated groups. Dose-dependent decrease of testosterone concentration in serum (sign. at high dose).	REACH registration dossier: Oishi *S*, 2002	3
Non-standard guideline	CF-1 pregnant mice Exposure from GD 1 to GD 4, SC Doses: 0, 948.5 and 1084 mg/kg bw/d	No effect on implantation sites	No modifications on the number of implantation sites at any dose group. No other information available.	REACH registration dossier: Shaw and deCatanzaro, 2009	2
Non-standard guideline	SD female rats PND 21 – PND 40: daily oral gavage with MPB, EPB, PPB, IPPB, BPB and IBPB (62.5, 250 or 1000 mg/kg bw/day)	No effect on vaginal opening, oestrus cycle, bw and different organ weight	No sign. changes in vaginal opening day. No sign. changes in estrous cycle compared to normal 4-day cycle.	Vo *et al*., 2010	2

	Paraben doses adjusted daily for weight changes.	Significant effect on adrenal weight No effect on ovaries Significant effect on myometer No effect on different hormones Significant effect on T4	No sign. changes in body weight, uterus wet weight, pituitary gland weight, ovary weight, thyroid weight, kidney weight and liver weight. Increase adrenal weight with 0.32 mg/g bw (± 0.03) at 1000 mg/kg bw/d of PPB ($p<0.05$). No sign. histopathological effects on the ovaries. Myometrial hypertrophy uterus at oral concentration of 1000 mg/kg bw/d of PPB ($p<0.05$). No sign. effect on serum levels of estradiol, prolactin and TSH. Alteration of serum levels of tetra-iodothyronine (T4) at medium dose of 250 mg/kg bw/d of PPB ($p<0.01$)		
Non-standard guideline	SD neonatal female rats, 11 groups of 5 rats SC, daily exposure of 62.5, 250, 1000 mg/kg/d PPB for 7 days	No effect on uterine weight Sign. Effect in primordial follicles Sign. Effect in primary follicles	No sign. increase in uterine weight Sign. increase of number of primordial follicles at 1000 mg/kg bw/d of PPB ($p<0.05$). Sign. decrease in number of	Ahn *et al*., 2012	2

		Sign. effects on gene expression	primary follicles at 250 and 1000 mg/kg bw/d of PPB ($p<0.05$) <u>Expression of genes associated with follicle development</u> Sign. increase of **CaBP-9k** gene expression at dose 250 and 1000 mg/kg bw/d ($p<0.05$). <u>Expression of ovarian genes</u> mRNA expression of ***AMH*** sign. increased at 250 and 1000 mg/kg bw/d ($p<0.05$). No differences in ***KILT*** mRNA expression. Sign. increase in ***Foxl2*** mRNA expression at 250 mg/kg bw/d. <u>Expression of steroidogenic enzymes</u> Levels of ***StAR*** mRNA were sign. decreased at all doses. At mid dose (250 mg/kg bw/d) increased **cyp11a1** mRNA expression ($p<0.05$) (also sign. increase compared to the estradiol positive group ($p<0.05$). No information about changes in body weight.		

Non-standard guideline	Wistar male rats 8 weeks exposure (from PND 21 to PND 77) + 26 weeks recovery, treatment-free period Oral: gavage Doses: 0, 3, 10, 100 and 1000 mg/kg bw/d	Effect on the morphology of the testis No effect on sperm	No effect on time of sexual maturation, nor in weight of reproductive organs. Sign. higher weights of right testis in 100 and 1000 mg/kg bw/day groups at the end of the recovery period. Sign. higher weights of the left epididymis in all groups at the end of the recovery period. No effect on spermatid count, sperm count nor sperm motility.	REACH Registration dossier: Gazin *et al*., 2013	4 However, eMSCA disagrees and considers a reliability of 2 (No guideline followed, but GLP, 4 tested doses and control group, Well documented non-guideline study)
Non-standard guideline	5 weeks exposure of female rats to 100 mg/kg/day PPB to assess effect on ovarian dysfunction	Affected estrous cycle Affected folliculogenesis Affected hormone levels	Irregular estrus cycle. Decreased number of ovarian follicles. Increased FSH blood level. Changed mRNA pattern of genes involved in steroidogenesis and hormone receptors.	Lee *et al*., 2017	3
Similar to combined OECD TG 408 and TG 415	Reproductive developmental study after 90-days exposure	Early vaginal opening Affected fertility Affected uterus weight	No effect observed, but some observations not considered: - Earlier vaginal opening (significant) - Delayed preputial	REACH Registration dossier: Sivaraman *et al*., 2018	2

			separation - Decreased fertility index and conception rate (treated males) - Higher uterine weight (rela: sign., abs: not sign.)		
Non-standard guideline	BALB/c mice 21 days (4 weeks-old mice) Subcutaneous injection	Affected estrous cycle No effect on ovaries Affected hormone levels	Disturbed estrous cycle. Reduced number of corpus luteum and oocytes retrieved. No effect on ovaries nor folliculogenesis was observed. Decreased testosterone and increased estradiol levels in serum. Increased expression of Star and Cyp11A1.	Jiao *et al.*, 2021	3
Non-standard guideline	BALB/c mice GD 1 to PND 21: daily oral (0, 20, 100 or 10000 µg/kg bw/d) To assess effects on mammary gland	Affected mammary gland morphology	Changed mammary gland morphology.	Mogus *et al.*, 2021	2
Non-standard guideline	CD1 mice GD 1 to GD 8/9: oral gavage (0, 625, 1250 and 2500 mg/kg bw/d) To assess embryo implantation and fertility	Affected fertility	Reduced uterus weight at high dose, reduced number of implantation sites, reduced number of pups delivered.	Wang *et al.*, 2021	3

		Affected hormone levels	Increased level of estradiol and progesterone.		
Non-standard guideline	ICR mice GD 7.5 to GD 13.5 intraperitoneal injection (0, 7.5, 90 and 450 mg/kg bw/d) To assess late ovary aging (46 w-old)	Early ovary aging Affected hormone levels	Increase of atretic follicles and decrease of primordial follicles in 46 weeks-old mice. Reduced estradiol and progesterone serum levels. Reduced expression of steroidogenic enzymes (Star, Cyp11A1).	Li *et al.*, 2021	2
Non-standard guideline	C57BL/6J mice 8-months exposure (from 6 to 38 w) Oral (via diet) To assess late ovary aging	Early ovary aging Affected estrous cycle Affected fertility Affected hormone levels	Increase atretic follicles and decrease primordial follicles in 38 weeks-old mice. Estrous cycle more disturbed than control. Fertility index and number of pups delivered more reduced than control. Reduced estradiol and progesterone serum levels. Reduced expression of steroidogenic enzymes (Star, Cyp11A1, Cyp19A1).	Yan *et al.*, 2022	3

Female and male rodents

Dose Range-Finding Study for Reproduction/Developmental Toxicity Screening Test, OECD TG 421 (REACH registration dossier: Unpublished study report, 2018)

Method and results are described in detail in section 7.9.7.1 Fertility

Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, OECD TG 422 (REACH registration dossier: Unpublished study report, 2012)

Method and results are described in detail in section 7.9.7.1 Fertility

Sivaraman *et al*. (2018)

The aim of the study was to assess the effect of propylparaben exposure in juvenile rats on reproductive development and function. The authors performed a reproductive study after 90 days of exposure (from PND 4 to 90) including a toxicokinetics study from PND 7 and PND 21, for which blood was collected at 0.25, 0.5, 1, 4, 8 and 24 h following dosing on PND 7 or PND 21 (5 animals/sex/2 time points). Additionally, a conventional uterotrophic assay has been performed in immature female rats after exposure on PND 21 to 23 (see OECD level 3).

Sprague-Dawley newborns (F1) were assigned to 4 groups exposed orally (via gavage) to 0, 10, 100 and 1000 mg/kg bw/d propylparaben from PND 3 to 90 (25 pups/sex/group, with 7 – 8 litters/group). During this time developmental landmarks were observed (including sexual maturation). At PND 90, the subset animals dedicated to necropsy were euthanized and analyzed (10 rats/sex/group). The other subset animals (15 rats/sex/group) were individually cohabitated with untreated animals of the opposite sex for mating (i.e. exposed males with unexposed females and vice versa) for a maximum of 14 days. On day of copulation (GD 0), animals were separated and returned to individual housing. On GD 13, all untreated females underwent cesarean-section, whereas the treated animals were allowed to litter. F1-generation rats (males and females) were euthanized on PND 132. F2 generation pups were maintained till PND 4-6.

Toxicokinetics study

Propylparaben, which is the only estrogenic active substance, is a minor circulating analyte (<1 %). PHBA is the principal metabolite (83 to 95 %). No sex difference has been observed. The mean propylparaben and its metabolites exposure increases with dose. The highest mean exposure was reported on PND 7, due to lower maturation of metabolic pathways and no accumulation is observed.

Table 99: mean AUC

MEAN AUC (ng x h/mL)	**PND 7**			**PND 21**			**PND 83**		
Dose (mg/kg/d)	**10**	**100**	**1000**	**10**	**100**	**1000**	**10**	**100**	**1000**
PPB	60.7	509	13400	2.76	167	895	5.23	n.a.	69.9
PHBA	1870	28100	2250000	230	7860	429000	n.a.	8300	267000
SPP	7300	28800	95200	2290	13500	101000	375	6800	31300
SHBA	1920	10300	77200	465	5710	50600	379	11300	61100

PHBA = 4-hydroxybenzoic acid, SPP = Sulfate of propylparaben, SHBA= Sulfate of hydroxybenzoic acid

Reproductive development and function

Exposure to propylparaben did not change the body weight nor the food consumption at 10 or 100 mg/kg/day. A slight increase of body weight was observed in males only

(maximum 7 %) at 1000 mg/kg/day. At this dose, the food consumption increased also in males and females.

Propylparaben did not affect the vaginal opening, excepted at 1000 mg/kg/day, at which the onset occurred statistically earlier (mean 31.2 days instead 33.9 days in control). However, the authors did not consider this value was in the historical control range (from 29.0 and 33.9 days). Interestingly on days of vaginal opening, the body weight was significantly lower in females exposed to 1000 mg/kg/day. No effect on the preputial separation was observed in males.

Table 100: Vaginal opening and preputial separation data

	Vaginal opening		Preputial separation	
Dose (mg/kg/day)	**PND (days)**	**Bw (g)**	**PND (days)**	**Bw (g)**
0	33.9 ± 3.2	128.6 ±18.3	42.1 ± 1.9	221.1 ± 18.6
10	32.4 ± 1.5	125.2 ± 12.9	42.3 ± 1.6	224.1 ± 19.5
100	32.7 ± 1.3	122.1 ± 10.0	42.3 ± 1.7	224.3 ± 17.7
1000	31.2* ± 2.2	115.2* ± 14.9	43.2 ± 1.5	243.4**** ± 16.0

**** $p < 0.01$

Propylparaben exposure did not affect estrous cyclicity.

Table 101: Estrous cyclicity

Dose (mg/kg/day)	Mean cycle length (days)
0	4.19 ± 0.50
10	4.43 ± 1.42
100	4.29 ± 0.66
1000	4.29 ± 0.56

Females exposed to propylparaben did not show any substance related effects on mating, fertility, gestation, or reproduction.

Table 102: fertility and developmental data (females exposed to PPB)

Females exposed to propylparaben						
Dose (mg/kg/d)	**Fertility index (%)**	**Conception rate (%)**	**Gestation length (days)**	**Sex ratio (% males)**	**Live birth index (%)**	**Viability index (Day 4)**
0	86.7	92.9	21.8 ± 04	49.33 ± 18.06	88.96 ± 13.73	100.0 ± 0.00
10	80.0	92.3	21.8 ± 0.4	48.22 ± 13.27	88.20 ± 9.84	99.56 ± 1.53
100	93.3	100.0	22.2 ± 0.4	48.06 ± 13.94	89.72 ± 10.13	98.59 ± 2.83
1000	93.3	100.0	21.9 ± 0.4	43.69 ± 13.68	92.36 ± 6.43	99.04 ± 2.43

Similarly, naïve females mated with males exposed to propylparaben did not show any substance related effects on mating, fertility, gestation, or reproduction. At 100 and 1000 mg/kg/day there were minor reduction in fertility index and conception rate but were not considered relevant because they were within the historical control range.

Table 103: fertility and developmental data (females mated with exposed males)

Naïve females mated with males exposed to propylparaben						
Dose (mg/kg/d)	**Fertility index (%)**	**Conception rate (%)**	**Implantation sites (n)**	**Live embryos (n)**	**Pre-implantation loss (%)**	**Post-implantation loss (%)**
0	92.9	92.9	16.8 ± 2.0	15.8 ± 1.7	5.74 ± 5.10	5.15 ± 7.21
10	93.3	93.3	17.0 ± 1.7	15.6 ± 2.1	5.06 ± 4.48	8.15 ± 6.15
100	80.0	80.0	17.4 ± 1.5	16.5 ± 1.2	7.76 ± 6.78	5.10 ± 4.77
1000	86.7	86.7	15.8 ± 3.8	15.3 ± 3.8	7.88 ± 10.74	3.68 ± 3.50

After 90 days of exposure, there was an important increase of mean absolute uterine weight (36.0 % higher) and relative uterine weight in females dosed at 1000 mg/kg/day (43.0 %, significant). However, the authors did not consider this increase as relevant because more females were in the proestrus or estrous on the day of necropsy (6 vs 4). Interestingly such increase does not correlate with the number of rats in proestrus and estrous in intermediate doses (10 and 100 mg/kg/day). No other substance related change on the reproductive organ weight were observed.

Table 104: Estrous and uterus weight

Dose (mg/kg/day)	Rats in proestrus (n)	Rats in estrous (n)	Absolute uterine weight (g)	Relative uterine weight (%)
0	3 (/10)	1 (/10)	0.6357	0.22622
10	2 (/10)	3 (/10)	0.6051	0.22682
100	2 (/10)	2 (/10)	0.5451	0.20641
1000	4 (/10)	2 (/10)	0.8651	0.32355[A]

[A]: $p < 0.005$

Some histological findings were observed in animals exposed to propylparaben (seminiferous tubule atrophy, mammary adenocarcinoma), but were considered as an incidental finding (no effect on seminiferous tubule in highest dose, single animal mammary carcinoma). No changes were also found on hematology, coagulation, serum chemistry and urinary parameters.

Male rodents:

Oishi S. (2002)

The aim of the study was to observe the effect of propylparaben on the male reproductive function. 3-weeks-old Wistar rats were exposed to 0, 0.01, 0.1 and 1 % propylparaben, corresponding to 0, 12.4, 125 and 1290 mg/kg bw/d via food for 4 weeks (n= 8/group).

Exposure to propylparaben reduced dose-dependently the sperm reserves in the cauda epididymal (number of sperm/cauda epididymis and number of sperm/g cauda epididymis). The daily sperm production was also statistically significant reduced at all doses. No effect has been observed on male reproductive organs weight.

In parallel, the testosterone level in serum also showed a dose-dependent reduction, reaching a level of 64.6 % of the control value after exposure to 1 % propylparaben (statistically significant decrease).

Table 105: Sperm parameters

Dose in % (in mg/kg bw/day)	Sperm/cauda epididymis (x 10^7)	Sperm/g cauda epididymis (x 10^7/g)	Daily sperm count in testis (x 10^6)	Serum Testosterone (ng/mL)
0 % (0)	43.6 ± 18.2	108 ± 43.6	37.5 ± 5.32	9.08 ± 2.12
0.01 % (12.4)	31.1 ± 10.5	70.8 ± 18.7	26.3 ± 2.34**	8.20 ± 2.29
0.1 % (125)	25.7 ± 6.97**	63.1± 17.7**	27.0 ± 9.07**	7.17 ± 3.20
1 % (1290)	22.5 ± 11.9**	48.8 ± 26.6**	25.9 ± 3.90**	5.86 ± 2.82**

** $p < 0.05$

Gazin *et al*. (2013)

The authors exposed juvenile male rats to propylparaben to assess its effect on the male reproductive system. Wistar rats were exposed per gavage from PND 21 to 0, 3, 10, 100 or 1000 mg/kg/d propylparaben for 8 weeks (n= 10/group + 10/group subjected to recovery period of 26 weeks).

Propylparaben exposure had no statistically significant effect on the sexual maturation, sperm (count and motility), weight of reproductive organs (epididymis, prostate, seminal vesicle, testis), not on hormones level (LH, FSH and testosterone). The slight earlier sexual maturation remains in the historical data.

Table 106: Sperm parameters and hormones level

Dose (mg/kg bw/day)	Sexual maturation (d)	Sperm count (n)	Motile sperm (%)	Testoste-rone (nmol/L)	LH (ng/mL)	FSH (ng/mL)
0	44 ± 1	428 ± 202	81.1 ± 12.5	16.9 ± 13.9	0.67 ± 0.23	13.6 ± 3.20
3	44 ± 2	501 ± 504	88.2 ± 5.4	17.6 ± 10.5	0.66 ± 0.28	12.7 ± 2.66
10	44 ± 2	449 ± 271	71.4 ± 28.8	21.2 ± 11.9	0.71 ± 0.21	12.4 ± 3.37
100	43 ± 2	473 ± 212	85.5 ± 9.5	22.9 ± 14.0	0.51 ± 0.23	13.4 ± 2.03
1000	43 ± 2	547 ± 198	85.8 ± 9.6	18.9 ± 11.1	0.62 ± 0.33	12.5 ± 3.60

Few animals showed atrophy/hypoplasia of the testes, but the lack of consistency between left/right organs, dose-dependency, or correlation with organ weight, indicates that these findings were incidental and not substance-related.

Female rodents:

Shaw and deCatanzaro (2009)

This experiment was conducted to determine if high doses of propylparaben would impact embryo implantation and therefore affect the pregnancy. CF-1 pregnant female mice were injected subcutaneously to 0, 35 or 40 mg of propylparaben dissolved in 0.05 mL DMSO (corresponding to 0, 948.5 and 1084 mg/kg bw/d) on each day from GD1 to GD 4 (n= 5 to 7 per group). Mice were sacrificed on GD 6 to determine the number of visible implantation sites.

Propylparaben did not affect the number of implantation sites at either dose.

Vo *et al*. (2010)

The authors performed a female pubertal assay with different parabens to assess their impact on puberty onset and reproductive organs. Prepubertal Sprague-Dawley female rats were exposed to 0, 62.5, 250 or 1000 mg/kg bw/d propylparaben from PND 21 to PND 40 by daily oral gavage (n= 10/group).

Propylparaben delayed only slightly the vaginal opening and there was no significant change in the estrous cycle. Regarding the organs weight, only the adrenal weight was significantly increased at the highest dose, whereas the ovary weight was slightly reduced and the uterus weight slightly increased. Histopathological analysis confirmed that the uterus was affected, showing an increased thickness, characteristic of myometrial hypertrophy.

Table 107: Vaginal opening and organ weight

Dose (mg/kg bw/day)	Vaginal opening (d)	Adrenal weight (mg/g bw)	Ovary weight (mg/g bw)	Uterus weight (mg/g bw)	Uterus thickness (µm)
0	33.6 ± 3.23	0.24 ± 0.01	0.51 ± 0.06	1.29 ± 0.11	126.64 ± 4.44
62.5	33.0 ± 2.49	0.26 ± 0.01	0.48 ± 0.08	1.41 ± 0.07	135.90 ± 8.88
250	34.0 ± 1.33	0.25 ± 0.03	0.49 ± 0.07	1.42 ± 0.12	153.85 ± 15.38
1000	34.6 ± 1.78	0.32 ± 0.03**	0.42 ± 0.09	1.46 ± 0.10	197.44 ± 8.88**

** $p < 0.05$

Serum hormone levels were also quantified but showed strong variability and the changes observed were not dose-dependent.

Table 108: Hormones level

Dose (mg/kg bw/day)	Estradiol (pg/mL)	Prolactin (ng/mL)	T4 (ng/mL)
0	47.07 ± 14.72	9.81 ± 3.12	3.00 ± 0.32
62.5	20.30 ± 6.47	61.73 ± 67.59	2.34 ± 0.24
250	24.69 ± 5.74	41.26 ± 13.29	1.74 ± 0.20****
1000	40.08 ± 9.00	19.97 ± 3.78	2.54 ± 0.45

**** $p < 0.01$

Ahn *et al*. (2012)

The aim of this study was to assess the impact of propylparaben on early folliculogenesis. Sprague-Dawley neonatal female rats were injected subcutaneously from PND 1 to PND 7 with doses of 0, 62.5, 250, or 1000 mg/kg bw/d of propylparaben (n= 5/group). On PND 8, the rats were sacrificed and their ovaries analysed to determine the number of primordial follicles, early primary follicles and primary follicles.

Propylparaben exposure did not induce significant change of the body weight, nor reproductive organs weight. However, a slight increase of the uterus weight and a slight reduction of the ovary weight was observed.

Table 109: Uterus and ovary weight

Dose (mg/kg /day)	Body weight (g)	Uterus weight (mg/g bw)	Ovary weight (mg/g bw)
0	14.9 ± 3.36	0.23 ± 0.108	0.32 ± 0.071
62.5	20.78 ± 1.58	0.28 ± 0.086	0.24 ± 0.019
250	19.81 ± 1.02	0.36 ± 0.097	0.27 ± 0.045
1000	19.21 ± 2.43	0.38 ± 0.066	0.27 ± 0.008

Histological analysis of the ovaries showed a significant increase of primordial follicles at high dose, and a significant decrease of early primary follicles after exposure to 250 and 1000 mg/kg bw/d. The number of primary follicles was not affected.

Exposure to propylparaben increased the expression of AMH and Foxl2 mRNA, which are associated with follicle development. On the other hand, the expression of the steroidogenic enzyme StAR was significantly reduced at all doses, whereas the expression of Cyp11a1 mRNA was not consistent, showing a significant increase after exposure to 250 mg/kg bw/d and a significant reduction after exposure to 1000 mg/kg bw/d.

Lee *et al*. (2017)

The aim of this study was to assess the effect of parabens (methyl-, butyl- and propylparaben) on ovarian folliculogenesis and steroidogenesis. Female 8-weeks-old Sprague-Dawley rats (n= 6/group) were exposed to 100 mg/kg bw/day for 5 weeks, in presence or in absence of 4-vinylcyclohexen diepoxide (VCD), a known disruptor of ovarian follicles.

Exposure to propylparaben deregulated the estrus cycle, showing rapidly a constant diestrus phase.

Histological analysis after 5 weeks of exposure showed a decreased number of total follicles, including a significantly decrease of secondary and preovulatory follicles. Number of primary follicles was also lower, but not significantly.

Blood serum analysis revealed an increased level of FSH after propylparaben exposure. Finally, the modified mRNA pattern of genes involved in steroidogenesis in the exposed ovaries indicated a downregulated steroidogenesis, as well as a decreased expression of hormone receptors for FSH and LH.

Effects of propylparaben exposure alone were almost similar to VCD (or even stronger), and co-exposure to propylparaben and VCD accelerated the VCD-induced ovarian failure. This indicates that propylparaben could provoke ovarian failure.

Jiao *et al.* (2021)

The aim of this study was to determine the effects of propylparaben on the reproduction. BALB/c mice (4 weeks-old) were exposed to 100 mg/kg day of propylparaben via subcutaneous injection for 21 consecutive days and compared to a control unexposed group (n= 10/group). Estrous cyclicity was assessed during the last 8 days. All mice were

sacrificed after 21 days.

The estrous cycle was affected, showing a prolonged estrous period and a shortened proestrus. Serum analysis revealed a reduced level of testosterone and an increased level of estradiol ($p<0.05$), whereas no effect has been measured on progesterone. The expression of the steroidogenic enzymes StAR and CYP19A1 were increased ($p<0.05$). analysing the ovaries, the authors did not observe any significant change in the number of preantral and antral follicles. However, the number of corpus luteum were significantly decreased.

Additionally, the authors assessed the effects of propylparaben on enhanced ovulation. Mice (n= 5/group) were injected subcutaneously with 0, 100 or 1000 mg/kg bw/d propylparaben, and intraperitoneally with 15 IU of pregnant mare serum gonadotropin, followed by 10 IU of hCG to provoke a superovulation. However, the mice exposed to propylparaben showed a dose-dependent decrease of oocytes retrieved, linked to reduced number of corpus luteum.

Mogus *et al.* (2021)

The aim of this study was to assess the effect of propylparaben on mammary gland. Female BALB/c mice (6 to 8 weeks-old) were exposed to 0, 20, 100 or 10000 µg/kg bw/day (n= 15 - 18/group) orally (via pipette feeding) from GD 0 to PND 21. After separation of the pups, dams were then untreated for 5 weeks to ensure the completion of mammary gland involution (17 - 20 weeks old) and euthanized. Nulliparous unexposed mice were also analyzed as additional control group.

The pregnancy outcome was only modestly affected by propylparaben, showing a slight prolongation of the pregnancy length (0.5 day a all doses). Moreover, whereas exposure to 20 and 100 µg/kg bw/d resulted in fewer pups per litter, more pups were obtained at the highest dose, but these values are not statistically different from the control.

Propylparaben exposure altered the morphology of the post-involution mammary gland. Indeed, analysis of the mammary gland showed that the ductal epithelium volume increase expected after a pregnancy is reduced when the mice are exposed to 20 and 100 µg/kg bw/d propylparaben (statistically significantly higher than the nulliparous control and lower than the parous control, no effect observed in the highest dose group). This was confirmed by looking at the mammary epithelium proliferation which was also statistically significant reduced at low and medium doses.

Wang *et al.* (2021)

The authors assessed the effect of propylparaben on embryo implantation in early pregnant mice. Pregnant CD1 mice (n= 59 in total) were dosed daily by oral gavage with 0, 625, 1250 and 2500 mg/kg bw/d propylparaben from GD 1 until sacrifice. Mice were then sacrificed at GD 5, GD 7, or GD 8. There was no difference in early pregnancy (GD 5). But on GD 7, whereas the number of implantation sites was not significantly different, the rate of pregnant mice with impaired embryo implantation was significantly increased after exposure to 2500 mg/kg bw/d (36 % of mice with less than 7 implantation sites vs 0 % in control). Accordingly, the weight of uterus was also reduced in mice treated with 2500 mg/kg bw/d propylparaben. Additionally, the levels of estradiol and progesterone were significantly higher after exposure to high dose of propylparaben.

Accordingly, the authors assessed the effects on endometrial decidualization, i.e. when uterus stromal cells transform in decidual cells, a phenomenon occurring between GD 6 and GD 8. CD1 mice (n= 14) were mated with vasectomized males to establish pseudopregnancy. Mice were then exposed to 0 or 2500 mg/kg bw/d from pseudo-GD 1 to pseudo-GD 8. On pseudo-GD 4, artificial decidualization was experimentally induced by injecting 25 µL corn oil into one horn of the uterus, whereas the other horn was not injected

to serve as control. Weight of the stimulized horn was slightly lower after propylparaben exposure, and its morphology affected. Expression of different markers of decidualization were also significantly reduced, confirming that the decidualization was compromised.

Finally, authors assessed the impact of propylparaben on fertility, by mating CD1 mice (n= 27) with males and then exposing them to 0 or 2500 mg/kg bw/d propylparaben from GD 1 to GD 9. The dams were then unexposed until parturition. Whereas the global number of pups and their weight was not affected after propylparaben exposure, 38 % of pregnant mice had less than 7 pups (0 % in control). This confirms that propylparaben exposure during early pregnancy affects the fertility.

Li *et al.* (2021)

The authors assessed the effect of prenatal propylparaben exposure on ovarian aging, in adult mice 46 weeks-old. Pregnant ICR mice were exposed to 0, 7.5, 90 and 450 mg/kg bw/d propylparaben via intra-peritoneal injections from GD 7.5 to GD 13.5, i.e. during the fetal sex determination period (n= 8/group). At PND 3, 5 female pups from different dams were sacrificed in each group. To assess the ovarian reserve of neonatal offspring. The others female pups were maintained for 46 weeks. In each group, the estrous cyclicity of 8 offspring has been checked for 2 weeks and then sacrificed at diestrus to analyse their ovary and hormone levels.

Whereas 46 weeks-old mice (corresponding to 40 years-old in human), already show disturbed estrous cyclicity, the perinatal exposure exacerbated the loss of estrous cycle (not significant). Hormone analysis showed a reduced serum level of E2 (significant in the highest dose group only) and progesterone (significant at all doses). The level of protein expression of steroidogenic enzymes StAR and CYP11A1 was also significantly reduced in ovaries of mice exposed to propylparaben.

At PND 3, there was no significant difference in the size of primordial follicle pool. However, at 46 W, the number of primordial follicles was reduced at all doses ($p<0.05$). Consistently with this finding, the proportion of growing follicles was increased, ($p<0.05$) indicating an overactivation of primordial follicles. Furthermore, the proportion of atretic follicles was significantly increased in most exposed groups ($p<0.05$). mRNA analysis confirmed that the prenatal propylparaben exposure induced the signaling pathway responsible for the depletion of primordial follicles, triggered ovarian inflammation and fibrosis, and oxidative stress.

All these data indicate that prenatal propylparaben exposure accelerate ovarian aging.

Yan *et al.* (2022)

The aim of this article was to assess the effect of chronic exposure at the human relevant doses of propylparaben on ovarian aging in mice. C57BL/6J mice (n= 20/group) were exposed orally (via diet) to 0 or 7.5 mg/kg bw/d for 8 months (from 6 weeks to 38 weeks-old). Estrous cyclicity has been checked the 2 weeks before sacrifice. Ovaries were then examined to assess the impact of propylparaben of folliculogenesis. Additionally, a fertility status test was performed. 12 mice per group were separated after 6 weeks of treatment and mated continuously with unexposed male mice until sacrifice. Their number of litters and litter size were recorded.

Neither body nor ovary weight have been affected by propylparaben exposure. The number of primordial follicles was significantly lower, whereas the number of atretic follicles showed an upward trend (non-significant). This suggests an acceleration of the recruitment of primordial follicles, reducing the ovarian reserve.

The mice exposed to propylparaben had also an estrous cycle more disturbed as the control ones, as 25 % of the unexposed 8 months-old mice still showed regular cycles, whereas

all mice exposed to propylparaben were irregular. Serum analysis showed a reduced level of estradiol and progesterone, whereas FSH was not affected. The levels of StAR, CYP11A1 and CYP19A1 was also significantly reduced in comparison to control.

The fertility status test showed a reduction of the fertility index in mice exposed to propylparaben (50 % vs 72.2 % in control) and a reduced total number of pups (114 vs 149 in control).

Further analyses showed that chronic exposure to propylparaben at humanly relevant dose increases oxidative phosphorylation, mitochondrial damages, and oxidative stress in the ovaries.

Conclusion OECD CF Level 4

Female reproductive system:

The studies assessing effects of propylparaben on the female reproductive system showed in most cases that this substance affects the folliculogenesis in ovaries (disturbed in four of six studies). Effect on uterus is unclear, as there is a trend increased uterus weight in most studies, but significant only in one. The reproductive capacities of the rodents were however not clearly affected.

Male reproductive system:

Only few studies assessed the effect of propylparaben on the male reproductive system. They get contradictory results, only one study (of three) showing a reduced sperm count.

OECD CF Level 5: *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism

Extended One-Generation Reproductive Toxicity Study with DNT and DIT (REACH registration dossier: Unpublished study report, 2021)

Method and results are described in detail in section 7.9.7.1 Fertility

Conclusion OECD CF Level 5

The adverse effects on sperm, ovaries and uterus were not confirmed in the EOGRTS.

A dose-dependent decrease of anogenital distance/index has been shown in male pups of both generations, and this decrease was slight but statistically significant in F2. Also observed in F2, a slight but statistically significant delayed nipple retention has been observed. However, these effects are too weak and give only indications of adverse effects.

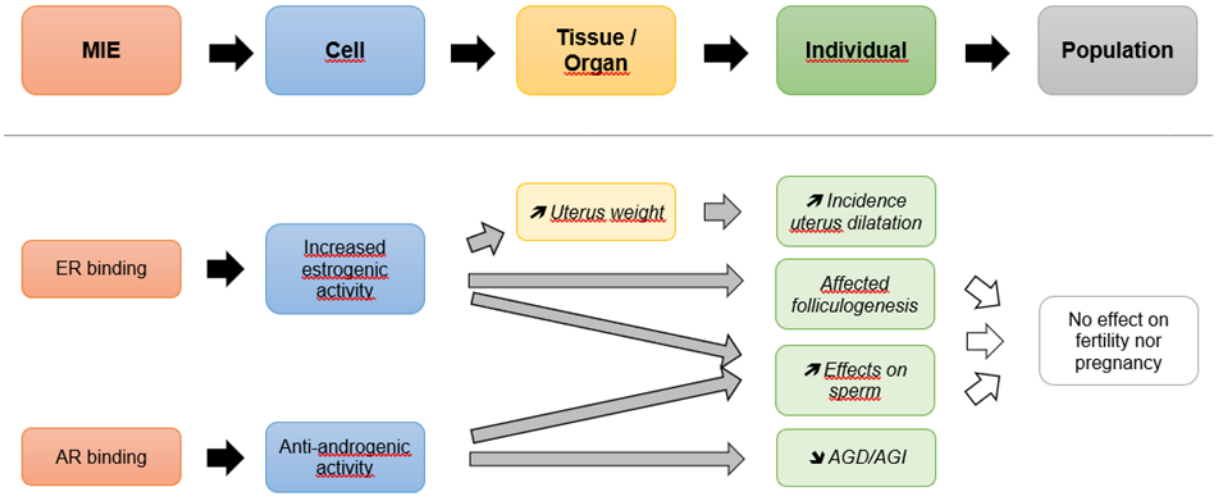
Conclusion of ED properties human health:

Based on the available data, propylparaben shows estrogenic and anti-androgenic activity. Slight effects have been observed on uterus (increased weight and incidence of uterus dilatation), affected folliculogenesis, affected sperm (motility) and reduced anogenital distance in male pups. However, these effects are weak and/or not coherent between the different studies and did not affect the fertility nor the pregnancy of rodents exposed to propylparaben. Therefore, they are not considered as sufficient to demonstrate an endocrine adverse effect relevant for human health.

There is evidence of endocrine disrupting activity but only indications are available for adverse effect.

Figure 5: scheme showing the postulated modality but in absence of adverse effect on reproduction

The MoA presented here does not describe every detail of the biology but instead focuses on describing critical steps, acknowledging that other activities could also influence each of the key events described.



7.10.4 Endocrine disruption – Human information

Data from human biomonitoring studies

The human exposure studies include two types of surveys:

1) **human biomonitoring studies**: that assess human exposure to propylparaben (not the purpose of this substance evaluation and therefore not included in the SEv report)

2) **human dose-response studies**: epidemiological studies that estimate associations between exposure to propylparaben and endocrine endpoints (included in this section)

Human dose-response studies

Different surveys reporting on human dose-response relationships, i.e. the association between exposure to propylparaben and endocrine endpoints are reported in Table 110.

The outcome of the studies is evaluated as follows:
p<0.05 = significant outcome in line with hypothesis;
p between 0.05 and 0.10=borderline non-significant outcome in line with hypothesis;
p>0.10 = no confirmation of the hypothesis or opposite direction.

Table 110: Review literature on dose-response studies in humans for propylparaben

Reference	Short method description	Description of dose-response relationships
Smith *et al*., 2013	**Type of study:** prospective cohort study **Subjects:** 192 women patients fertility clinic age range: 18 - 46 Yrs. exclusion: oophorectomy period: Aug. '05 - Nov. '10 **PPB exposure:** spot urine samples 1 - 14 samples per person 0 - 1145 days before outcome analytical method: SPE-HPLC-MSMS LOD: 0.2 µg/L	**Borderline non-significant association between PPB exposure and serum FSH level:** N= 110 Median (IQR) PPB exposure: 49.6 (13.0 - 89.3) µg/L Higher day-3 FSH level indicates diminished ovarian reserve. RESULTS: a higher day-3 FSH was observed for higher urinary PPB tertiles: mean change (IC95) for tertiles 2 and 3 compared with tertile 1 were 1.16 IU/L (-0.26, 2.57) and 1.02 IU/L (-0.40, 2.43); p_{trend} = 0.16. **Borderline non-significant association between PPB exposure and antral follicle count (AFC):** N= 142 Median (IQR) PPB exposure: 37.1 (17.4 - 83.4) µg/L Lower AFC (sum of left and right, measured by transvaginal ultrasound) indicates a diminished ovarian reserve. RESULTS: a lower AFC was observed for higher urinary PPB tertiles: mean change (IC95) for tertiles 2 and 3 compared with tertile 1 were -5.0 % (-23.7, 18.4) and -16.3 % (-30.8, 1.3); p_{trend} = 0.07. **No association between PPB exposure and ovarian volume (OV):** N= 109 Median (IQR) PPB exposure: 35.5 (11.6 - 88.7) µg/L Lower OV (sum of left and right, measured by transvaginal ultrasound) indicates a diminished ovarian reserve. RESULTS: no association between tertiles of PPB exposure and OV (p_{trend} = 0.96).

Reference	Short method description	Description of dose-response relationships
Koeppe *et al.*, 2013	**Type of study:** cross-sectional, multistage, stratified, cluster sampling survey (NHANES) **Subjects:** Adolescents (age 12 - 19 yrs.): N= 1493 Adults (age: 20+): N= 356 general population period: 2007 - 2008 **PPB exposure:** spot urine samples analytical method: SPE-HPLC-MSMS LOD: 0.2 µg/L	**Levels of PPB exposure:** Male adolescents (N= 185): median (IQR): 1.75 (0.59 - 5.65) µg/g crt; max.: 480 µg/g crt; Female adolescents (N= 171): median (IQR): 26.1 (6.29 - 86.2) µg/g crt; max.: 1002 µg/g crt; Male adults (N= 785): median (IQR): 1.65 (0.50 - 11.4) µg/g crt; max.: 1486 µg/g crt; Female adults (N= 708): median (IQR): 39.1 (7.98 - 110) µg/g crt; max.: 990 µg/g crt; **Significant association between PPB exposure and serum thyroid hormones:** Serum thyroid hormones: total T3, total T4, fT3, fT4, TSH RESULTS: - Adults (N= 1479): sign. association between urinary PPB and total T4: β= -0.05; p= 0.04 - Female adults (N= 702): sign. association between urinary PPB and fT3: β= -0.006; p= 0.02 - Female adults (N= 702): sign. association between urinary PPB and fT4: β= -0.01; p= 0.01
Sprague *et al.*, 2013	**Type of study:** prospective cohort study **Subjects:** 268 women patients at mammogram screening mean (St.Dev.) age: 60.6 (4.4) Yrs. period: June 2008 – June 2008 **PPB exposure:** serum samples analytical method: SPE-HPLC-MSMS LOD: 0.7 ng/mL	**Levels of PPB exposure:** N= 268 Detection rate: 66 %; median PPB exposure: 0.46 ng/mL; max. 630 ng/mL **No association between PPB exposure and breast density:** Mammographic breast density was measured. RESULTS: no sign. association between PPB and breast density.

Reference	Short method description	Description of dose-response relationships
Philippat *et al.*, 2012	**Type of study:** nested case-control **Subjects:** 191 newborn-mother pairs mean age of mothers: 29.3 Yrs. period: 2002 - 2006 **PPB exposure:** spot urine samples (prenatal) analytical method: SPE-HPLC-MSMS LOD: 0.2 µg/L	**Levels of PPB exposure:** N= 268 Detection rate: 96.9 %; median (P5 - P95) PPB exposure: 12.5 (0.5 - 402) µg/L **No association between prenatal PPB exposure in mother and birth outcome of baby:** Birth parameters: weight, length, head circumference RESULTS: no sign. association between prenatal PPB exposure and birth outcome
Meeker *et al.*, 2011	**Type of study:** prospective cohort study **Subjects:** 194 men patients fertility clinic age range: 18 - 55 Yrs. exclusion: vasectomy period: 2000 - 2004 **PPB exposure:** spot urine samples 1 sample per person urine on day of blood/semen sampling analytical method: SPE-HPLC-MSMS LOD: 0.2 µg/L	**Levels of PPB exposure:** N= 194 Median (IQR) PPB exposure: 3.45 (0.80 - 17.1) µg/L **No association between PPB exposure and serum hormone levels:** Serum hormones: FSH, LH, inhibin B, FSH/inhibin B ratio, T, E2, SHBG, free androgen index (FAI), free T, T/LH ratio, E2/T ratio, prolactin, free T4, free T3, TSH RESULTS: no sign. associations between PPB and serum hormone levels (p_{trend} between 0.10 and 0.94). **No association between PPB exposure and semen quality:** semen analysis: total count, concentration, motility, morphology, motion parameters RESULTS: no sign. associations between PPB and semen quality (p_{trend} between 0.27 and 1.00). **Borderline association between PPB exposure and sperm DNA damage:** sperm DNA damage (comet assay): comet extent, tail distributed moment (TDM), %DNA located in tail RESULTS: change in TDM (µm) associated with an ln-unit increase of PPB (n= 190): -1.42 (-2.88 to 0.04); p= 0.06

Reference	Short method description	Description of dose-response relationships
Buttke *et al*. 2012	**Type of study:** cross-sectional, multistage, stratified, cluster sampling survey (NHANES) **Subjects:** 287 girls age range: 12 - 16 Yrs. general population period: 2005 - 2008 **Total paraben exposure:** spot urine samples 1 sample per person urine on day of health questionnaire sum of parabens: propyl-, butyl-, ethyl- and methylparaben analytical method: SPE-HPLC-MSMS LOD: 0.2 µg/L	**Levels of paraben exposure (sum of PPB, BPB, EPB and MPB):** N= 287 Median (IQR) total paraben exposure: 668 (527 - 847) µg/g crt **No association between paraben exposure and age of menarche:** Age of menarche was assessed by self-assessment health questionnaire RESULTS: no sign. associations between total paraben exposure and age of menarche: hazard ratio= 1.06 (0.94 – 1.18); p= 0.47

Smith *et al*. (2013) studied 192 women from a fertility clinic. Propylparaben exposure was assessed by means of one or multiple (maximum 14 over a period of 3 years) urine collections. A single measurement of urinary propylparaben levels represents short-time exposure (order of magnitude of hours/days); by considering multiple urine collections over time, a better estimate of the 'usual' exposure can be obtained. In the study of Smith *et al.*, urinary propylparaben levels were associated with different outcomes of ovarian function. Borderline non-significant associations were observed between urinary propylparaben levels and antral follicle count (p= 0.07) and between urinary propylparaben levels and serum FSH levels (p= 0.16), both demonstrating that exposure to propylparaben may be associated with diminished ovarian reserve. No consistent results were obtained for methyl- or butylparaben.

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

Sprague *et al*. (2013) measured serum levels of propylparaben in 268 elderly women (mean age: 60.6 yrs.) who visited the clinic for a mammogram screening. Serum levels were low in comparison with urinary level (median: 0.46 ng/mL), but levels were detectable in 66.5% of the participants. No significant differences (p= 0.41) in breast density were observed between women with propylparaben plasma levels below the LOD (N= 121), women with levels below the median (N= 72) or women with levels above the median (N= 71). In the same group of women, a significant and positive association was observed between serum levels of bisphenol A (BPA) and breast density.

Philippat *et al*. (2012) performed a nested case-control study. 48 newborns that were diagnosed with hypospadias or cryptorchidism (cases) and 143 matched controls were selected from the French birth cohort 'EDEN'. Birth parameters (weight, length, and head circumference) were related to the prenatal urinary propylparaben concentrations of the mother. No significant associations were observed.

Meeker *et al*. (2011) studied 194 men from a fertility clinic. Urinary propylparaben levels were associated with male reproductive outcome parameters. Serum reproductive or thyroid hormone levels and the 'classical' sperm quality parameters (i.e. concentration, motility and morphology) were not significantly related to propylparaben exposure. Borderline non-significant (p=0.06) associations were observed between propylparaben exposure and sperm DNA damage, assessed by the comet assay, i.e. higher urinary propylparaben levels were associated with more DNA damage in sperm, obtained on the same day. Urinary butylparaben levels were significantly (p= 0.03) and positively associated with sperm DNA damage.

In a recent study of **Buttke *et al*. (2012)**, based on the data of the National Health and Nutrition Examination Survey (NHANES) from the general population of the U.S., urinary paraben levels were associated with age of menarche, obtained through a self-assessment questionnaire. Urinary paraben levels were reported as the sum of propyl-, butyl-, ethyl- and methylparaben, thus not allowing to appraise the separate effect of propylparaben. The sum of parabens was not associated significantly with age at menarche

In a case report on three prepubertal boys, topical application of body care products containing lavender and tea tree oils was associated with the appearance of gynecomastia, which resolved after the use of the products was discontinued (Henley *et al.*, 2007). The effect was assigned to estrogenic and anti-androgenic activities of the components in the personal care products, but components like parabens, phthalates or bisphenol were not measured.

In conclusion, in five epidemiological studies, endocrine endpoints (reproductive and/or thyroid hormone levels, semen parameters, ovarian reserve parameters, breast density and birth outcome) were compared with urinary or serum propylparaben levels.
Exposure to propylparaben was significantly ($p<0.05$) associated with a decrease of thyroid hormone levels (Koeppe *et al*., 2013). These findings could be compared to animal research *(*Vo *et al.*, 2010: effects on T4), while another epidemiological study (Meeker *et al.*, 2011) could not give indication of possible association between propylparaben exposure and serum thyroid hormone levels.

A trend was observed for a negative effect of propylparaben on the female and male reproductive system. Higher propylparaben exposure was borderline non-significantly associated with diminished ovarian reserve ($p= 0.07$) (Smith *et al*., 2013) and with sperm DNA-damage ($p= 0.06$) *(*Meeker *et al.*, 2011).

No significant relation was found between propylparaben exposure and breast density in elderly women (Sprague *et al.*, 2013) or between prenatal propylparaben exposure and birth outcome parameters in babies (Philippat *et al*., 2013).

Epidemiological, observational studies can provide supporting evidence for relationships between exposure and biological effects but do not show causal relationships. Evidence for causality can be obtained if several epidemiological trials provide similar results, and if these associations are supported by mechanistic results from *in vivo* or *in vitro* studies.

7.10.5 Conclusion on endocrine disrupting properties (combined/separate)

Several *in vitro* studies demonstrate a clear estrogenic agonistic activity of propylparaben via ER-binding, ER activation and cell proliferation. The estrogen agonistic activity was also confirmed *in vivo* in a reliable uterotrophic assay.

Although *in vitro* studies on anti-androgen activity show more diverging results, the majority of the studies show positive results. However, it was clearly demonstrated *in vivo* in rodents in a hershberger assay and in embryonic zebrafish, in which significant downregulation of the AR receptor was observed.

Furthermore in vitro data suggest also other ED modes of actions like glucocorticoid-like and PPARy activity.

Human health:

In rodents slight effects have been observed on uterus (increased weight and incidence of uterus dilatation), affected folliculogenesis, affected sperm (motility) and reduced anogenital distance in male pups. However, these effects are weak and/or not coherent between the different studies and did not affect the fertility nor the pregnancy of rodents exposed to propylparaben.

There is evidence of endocrine disrupting activity but only indications are available for adverse effect.

Environment:

Several non-guideline studies demonstrate, a clear induction of vitellogenin in fish after exposure to propylparaben. Results of the Fish Sexual Development Test (OECD TG 234) also confirm this weak estrogen activity of propylparaben whereby a non statistically significant increase in VTG in males and females was observed. It is however not surprisingly for a weak estrogen that this increase in VTG is not statistically significant. Furthermore, propylparaben downregulated the androgen receptor gene (ar), supporting an anti-androgen activity. In support, estrogenic and anti-androgen activity of

propylparaben in rodents is confirmed resp. in 3 of 4 uterotrophic assays and in a hershberger assay.

Propylparaben disrupts gene transcription of the HPG-axis and those involved in steroidogenesis in embryonic zebrafish and in adult male mosquitofish and consequently changes steroid hormone levels (decrease of testosterone) in embryonic zebrafish demonstrating estrogenic and anti-androgenic effect as well as abnormal steroidogenesis after exposure to environmental relevant concentration of propylparaben. Additionally, several literature studies show that propylparaben also affect steroidogenic gene transcription and testosterone level in rodents, although not in a consistent matter.

A sex shift towards females (significant at the highest concentration) was observed in the FSDT, in absence of general toxicity. In a supportive feeding study with juvenile zebrafish, feminisation was seen at all concentrations but was only significant at the lowest concentration (71 % females compared to 40% in control). This feminisation is further supported by the findings with the invertebrate marine copepod *Tigriopus japonicus* after exposure to a single concentration of 50 µg/L propylparaben.

Furthermore, propylparaben induced oocyte maturation in zebrafish accompanied by increasing ovarian lesions (egg debris and granulomatomous inflammation). An increase in oocyte atresia was also observed in rodents.

Moreover, a significant dose-dependent increase in mRNA expression of choriogenins CHG-L and CHG, an estrogen-responsive gene, was seen in adult male medaka (*Oryzias latipes)* in a supportive study.

Although no effect on hatching success was seen in the FSDT, literature studies demonstrate that propylparaben can decrease the hatching rate and delay the time to hatch in fish as well as in invertebrates. Furthermore, propylparaben reduced fecundity in invertebrates (fruit fly *Drosophila melanogaster*, dengue mosquito *Aedes aegypti*, nematode *Caenorhabditis elegans* and the marine copepod *Tigriopus japonicus*).

It should be noted that estrogen agonistic activity is a common endocrine modality amongst the members of the paraben family (SVHC identification as ED HH of butylparaben[1] and isobutylparaben[2]; available data demonstrating clear evidence for methylparaben). Furthermore, as indicated by (Darbre and Harvey, 2014) estrogen receptor binding activity of parabens may increase with increasing alkyl chain length and thus there might be a higher potential for estrogenic activity for long-chain length molecules (methyl<ethyl<propyl<butyl 4-hydroxybenzoate).

Furthermore, in fish, similar effects were seen for methylparaben in an FSDT (OECD TG 234), namely a significant increase in VTG and a trend towards feminisation.

Considering all relevant and reliable information in a Weight of Evidence Approach, it is concluded that there is scientific evidence that propylparaben can be identified as a endocrine disruptor for the environment according to the WHO/IPCS (2002) definition.

[1] Member State Committee support document for identification of butyl 4-hydroxybenzoate as a substance of very high concern because of its endocrine disrupting properties (article 57 (f) – human health properties): https://echa.europa.eu/registry-of-svhc-intentions/-/dislist/details/0b0236e18442b804

[2] Member State Committee support document for identification of isobutyl 4-hydroxybenzoate as a substance of very high concern because of its endocrine disrupting properties (article 57 (f) – human health properties): https://echa.europa.eu/registry-of-svhc-intentions/-/dislist/details/0b0236e1875e6bc9

7.11 PBT and vPvB assessment

The substance does not meet the screening criteria for P (readily biodegradable) and B (log Pow<4.5). The substance is therefore not to be considered as a potential PBT or vPvB.

7.12 Exposure assessment

7.12.1. Human health

As the initial concerns for human health were refuted, an exposure assessment for the consumer and the sensitive populations was not undertaken by the eMSCA in the context of this substance evaluation.

7.12.1.1. Worker

7.12.1.1.1. Exposure assessment performed by the eMSCA

The eMSCA has used the Chesar tool (CHESAR, version 3) in order to perform the exposure assessment for the Substance. This is an assessment performed by the eMSCA. Some parameters were taken from a worst-case eMSCA perspective, which is sometimes stricter than what the Registrants indicated in their assessment.

Table 111 gives an overview of the physicochemical properties and reference values that the eMSCA used as input values into the model.

Table 111: Physicochemical properties and reference values used as input values for the CHESAR assessment

Physicochemical properties	
Molecular weight	≥ 180.2 g/mol
Vapour pressure	3.4E-4 Pa at 20 °C
Water solubility	500 mg/L at 25 °C
Partition coefficient octanol-water (Log K_{ow})	2.8 at 20 °C
Biodegradability test result	Readily biodegradable
Adsorption / Desorption (Koc)	286.6 at 20 °C
Reference values - DNELs	
Reference value long-term, inhalation, systemic, workers	88.2 mg/m^3
Reference value long-term, dermal, systemic, workers	675.6 mg/kg bw/day

7.12.1.1.1.1. Exposure Scenario 1: Formulation or Re-packing – Manufacturing of cosmetic products

The manufacturing of the substance, as part of the Formulation or Re-packing technique, is described by PROCs 1, 2, 3, 5, 8a, 8b, 9, 14 and 15[9]. No dermal or respiratory protection is indicated by the Registrants for all the PROCs. This process takes place in an industrial setting, with indoor use. No use of LEV (Local Exhaust Ventilation) is indicated by the Registrants.

The eMSCA has set up a worst-case approach (duration of activity inferior or equal to 8 hours/day; use of the substance indoors (without LEV); no use of respiratory or dermal protection for all PROCs.

[9] For more elaborate descriptions on the PROCs: ECHA Guidance Chapter R.12 on Use description (https://echa.europa.eu/documents/10162/17224/information_requirements_r12_en.pdf/ea8fa5a6-6ba1-47f4-9e47-c7216e180197)

The product category formulated is PC 39: Cosmetics, personal care products.

Table 112: Exposure values for "Formulation or Re-packing – Manufacturing of cosmetics products", estimated by the eMSCA

Industrial						
Contributing scenario	Assessment parameters	Solid (dustiness)	Respiratory protection	Dermal Personal Protective Equipment	Long-term Inhalation Exposure Estimate (mg/m^3)[10]	Long-term Dermal Exposure Estimate ($mg/kg/day$)[2]
PROC 1	Duration of activity ≤ 8 hours/day, indoor use (no LEV), basic room ventilation (up to 3 Air Change per hour), operating temperature ≤ 40 °C, 100 % substance in mixture	Yes (very high dustiness)	No	No	0.01	0.034
PROC 2					1	1.37
PROC 3					1	0.69
PROC 5					25	13.71
PROC 8a					50	13.71
PROC 8b					25	13.71
PROC 9					20	6.86
PROC 14					10	3.43
PROC 15					5	0.34

7.12.1.1.1.2. Exposure Scenario 2: Formulation or Re-packing –Manufacturing of blends

The manufacturing of blends, as part of the Formulation or Re-packing technique, is described by PROCs 1, 2, 3, 5, 8a, 8b, 9, 14 and 15. No respiratory or dermal protection is indicated by the Registrants, nor for any of the contributing scenarios. This process takes place in an industrial setting, with indoor use. No use of LEV (Local Exhaust Ventilation) is indicated by the Registrants. The duration during which the activity of the different contributing scenarios takes place, is generally < or = to 8 hours.

The eMSCA has set up a worst-case approach (duration of activity inferior or equal to 8 hours/day; use of the substance indoors (without LEV); no use of respiratory or dermal protection for all PROCs.

The Product category formulated is PC 39: Cosmetics, personal care products.

Table 113: Exposure values for "Formulation or Re-packing – Formulation of End-products", estimated by the eMSCA

Industrial						
Contributing scenario	Assessment parameters	Solid (dustiness)	Substance in preparation	Dermal Personal Protective Equipment	Long-term Inhalation Exposure Estimate (mg/m^3)	Long-term Dermal Exposure Estimate ($mg/kg/day$)
PROC 1	Duration of activity ≤ 8	Yes (very high dustiness)	No	No	0.01	0.034

[10]Using TRA workers

PROC 2	hours/day, indoor use (no LEV), basic room ventilation (up to 3 Air Change per hour), operating temperature ≤ 40 °C, 100 % substance in mixture				1	1.37
PROC 3					1	0.69
PROC 5					25	13.71
PROC 8a					50	13.71
PROC 8b					25	13.71
PROC 9					20	6.86
PROC 14					10	3.43
PROC 15					5	0.34

7.12.2 Environment

A full exposure assessment for environment was not undertaken by the eMSCA in the context of this substance evaluation. Data on occurrence in the environment indicates that, due to its widespread uses in cosmetics and personal care products, and thus continuous introduction in the environment, propylparaben is found with increasing concentrations in water, soil, sludge, and indoor dust.

7.12.3 Occurrence data

Only literature since the year 2000 is considered underneath.

7.12.3.1 Occurrence in articles and products

Propylparaben is used in several articles and products like personal care products, cosmetics, pharmaceuticals, veterinary products, medical devices, (baby) food, baby teethers, finger paint, paper products and detergents and can be found depending on the product category from ng/g to mg/g (Europe: Vinnegaard *et al*., 2000; Kant Laboratorium BS, 2006; Eriksson *et al*., 2008; Márquez-Sillero *et al*., 2010; Yazar *et al*., 2010; Gosetti *et al*., 2018; Potouridis *et al*., 2019; Freire *et al*., 2019; Nobile *et al*., 2020; SCSS opinion, 2021 / Outside Europe: Wang *et al*., 2013; Guo and Kannan, 2013; Liao *et al*., 2013a; Liao *et al*., 2013b; Guo *et al*., 2014; Park *et al*., 2014; Liao and Kannan, 2014; Dodge *et al*., 2015; Ma *et al*., 2016; Messerlian *et al*., 2017; Lu *et al*., 2017; Li and Kannan, 2018; Zhu and Kannan, 2020; Levasseur *et al*., 2021).

In the latest opinion of the SCCS (2021)[11] safe use of propylparaben in cosmetic products is considered up to a maximum concentration of 0.14 %. Additionally, the safe concentration for mixtures of parabens was set where the sum of the individual concentrations should not exceed 0.8 % (as acid). In such mixtures, however, the sum of the individual concentrations of butyl- and propylparaben and their salts should not exceed 0.14 %.

7.12.3.2 Occurrence in indoor dust/air

Outside Europe:

Wang *et al*. (2012) determined the concentration of a. o. 6 parabens in 158 indoor dust samples collected in US, China, Japan, and Korea. Quantification was done by using the

[11] SCCS (Scientific Committee on Consumer safety) (2021). Opinion on propylparaben (PP), 30-31 March 2021 (SCCS/1623/20)

isotope-dilution method. The detection rate of propylparaben in indoor dust was 100 % and it was present in geometric mean concentrations between 123 to 761 ng/g. The mean Estimated Daily Intake (EDI) for infants is estimated to be 2.56 ng/kg bw/d, 3.05 ng/kg bw/d for toddlers and 0.60 ng/kg bw/d for teanagers. Depending on the country, EDI ranged between 0.29 and 1.78 ng/kg bw/d for children and between 0.06 and 0.36 ng/kg bw/d for adults.

Levasseur *et al*. (2021) detected median concentrations of 1048 ng/g in dust collected between 2014 and 2016 in the living area of homes of families participating to the TESIE study (Toddler's Exposure to SVOCs in the Indoor Environment). This study examined children of mothers of North Carolina who participated in the Newborn Epigenetics Study (NEST) for exposure to 12 phthalates. The detection frequency of propylparaben in dust was 98 %.

7.12.3.3 Human biomonitoring data

Propylparaben is frequently detected in human urine, breast milk and blood serum at ng/L to µg/L and was also found in saliva and adipose tissue (Europe: Schlumpf *et al*., 2010; Philippa *et al*., 2014; Azzouz *et al*., 2016b; Frederikson *et al*., 2013; Frederikson *et al*., 2014; Rodríguez-Gómez *et al*., 2014, Artacho-Cordón *et al*., 2018; Vela-Soria *et al*., 2018; Aylward *et al*., 2020; Dualde *et al*., 2020; Rolland *et al*., 2020; Leppert *et al*., 2020; Strømmen et al., 2021 / Outside Europe: Ye et al., 2008; Kang et al., 2013; Parke *et al*., 2014; Dodge *et al*., 2015; Pycke *et al*., 2015; Hines *et al*., 2015; Pollack *et al*., 2016; Souza *et al*., 2016; Fisher *et al*., 2017; Guo *et al*., 2017; Wu *et al*., 2017a; Geer *et al*., 2017; Messerlian *et al*., 2017; Messerlian *et al*., 2018; Honda *et al*., 2018; Berger *et al*., 2018; Ferguson *et al*., 2018; Lu *et al*., 2019; Aker *et al*., 2019; Park *et al*., 2019; Lee *et al*., 2021, Levasseur *et al*., 2021; Wu *et al*., 2022).

7.12.4 Occurrence in environment

Although propylparaben is biodegradable under aerobic conditions, it degrades slowly under anaerobic conditions. Due to its widespread uses in cosmetics and personal care products, and thus continuous introduction in the environment, propylparaben is found with increasing concentrations in different environmental compartments (water, soil, sludge, indoor dust) (Nagar *et al*., 2020 and Nguyen *et al*., 2021).

Parabens in air samples ranged from not detected to pg/m^3 (Outside Europe: Chen *et al*., 2021). Parabens are ubiquitous in surface waters and sediments (Europe: Kasprzyk-Hordern *et al*., 2008; González-Mariño *et al*., 2009; Reguiro *et al*., 2009; Jonckers *et al*., 2009 and 2010; Kinani et al., 2010; Ferreira et al., 2011; Bledzka *et al*., 2014; Carmona *et al*., 2014; Haman *et al*., 2015; Gorga *et al*., 2015 / Outside Europe: Liao *et al*., 2013c, Lv *et al*., 2014; Zhang *et al*., 2015; Liu *et al*., 2015; Liu *et al*., 2018; Huang *et al*., 2018; Liao *et al*., 2018; Liao et al., 2019; Lee *et al*., 2019; Radwan *et al*., 2020; Feng *et al*., 2019).

Furthermore, propylparaben was found in forestry and agricultural soils at ng/kg (Europe: Núñez *et al*., 2008; Camino-Sánchez *et al*., 2016 / Outside Europe: Chen *et al*., 2021).

They can come into the environment via sludge from WWTP used as fertiliser and wastewater irrigation (Kim *et al*., 2020). Concentrations of propylparaben in effluent and sludge of WWTP were in the range of ng/L and ng/g resp. (Europe: Ramberger *et al*., 2006; Reguiro *et al*., 2009; Nieto *et al*., 2009; Fereirra *et al*., 2011; Albero *et al*., 2012, Bledzka *et al*., 2014; Carmona *et al*., 2014; Gasperi *et al*., 2014; Molins-Delgado *et al*., 2016; Malvar *et al*., 2019; Golovko *et al*., 2021 / Outside Europe: Song *et al*., 2017; Chen *et al*., 2017, Nguyen *et al*., 2021).

Parabens have been detected in the aquatic ecosystem in the ng/L or µg/L range and propylparaben is one of the most omnipresent of the parabens in the environment (Torres

et al., 2016). Elimination of propylparaben in greywater (water from households) was found to be by sorption to biomass followed by biodegradation (Song *et al*., 2017). The exposure pathway of propylparaben can be changed by soil particle adsorption (Kim *et al*., 2020). Furthermore, propylparaben contains a phenolic hydroxyl groups and can react easily with free chlorine, generating halogenated by-products. These chlorinated by-products are more stable and persistent than the parent species (Haman *et al*., 2015).

Furthermore, propylparaben is detected in fish in ng/g (Europe: Jakimska *et al*., 2013)

7.13 Risk characterisation

7.13.1. Human health

7.13.1.1. Worker

7.13.1.1.1. Exposure assessment performed by the eMSCA

Based on the Registrants' exposure calculations and the DNELs proposed by the Registrants, all obtained RCRs (risk characterisation ratios) are < 1 for every exposure scenario. The eMSCA has also performed its own exposure assessment. This risk characterisation is based on the eMSCA's exposure estimates. No RCRs are > 1.

7.13.1.1.1.1. Exposure Scenario 1: - Formulation or Re-packing – Recrystallisation of Substance

Table 114: Risk characterization ratios for "Formulation or Re-packing – Manufacturing of cosmetics products", estimated by the eMSCA

Industrial			
Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 1	< 0.01	< 0.01	< 0.01
PROC 2	0.011	< 0.01	0.013
PROC 3	0.011	< 0.01	0.012
PROC 5	0.283	0.02	0.304
PROC 8a	0.567	0.02	0.587
PROC 8b	0.283	0.02	0.304
PROC 9	0.277	0.01	0.237
PROC 14	0.113	< 0.01	0.118
PROC 15	0.057	< 0.01	0.057

All RCRs for long-term dermal, long-term inhalation and combined are below 1.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 1.

7.13.1.1.1.2. Exposure Scenario 2: Formulation or Re-packing – Manufacturing of blends

Table 115: Risk characterization ratios for "Formulation or Re-packing – Manufacturing of blends", estimated by the eMSCA

Industrial			
Contributing scenario	RCR Long-term Inhalation	RCR Long-term Dermal	RCR Combined
PROC 1	< 0.01	< 0.01	< 0.01

PROC 2	0.011	< 0.01	0.013
PROC 3	0.011	< 0.01	0.012
PROC 5	0.283	0.02	0.304
PROC 8a	0.567	0.02	0.587
PROC 8b	0.283	0.02	0.304
PROC 9	0.227	0.01	0.237
PROC 14	0.113	< 0.01	0.118
PROC 15	0.057	< 0.01	0.057

All RCRs for long-term dermal, long-term inhalation and combined are below 1.

Conclusion: The risk management measures presented by the Registrants are sufficient for Exposure Scenario 2.

7.13.2 Environment

Not assessed.

17.14 References

Ahn H.J., An B.S., Jung E.U., Yang H., Choi K.C. and Jeung E.B. (2012). *Parabens Inhibit the Early Phase of Folliculogenesis and Steroidogenesis in the Ovaries of Neonatal Rats*. Mol. Reprod. Dev., 79, 626 – 636.

Aker A.M., Ferguson K.K., Rosario Z.Y., Mukherjee B., Alshawabkeh A.N., Calafat A.M., Cordero J.F. and Meeker J.D. (2019). *A repeated measures study of phenol, paraben and Triclocarban urinary biomarkers and circulating maternal hormones during gestation in the Puerto Rico PROTECT cohort*. Environmental Health, 18, 28.

Albero B., Pérez R.A, Sánchez-Brunete C. and Tadeo J.L. (2012). *Occurrence and analysis of parabens in municipal sewage sludge from wastewater treatment plants in Madrid (Spain).* Journal of Hazardous Materials, 239 – 240, 48 – 55.

Alslev B., Korsgaard B., and Bjerregaard P. (2005). *Estrogenicity of butylparaben in rainbow trout Oncorhynchus mykiss exposed via food and water.* Environmental Toxicology, 72, 295 - 304.

An Y.J., Kim S.W. and Lee W.M. (2013). *The collembola Lobella sokamensis juvenile as a new soil quality indicator of heavy metal pollution*. Ecol. Indicat., 27– 56 - 60.

Angelov T., Vlasenko A., and Taskov W. (2008). *HPLC Determination of Parabens and Investigation on their Lipophilicity Parameters*. Journal of Liquid Chromatography & related Technologies, 31, 188 - 197.

Ankley G.T., Mihaich E., Stahl M.R., Tillitt D., Colborn T., McMaster S., Miller R., Bantle J., Campbell P., Denslow N.D., Dickerson R., Folmar L.C., Fry R., Giesy J.P., Gray L.E., Guiney P., Hutchinson T., Kennedy S., Kramer V., Leblanc G.A., Mayes M., Nimrod A.C., Patino R., Peterson R., Purdy R., Ringer R., Thomas P., Touart L., Van Der Kraak G.J. and Zacharewski T. (1998). *Overview of a workshop on screening methods for detecting potential (anti-) oestrogenic/androgenic chemicals in wildlife*. Env. Toxicol. Chem., 17, 68 – 87.

Artacho-Cordón F., Ríos-Arrabal S., León J., Frederiksen H., Sáenz J.M., Martín-Olmedo P., Fernández M.F., Olea N. and Arrebola J.P. (2018). *Adipose tissue concentrations of non-persistent environmental phenols and local redox balance in adults from Southern Spain*. Environment International, 133, 105118.

Atli E. (2022). *The effects of ethylparaben and propylparaben on the development and fecundity of Drosophila melanogaster*. Environmental Toxicology and Pharmacology, 9, 103856.

Aubert N., Ameller T., and Legrand J.J. (2012). *Systemic exposure to parabens: Pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical, or subcutaneous administration*. Food and Chemical Toxicology, 50 (3 – 4), 445 – 454.

Aylward L., Vilone G., Cowan-Ellsberry C., Arnot J.A., Westgate J.N., O'Mahony C. and Hays S.M. (2020). *Exposure to selected preservatives in personal care products: case study comparison of exposure models and observational biomonitoring data*. Journal of Exposure Science and Environmental Epidemiology, 30, 28 – 41.

Azzouz A., Rascón A.J. and Ballesteros E. (2016b). *Simultaneous determination of parabens, alkylphenols,phenylphenols, bisphenol A and triclosan in human urine, blood andbreast milk by continuous solid-phase extraction and gaschromatography–mass spectrometry*. Journal of Pharmaceutical and Biomedical Analysis, 119, 16 – 26.

Barr L., Metaxas G., Harbach C.A., Savoy L.A. and Darbre P.D. (2012). *Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum*. Journal of Applied Toxicology, 32, 219 – 232.

Basketter D.A., Schole E.W., Kimber I., Botham P.A., Hilton J., Miller K., Robbins M.C., Harrison P.T.C. and Waite S.J. (1991). *Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig test data*. Toxicology Methods, 1(1)– 30 - 43.

Basketter D.A. Scholes E.W. and Kimber I. (1994). *The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximisation test*. Fd. Chem. Toxic., 32(6), 543 - 547.

Basketter D.A. and Scholes E.W. (1992). *Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens*. Fd. Chem. Toxic., 30(1), 65 – 69.

Bereketoglu C. and Pradhan A. (2019). *Comparative transcriptional analysis of methylparaben and propylparaben in zebrafish*. Sci Total Environ., 671, 129 - 139.

Berger R.G., Shaw J. and deCatanzaro D. (2008). *Impact of acute bisphenol A exposure upon intrauterine implantation of fertilized ova and urinary 17β-estradiol and progesterone levels.* Reproductive toxicology, 26, 94 – 99.

Berger E., Potouridis T., Haeger A., Püttmann W. and Wagner M. (2015). *Effect-directed identification of endocrine disruptors in plastic baby teethers*. Journal of Applied Toxicology, 35(11), –254 - 1261.

Berger K., Gunier R.B., Chevrier J., Calafat A.M., Ye X., Eskenazi B., and Harley K.G. (2018). *Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels*. Environ Res., 165, 379 – 386.

Bergfield W.F. et al. (2006). Amended Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben and Benzylparaben. Final report of the Cosmetic Ingredient Review Expert Panel.

Bjerregaard P., Andersen D.N., Pedersen K.L., Pedersen S.N. and Korsgaard B. (2003). *Estrogenic effect of propylparaben (propylhydroxybenzoate) in rainbow trout Oncorhynchus mykiss after exposure via food and water*. Comparative Biochemistry and Physiology, Part C 136, 309 - 317.

Blair R.M., Fang H., Branham W.S., Hass B.S., Dial S.L., Moland C.L., Tong W., Shi L., Perkins R., and Sheehan D.M. (2000). *The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands*. Toxicological Sciences, 54, 138 - 153.

Błedzka D., Gromadzińska and Wąsowicz W. (2014). Parabens. *From environmental studies to human health*. Environment International, 67, 27 – 42.

Buttke D E, Kanta S and Martin C (2012). *Exposures to endocrine disrupting chemicals and age of menarche in adolescent girls in NHANES (2003-2008)*. Environmental health Perspectives, 120(11), 613 - 1618.

Byford J R, Shawa L E, Drewb M G B, Pope G S, Sauer M J, Darbre P D (2002). *Oestrogenic activity of parabens in MCF7 human breast cancer cells*. Journal of Steroid Biochemistry and Molecular Biology, 80, 49 – 60.

Calafat A M, Weuve J, Ye X, Jia L T, HU H, Ringer S (2009). *Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants*. Environ. Health perspect., 117, 639 - 644.

Calma M.L. and Medina P.M.B. (2020). *Acute and chronic exposure of the holometabolous life cycle of Aedes aegypti L. to emerging contaminants naproxen and propylparaben*. Environmental Pollution, 266, 115275.

Camino-Sánchez F J, Zafra-Gómez A, Dorival-García, Juárez-Jiménez B and Vílchez J L (2016). *Determination of selected parabens, benzophenones, triclosan and triclocarban in agricultural soils after and before treatment with compost from sewage sludge:A lixiviationstudy.* Talanta, 150, 415 - 424.

Carlsson G, Pohl J, Athanassiadis I, Norrgren L, Weiss J (2019). *Thyroid disruption properties of three indoor dust chemicals tested in Silurana tropicalis tadpoles*. J. Appl. Toxicol., 39(9), 248 - 1256.

Carmona E, Andreu V and Picó Y (2014). *Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water*. Science of the Total Environment, 484, 53 63.

Chen J, Ahn K C, Gee N A, Gee S J, Hammock B D, Lasley B L (2007). *Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products*. Toxicology and Applied Pharmacology, 221, 278 – 284.

Chen J, Pycke B F G, Brownawell B J, Kinney C A, Furlong E T, Kolpin D W and Halden R U (2017). *Occurrence, temporal variation, and estrogenic burden of five parabens in sewage sludge collected across the United States*. Science of the Total Environment, 593 – 594, 368 – 374.

Chen M-H, Yu B, Zhang Z-F and Ma W-L (2021). *Occurrence of parabens in outdoor environments: Implications for human exposure assessment*. Environmental Pollution, 282, 117058.

Dang Z and Kienzler A (2019). *Changes in fish sex ratio as a basis for regulating endocrine disruptors*. Environment International, 130, 104928.

Darbre P D, Aljarrah A, Miller W R, Coldham N G, Sauer M J, Pope G S (2004). *Concentrations of parabens in human breast tumours*. Journal of Applied Toxicology, 24, 5 – 13.

Darbre P D and Harvey P W (2008). *Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks*. Appl. Toxicol., 28(5), 561 - 578.

Derache R and Gourdon J (1963). *Metabolisme d'un conservateur alimentaire: l'acide parahydroxybenzoique et ses esters*. Fd. Cosmet. Toxicol., 1, 189 - 195.

Ding K, Kong X, Wang J, Lu L, Zhou W, Zhan T, Zhang C and Zhuang S (2017). *Side chains of parabens modulate antiandrogenic activity: in vitro and molecular docking studies*. Environmental Science and Technology, 51, 6452 - 6460.

Dobbins L L, Usenko S, Brain R A and Brooks B W (2009). *Probabilistic ecological hazard assessment of parabens using Daphnia magna and Pimephales promelas*. Environmental toxicology and chemistry, 28(12), 2744 - 2753.

Dodge L E, Kelley K E, Williams P L, Williams M A, Hernández-Díaz S, Missmer S A and Hauser R (2015). *Medications as a source of paraben exposure*. Reproductive Toxicology, 52, 93 – 100.

Dualde P, Pardo O, Corpas-Burgos F, Kuligowski J, Gromaz M, Vento M, Pastor A and Yusa V (2020). *Biomonitoring of parabens in human milk and estimated daily intake for breastfed infants*. Chemosphere, 240, 124829.

Dymicky M and Huhtanen C M (1979). *Inhibition Chlostridium botulinum by p-hydroxybenzoic acid n-alkyl esters*. Antimicrob agents Chemother, 15(6), 798 - 801.

Elmore S E, Cano-Sancho G, La Merrill M A (2020). *Disruption of normal adipocyte development and function by methyl- and propyl- paraben exposure*. Toxicol Lett., 334, 27 - 35.

Eriksson E, Andersen H R, Ledin A (2008). *Substance flow analysis of parabens in Denmark complemented with a survey of presence and frequency in various commodities*. Science Direct, 156, 240 - 259.

Fang H, Tong W, Shi L M, Blair R, Perkins R, Branham W, Hass B S, Xie Q, Dial S L, Moland C L and Sheenan D M (2001). *Structure-Activity Relationships for a Large Diverse Set of Natural, Synthetic, and Environmental Estrogens*. Chem. Res. Toxicol., 14, 280 - 294.

Feng J, Zhao J, Xi N, Guo W and Sun J (2019). *Parabens and their metabolite in surface water and sediment from the Yellow River and the Huai River in Henan Province: spatial distribution, seasonal variation, and risk assessment*. Ecotoxicol. Environ. Saf., 172, 480 – 487.

Feng Y and Grant D J W (2006). *Influence of Crystal Structure on the Compaction properties of n-Alkyl 4-Hydroxybenzoate Esters (Parabenes)*. Pharmaceutical Research, 23(7), 1608 - 1616.

Ferguson K K, Meeker J D, Cantonwine D E, Mukherjee B, Pace G G, Weller D and Mc Elrath (2018). *Environmental phenol associations with ultrasound and delivery measures of fetal growth*. Environment International, 112, 243 – 250.

Ferreira A M C, Möder M and Laespada M E F. (2011). *Stir bar sorptive extraction of parabens, triclosan and methyl triclosan from soil, sediment and sludge with in situ derivatization and determination by gas chromatography–mass spectrometry*. J Chromatogr A, 1218, 3837 – 44.

Fisher M, MacPherson S, Braun J M, Hauser R, Walker M, Feeley M, Mallick Ranjeeta, Bérubé R and Arbuckle T E (2017). *Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product use*. Environmental Science & Technology, 51, 4009 - 4017.

Frederiksen H, Nielsen J K S, Mørck T A , Hansen P W, Jensen J F, Nielsen O, Andersson A, Knudsen L E (2013). *Urinary excretion of phthalate metabolites, phenols, and parabens*

in rural and urban Danish motherechild pairs. Int. J. Hyg Environ. Health, 216, 772 - 783.

Frederiksen H, Jensen T K, Jørgensen N, Kyhl H B, Husby S, Skakkebæk N E, Main K M, Juul A, Andersson A (2014). *Human urinary excretion of nonpersistent environmental chemicals: an overview of Danish data collected between 2006 and 2012*. Reproduction, 147, 555 - 565.

Freire C, Molina-Molina J-M, Iribarne-Durán, Jiménez-Díaz I, Vela-Soria F, Mustieles V, Arrebola J P, Fernández M F, Artacho-Cordón F and Olea N (2019). *Concentrations of bisphenol A and parabens in socks for infants and young children in Spain and their hormone-like activities*. Environment International, 127, 592 – 600.

Fujino C, Watanabe Y, Sanoh S, Hattori S, Nakajima H, Uramaru N, Kojima H, Yoshinari K, Ohta S and Kitamura S (2019). *Comparative study of the effect of 17 parabens on PXR-, CAR- and PPARα-mediated transcriptional activation*. Food and Chemical Toxicology, 133, 110792.

Gal A, Gedeye K, Craig Z R and Ziv-Gal A (2019). *Propylparaben inhibits mouse cultured antral follicle growth, alters steroidogenesis, and upregulates levels of cell-cycle and apoptosis regulators*. Reproductive Toxicology, 89, 100 – 106.

García-Espiñeira M C, Tejeda- Benítez L P and Olivero-Verbel J (2018). Toxic Effects of Bisphenol A, Propyl Paraben, and Triclosan on Caenorhabditis elegans. Int. J. of Environ. Res. Public Health, 15, 684.

Gasperi J, Geara D, Lorgeoux C, Bressy A, Zedek S, Rocher V, El Samrani A, Chebbo G and Moillron R (2014). *First assessment of triclosan, triclocarban and paraben mass loads at a very large regional scale: Case of Paris conurbation (France)*. Science of the Total Environment, 493, 854 – 861.

Gazin V, Marsden E and Marguerite F (2013). *Oral propylparaben administration to juvenile male wistar rats did not induce toxicity in reproductive organs*. Toxicological sciences, 136(2), 392 - 401.

Geer L A, Pycke B F G, Waxenbaum J, Sherer D M, Abulafia O and Halden R U (2017). *Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York*. Journal of Hazardous Materials, 323, 177 – 183.

Giordano F, Bettini R, Donini C, Gazzangia A, Caira M R, Zhang G G and Grant D J (1999). *Physical Properties of Parabens and their mixtures: Solubility in water, Thermal Behaviour, and Crystal Structures*. Journal of Pharmaceutical Sciences, 88(11), 1210 - 1216.

Golden R, Gandy J and Vollmer G (2005). *A Review of the Endocrine Activity of parabens and Implications for Potential Risks to Human Health*. Crit Rev Toxicol., 35(5), 435 - 458.

Golovko O, Örn S, Sörengard M, Frieberg K, Nasszzi W, Lai F Y and Ahrens L (2021). *Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems*. Science of the Total Environment, 754, 142122.

González-Doncel M, García-Mauriño J E, Segundo L S, Beltrán E M, Sastre S and Torija C F (2014). *Embryonic exposure of medaka (Oryzias latipes) to propylparaben: Effects on early development and post hatching growth*. Environmental Pollution, 134, 360 - 369.

González-Mariño I, Quintana J B, Rodríguez I and Cela R (2009). *Simultaneous determination of parabens, triclosan and triclocarban in water by liquid*

chromatography/electrospray ionisation tandem mass spectrometry. Rapid Commun Mass Spectrom, 23, 1756 – 1766.

Gorga M, Insa S, Petrovic M and Barceló D (2015). *Occurrence and spatial distribution of EDCs and related compounds in waters and sediments of Iberian rivers*. Sci. Total Environ., 503, 69 – 86.

Gosetti F, Bolfi B, Robotti E., Manfredi M, Binotti M, Ferero F, Bona G and Marengo E (2018). *Study of endocrine disrupting compound release from different medical devices through an on-line SPE UHPLC-MS/MS method*. Analytca Chimica Acta, 1042, 141 - 154.

Guo J, Wu C, Lu D, Jiang S, Liang W, Chang X, Hu H, Wang G and Zhou Z (2017). *Urinary paraben concentrations and their associations with anthropometric measures of children aged 3 years*. Environmental Pollution, 222, 307 - 314.

Guo Y and Kannan K (2013). *A Survey of Phthalates and Parabens in Personal Care Products from the United States and Its Implications for Human Exposure*. Environ. Sci. Technol., 47, 14442 − 14449.

Guo Y, Wang L and Kannan K (2014). *Phthalates and Parabens in Personal Care Products From China: Concentrations and Human Exposure*. Arch Environ Contam Toxicol., 66, 113 – 119.

Hansch C, Leo A and Hoekman D (1995). *Exploring QSAR - Hydrophobic, electronic, and steric constants*. American Chemical Society, Vol 2.

Hayward D S, Kenley R A and Jenke D R (1990). *Interactions between polymer containers and parenteral solutions: the correlation of equilibrium constants for polymer-water partitioning with octanol-water partition coefficients*. International Journal of Pharmaceutics, 59, 245 - 253.

Haman C, Dauchy X, Rosin C and Munoz J-F (2015). *Occurrence, fate, and behavior of parabens in aquatic environments: A review*. Water research, 68, 1 - 11.

Henley D V, Lipson N, Korach K S and Clifford A B (2007). *Prepubertal gynecomastia linked to lavender and tea tree oils*. The New England Journal of Medicine, 356(5), 479 - 486.

Hines E P, Mendolab P, von Ehrenstein O S, Ye X, Calafat A M and Fenton S E (2015). *Concentrations of environmental phenols and parabens in milk, urine, and serum of lactating North Carolina women*. Reproductive Toxicology, 54, 120 – 128.

Hirose M, Inoue T, Asamoto M, Tagawa Y, and Ito N (1986). *Comparison of the effect of 13 phenolic compounds in induction of proliferative lesions of the forestomach and increase in the labelling indices of the glandular stomach and urinary bladder epithelium of Syrian golden hamsters*. Carcinongenesis, 7(8), 1285 - 1289.

Holbech H, Schröder K D, Nielsen M L, Brande-Lavridsen N, Holbech B F and Bjerregaard P (2013). *Estrogenic effect of the phytoestrogen biochanin A in zebrafish, Danio rerio, and brown trout, Salmo trutta*. Aquatic Toxicology 144-145, 19-25.

Honda M, Robinson M and Kannan K (2018). Parabens in human urine from several Asian countries, Greece, and the United States. Chemosphere, 201, 13 - 19.

Hossaini A, Larsen J-J and Larsen J C (2000). *Lack of Oestrogenic Effects of Food Preservatives (Parabens) in Uterotrophic Assays*. Food and Chemical Toxicology, 38, 319 - 323.

Hu P, Chen X, Whitener R J, Boder E T, Jones J O, Porollo A, Chen J and Zhao L (2013). *Effects of Parabens on Adipocyte Differentiation*. Toxicological sciences, 131(1), 56 – 70.

Huang C, Wu L-H, Liu G-Q, Shi L and Guo Y (2018). *Occurrence and Ecological Risk Assessment of Eight Endocrine-Disrupting Chemicals in Urban River Water and Sediments of South China*. Archives of Environmental Contamination and Toxicology, 75, 224 – 235.

Hutchinson T H, Shillabeer N, Winter M J and Pickford D B (2006). *Acute and chronic effects of carrier solvents in aquatic organisms, A critical review*. Aquatic Toxicology, 76, 69 – 92.

Inui M, Adachi T, Takenaka S, Inui H, Nakazawa M, Ueda M, Watanabe H, Mori C, Iguchi T., and Miyatake K (2003). *Effect of UV screens and preservatives on vitellogenin and choriogenin in male medaka (Oryzias latipes)*. Toxicology, 194, 43 - 50.

Ishidate M Hayashi M, Sawada M, Matsuoka A, Yoshikawa K, Ono M and Nakadate M (1978). *Cytotoxicity test on medical drugs-chromosome aberration tests with Chinese hamster cells in vitro*. Eisi Shikensho Hokoku, 96, 55 - 61.

Jakimska A, Huerta B, Bargańka Z, Ko-Wasik A, Rodríguez-Mozaz S and Barceló D (2013). *Development of a liquid chromatography-tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers*. Journal of Chromatography A, 1306, 44 - 58.

Janjua N R, Mortensen G K, Andersson A M, Kongshoj B, Skakkebæk N E, Wulf H C (2007). *Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormones in humans*. Environ. Sci. Technol., 41, 5564 – 5570.

Janjua N R, Frederiksen H, Skakkebæk N E, Andersson A-M and Wulf H C (2008). *Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans*. International Journal of Andrology, 31(2), 118 - 129.

Jiao L, Li S, Zhai J, Wang D, Li H, Chu W, Geng X and Du Y (2021). *Propylparaben concentrations in the urine of women and adverse effects on ovarian function in mice in vivo and ovarian cells in vitro*. Applied Toxicology, 41(11), 1719 - 1731.

Jones P S, Thigpen D, Morrison J L and Richardson A P (1956). *p-hydroxybenzoic acid esters as preservatives*. J. Am. Pharm. Assoc., 45(4), 268 - 273.

Jonkers N, Kohler H-P E, Dammshäuser A and Giger W (2009). *Mass flows of endocrine disruptors in the Glatt River during varying weather conditions*. Environ Pollut, 157, 714 – 23.

Jonkers N, Sousa A, Galante-Oliveira S, Barroso C M, Kohler H-P E and GigerW (2010). *Occurrence and sources of selected phenolic endocrine disruptors in Ria de Aveiro, Portugal*. Environ Sci Pollut Res Int, 17, 834 – 43.

Judson R S, Magpantay F M, Chickarmane V, Haskell C, Tania N, Taylor J, Xia M H, Huang R L, Rotroff D M, Filer D L, Houck K A, Martin M T, Sipes N, Richard A M, Mansouri K, Setzer R W, Knudsen T B, Crofton K M and Thomas R S (2015). *Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High-Throughput Screening Assays for the Estrogen Receptor*. Toxicological Sciences, 148, 137–154. https://doi.org/10.1093/toxsci/kfv168

Kang H M, Kim M S, Hwang U K, Jeong C B, Lee J S (2019). *Effects of methylparaben, ethylparaben, and propylparaben on life parameters and sex ratio in the marine copepod Tigriopus japonicus*. Chemosphere, 226, 388 - 394.

Kang S, Kim S, Park J, Kim H-J, Lee J, Choi G, Choi S, Kim S, Kim S Y, Moon H-B, Kim S, Kho YL and Choi K (2013). *Urinary paraben concentrations among pregnant women and their matching newborn infants of Korea, and the association with oxidative stress biomarkers*. Science of the Total Environment, 461– 462, 214 – 221.

Kant laboratorium BS (2006). *Finger paints/preservatives, primary aromatic amines, bittering agensts, colourants and declaration. Customs capmpagn by the Federal Office of Public Health (BAG) (6 sets and 30 indivdual samples)- measurement by Basel-City (specials laboratory) and Vaud (colourants). Further samples from the Cantons of Aargau (3/16) and Basel-City (5/26)*. https://www.yumpu.com/en/document/view/21547004/bericht-als-pdf-herunterladen

Kasprzyk-Hordern B, Dinsdale R M, Guwy A J (2008). *The occurrence of pharmaceuticals, personal care products, endocrine disruptors, and illicit drugs in surface water in SouthWales, UK*. Water Res., 42, 3498 – 3518.

Khanna S and Darbre P D (2013). *Parabens enable suspension growth of MCF-10A immortalized, non-transformed human breast epithelial cells*. J. Appl. Toxicol., 33(5), 378 - 82.

Kim D, Kim L, Kim D, Kim S W, Kwak J I, Cui R and An Y-J (2020). *Multispecies bioassay of propylparaben to derive protective concentrations for soil ecosystems using a species sensitivity distribution approach*. Environmental Pollution, 265, 114891.

Kim S W, Moon J, Jeong S W, An Y-J (2018). *Development of a nematode offspring counting assay for rapid and simple soil toxicity assessment*. Environ. Pollut., 236, 91 - 99.

Kim T S, Yoon C Y, Jung K K, Kim S S, Kang I H, Baek J H, Jo M S, Kim H S and Kang T S (2010). In vitro study of Organization for Economic Co-operation and Development (OECD) endocrine *disruptor screening and testing methods- establishment of a recombinant rat androgen receptor (rrAR) binding assay*. J. Toxicol. Sci., 35(2), 239 - 243.

Kim T S, Kim C Y, Lee H K, Kang I H, Kim M G, Jung K K, Kwon Y K, Nam H-S, Hong S K, Kim H S, Yoon H J and Rhee G S (2011). *Estrogenic Activity of Persistent Organic Pollutants and Parabens Based on the Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay (OECD TG 455)*. Toxicol. Res., 27(3), 181 - 184.

Kimmel C B, Ballard W W., Kimmel S R, Ullmann B, and Schilling T F (1995). *Stages of Embryonic Development of the Zebrafish*. Developmental Dynamics, 203, 255 - 310.

Kinani S, Bouchonnet S, Creusot N, Bourcier S, Balaguer P, Porcher J M, Aït-Aïssa S (2010). *Bioanalytical characterization of multiple endocrine- and dioxin-like activities in sediments from reference and impacted small rivers*. Eviron. Pollut, 158(1), 74 - 83.

Kjærstad M B, Taxvig C, Andersen H R and Nellemann C (2010). *Mixture effects of endocrine disrupting compounds in vitro*. International journal of andrology, 425 - 433.

Kleinstreuer N C, Ceger P, Watt E D, Martin M, Houck K, Browne P, Thomas R S, Casey W M, Dix D J, Allen D, Sakamuru S, Xia M, Huang R and Judson R (2017). *Development and Validation of a Computational Model for Androgen Receptor Activity*. Chemical Research in Toxicology, 30, 946–964. https://doi.org/10.1021/acs.chemrestox.6b00347

Klopčič I, Kolšek K, Dolenc M S (2015). *Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line*. Toxicology Letters, 232(2), 376 - 383.

Koeppe E S, Ferguson K K, Colacino J A and Meeker J D (2013). *Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007-2008*. Science of the Total Environment, 445 - 446, 299 - 305.

Kolšek K, Gobec M, Raščan I M, Dolenc M S MDA (2015). *Screening of bisphenol A, triclosan and paraben analogues as modulators of the glucocorticoid and androgen receptor activities*. Toxicology in vitro, 29, 815.

Korsgaard B and Petersen I (1976). *Natural occurance and experimental induction by estradiol-17-b of a lipophosphoprotein (vitellogenin) in flounder (Platichyrus flesus)*. Comparative Biochemistry and Physiology, 54, 443 - 446.

Kristensen D M, Skalkam M L, Audouze K, Lesné L, Desdoits-Lethimonier C, Frederiksen H, Brunak S, Skakkebæk N E, Jégou B, Hansen J B, Junker S and Leffers H (2011). *Many Putative Endocrine Disruptors Inhibit Prostaglandin Synthesis*. Environmental Health Perspectives, 119, 534 – 541.

Kurata Y, Fukushima S, Hasegawa R, Hirose M, Shibata M, Shirai T, and Ito N (1990). *Structure-activity relations in promotion of rat urinary bladder carcinogenesis by phenolic antioxidants*. Jpn. J. Cancer res., 81, 754 - 759.

Kurokawa H, Saito D, Nakamura S, Katoh-Fukui Y, Ohta K, Baba T, Morohashi K, Tanaka M (2007). *Germ cells are essential for sexual dimorphism in the medaka gonad*. Proc. Natl. Acad. Sci., 104, 16958 - 16963.

Kwak J I, Kim S W, An Y J (2014). *A new and sensitive method for measuring in vivo and in vitro cytotoxicity in earthworm coelomocytes by flow cytometry*. Environ. Res., 134, 118 - 126.

Lee C.H. and Kim H.J. (1994). *A study on the absorption mechanisms of drug through membranes*. Arch. Pharm. Res., 17, 182 - 189.

Lee J.H., Lee M., Ahn C., Kang H.Y., Tran D.N. and Jeung E.B. (2017). *Parabens Accelerate Ovarian Dysfunction in a 4-Vinylcyclohexene Diepoxide-Induced Ovarian Failure Model*. Int. J. Environ. Res. Public Health, 14(2), 161.

Lee J.W., Lee H.K. and Moon H.B. (2019). *Contamination and spatial distribution of parabens, their metabolites, and antimicrobials in sediment from Korean coastal waters*. Exotoxicology and Environmental Safety, 180, 185 – 191.

Lee S., Karvonen-Gutierrez, Mukherjee B., Herman W.H., Harlow S.D. and Park S.K. (2021). *Urinary concentrations of phenols and parabens and incident diabetes in midlife women*. Env. Epidemiology, 5(5), e171. doi: 10.1097/EE9.0000000000000171

Lehman A J, Laug E P, Woodard G, Draize J H, Fitzhugh O.G and Nelson AA (1949). *Procedures for the Apraisal of the Toxicity of Chemicals in Foods*. Food, drug, cosmetic law quarterly, 4(3), 412 - 434.

Lemini C, Jaimez R, Ávila M E, Franco Y, Larrea F and Lemus A E (2003). *In vivo and in vitro estrogen bioactivities of alkyl parabens*. Toxicol. Ind. Health, 19, 69 - 79.

Lemini C, Hernández A, Jaimez R, Franco Y, Avila M E and Castell A (2004). *Morphometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben*. Toxicology and Industrial Health, 20, 123 - 132.

Leppert B, Strunz S, Seiwert B, Schlittenbauer L, Schlichting R, Pfeiffer C, Röder S, Bauer M, Borte M, Stangl G I, Schöneberg T, Schulz A, Karkossa I, Rolle-Kampczyk U E, Thürmann L, von Bergen M, Escher B I, Junge K M, Reemtsma T, Lehmann I and Polte T (2020). *Maternal paraben exposure triggers childhood overweight develoment*. Nature communications, 11 (1), 561.

Levasseur J L, Hammel S C, Hoffman K, Phillips A L, Zhang S, Ye X, Calafat A M, Webstr T F and Stapleton H M (2021). *Young children's exposure to phenols in the home: Associations between house dust, hand wipes, silicone wristbands, and urinary biomarkers*. Environment International, 147, 106317.

Li A J and Kannan K (2018). *Elevated Concentrations of Bisphenols, Benzophenones, and Antimicrobials in Pantyhose Collected from Six Countries*. Environ. Sci. Technol., 52, 10812 - 10819.

Li M, Zhou S, Wu Y, li Y, Yan W, Guo Q, Xi Y, Chen Y, Li Y, Wu M, Zhang J, Wei J and Wang S (2021). *Prenatal exposure to propylparaben at human-relevant doses accelerates ovarian aging in adult mice*. Environmental Pollution, 285, 117254

Li S N and Fan D F (1997). *Activity of esterases from different tissues of freshwater fish and responses of their isoenzymes to inhibitors*. J. Toxicol. Environ. Health, 51, 149 – 157.

Li W, Gao L, Shi Y, Wang Y, Liu J and Cai Y (2016). Spatial distribution, temporal variation and risks of parabens and their chlorinated derivatives in urban surface water in Beijing, China. Science of the Total Environment, 539, 262 – 270.

Li, Y., Hou, X., Zhang, M., Gu, W. (2015). *Effects of propylparaben on fecundity and lifespan in Drosophila melanogaster.* Toxicol. Environ. Chem., 96 (7), 1064 – 1074.

Liang J, Yang X, Liu Q S, Sun Z, Ren Z, Wang X, Zhang Q, Ren X, Liu X, Zhou Q and Jiang G (2022). *Assessment of Thyroid Endocrine Disruption Effects of Parabens Using In Vivo, In Vitro, and In Silico Approaches*, Environ. Sci. Technol., 56 (1), 460 - 469.

Liang j, Liu Q S, Ren Z, Min K, Yang X, Hao F, Zhang Q, Liu Q, Zhou Q and Jiang G (2023). *Studying paraben-induced estrogen receptor- and steroid hormone-related endocrine disruption effects via multi-level approaches*. Science of Total Environment, 869, 161793.

Liao C and Kannan K (2014). *Concentrations and composition profiles of parabens in currency bills and paper products including sanitary wipes*. Science of the Total Environment, 475, 8 - 15.

Liao C, Chen L and Kannan K (2013a). *Occurrence of parabens in foodstuffs from China and its implications for human dietary exposure*. Environment International– 57 - 58– 68 - 74.

Liao C, Liu F and Kannan (2013b). Occurrence of and Dietary Exposure to Parabens in Foodstuffs from the United States. Environ. Sci. Technol., 47, 918 – 3925.

Liao C, Lee S, Moon H-B, Yamashita N and Kannan K (2013c). *Parabens in Sediment and Sewage Sludge from the United States, Japan, and Korea: Spatial Distribution and Temporal Trends*. Environ. Sci. Technol., 47, 1–895 - 10902.

Liao C, Shi J, Wang X, Zhu Q and Kannan K (2019). *Occurrence and distribution of parabens and bisphenols in sediment from northern Chinese coastal areas*. Environmental Pollution, 253, 759 - 767.

Lite C, Guru A, Juliet M and Arockiaraj J (2022). *Embryonic exposure to butylparaben and propylparaben induced developmental toxicity and triggered anxiety-like neurobehavioral response associated with oxidative stress and apoptosis in the head of zebrafish larvae*. Environmental Toxicology 37(8), 1988-2004.

Litton bionetics inc. (1974). *Summary of mutagenicity screening studies Host-mediated Assay, cytogenetics, dominant lethal assay*. Litton bioetics Inc., Kensingtion, Maryland, USA (NTIS Report PB 245 - 459).

Liu W-R, Zhao J-L, Liu Y-S, Chen Z-F, Yang Y-Y, Zhang Q-Q and Ying G-G (2015). *Biocides in the Yangtze River of China: Spatiotemporal distribution, mass load and risk assessment*. Environmental Pollution, 200, 53 - 63

Liu W-R, Yang Y-Y, Liu Y-S, Zhao J-L, Zhang Q-Q, Yao L, Zhang M, Jiang y-X, Wei X-D and Ying G-G (2018). *Biocides in the river system of a highly urbanized region: A*

systematic investigation involving runoff input. Science of the Total Environment, 624, 1023 – 1030.

Lobemeier C, Tschoetschel C, Westie S and Heymann E (1996). *Hydrolysis of parabens by extracts from differing layers of human skin*. Biol. Chem., 377, 647 - 651.

Lu J, Mao H, Li H, Wang Q and Yang Z (2017). *Occurrence of and human exposure to parabens, benzophenones, benzotriazoles, triclosan and triclocarban in outdoor swimming pool water in Changsha, China*. Science of the Total Environment, 605 – 606, 1064 – 1069.

Lu S, Ren L, Liu Y, Ma H, Liu S, Zhu Z, Tang Z, Kang L and Liao S (2019). *Urinary parabens in children from South China: Implications for human exposure and health risks*. Environmental Pollution, 254, 113007.

Lv M, Sun Q, Hu A, Hou L, Li J, Cai X and Yu C-P (2014). *Pharmaceuticals and personal care products in a mesoscalesubtropical watershed and their application as sewage markers*. Journal of Hazardous Materials, 280, 696 – 705.

Ma D, Chen L, Zhu X, Li F, Liu C, Liu R (2014). *Assessment of combined antiandrogenic effects of binary parabens mixtures in a yeast-based reporter assay*. Environ. Sci. Pollut. Res. Int., 21(10), 6482 - 6494.

Ma W-L, Zhao X, Lin Z-Y, Mohammed MOA, Zhang Z-F, Liu L-Y, Song W-W and Li Y-F (2016). *A survey of parabens in commercial pharmaceuticals from China and its implications for human exposure*. Environmental International, 95, 30 - 35.

Ma Y, Li Y, Song X, Yang T, Wang H, Liang Y, Huang L and Zeng H (2023). *Endocrine Disruption of Propylparaben in the Male Mosquitofish (Gambusia affinis): Tissue Injuries and Abnormal Gene Expressions of Hypothalamic-Pituitary-Gonadal-Liver Axis*. International Journal of Environmental Research and Public Health, 20, 3557

Maack G and Segner H (2004). *Life-stage-dependent sensitivity of zebrafish (Danio rerio) to estrogen exposure*. Comparative Biochemistry and Physiology, Part C 139, 47 – 55.

Madsen T, Boyd H B, Nylén D, Pedersen A R, Petersen G I and Simonsen F (2001). *Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products*. Environmental Project No. 615. 1 - 201.

Majhi P D, Sharma A, Roberts A L, Daniele E, Majewski A R, Chuong L M, Black A L, Vandenberg L N, Schneider S S, Dunphy K A and Jerry D J (2020). *Effects of Benzophenone-3 and Propylparaben on Estrogen Receptor–Dependent R-Loops and DNA Damage in Breast Epithelial Cells and Mice*. Environmental Health Perspectives, 128(1), 1 - 15.

Malvar J L, Santos J L, Martin J, Aparicio I and Alonso E (2019). *Routine analytical method for monitoring the main metabolites for a recurrent group of parabens and pharmaceuticals in wastewater and tap water*. Analytical and Bioabalytical Chamistry, 411, 6625—6635.

Marchese S and Silva E (2012). *Disruption of 3D MCF-12A breast cell cultures by estrogens--an in vitro model for ER-mediated changes indicative of hormonal carcinogenesis*. PLoS One. 7(10): e45767.

Márquez-Sillero I, Aguilera-Herrador E, Cárdenas S and Valcárel M (2010). *Determination of parabens in cosmetic products using multi-walled carbon nanotubes as solid phase extraction sorbent and corona-charged aerosol detection system*. Journal of Chromatography A, 1217, 1 - 6.

Marzulli F N, Carson T R and Malbach H I (1968). *Delayed contact hypersensitivity studies in man and animals*. Proc. Joint Conf. Cosmet. Sci., 107 - 122.

Matthews C, Davidson J, Bauer E, Morrison J L and Richardson A P (1956). *p-Hydroxybenzoic acid esters as preservatives II. Acute and chronic toxicity in dogs, rats, and mice*. J. Am. Pharm. Assoc. Sci. Ed., 45(4), 260 - 267.

McCann J, Choi E, Yamasaki E and Ames B N (1975). *Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals*. Proc. Nat. Acad. Sci. USA, 72(12), 5135 - 5139.

Meeker J D, Yang T, Ye X, Calafat A M and Hauser R (2011). *Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage*. Environmental Health Perspectives, 119(2), 252 - 257.

Messerlian C, Mustieles V, Wylie B J, Ford J B, Keller M, Ye X, Calafat A M, Williams P L and Hauser R (2017). *Ultrasound gel as an unrecognized source of exposure to phthalates and phenols among pregnant women undergoing routine scan*. Int. J. Hyg. Environ. Health, 220(8), 1285 - 1294.

Messerlian C, Mustieles V, Minguez-Alarcon L, Ford J B, Calafat A M, Souter I, Williams P L, Hauser R (2018). *Preconception and prenatal urinary concentrations of phenols and birth size of singleton infants born to mothers and fathers from the Environment and Reproductive Health (EARTH) study*. Environment International, 114, 60 – 68.

Mikula P, Dobšíková R, Svobodová Z and Jarkovský J (2006). *Evaluation of xenoestrogenic potential of propylparaben in zebrafish (Danio rerio)*. Neuro. Endocrinol. Lett, 27(suppl.2), 104 - 107.

Mikula P, Kružiková K, Dobšíková R, Haruštiaková D and Svobodová Z (2009). *Influence of propylparaben on vitellogenesis and sex ration in juvenile zebrafish (Danio rerio)*. Actavet. BRNO, 78, 319 - 326.

Miller D, Wheals B B, Beresford N and Sumpter J P (2001). *Estrogenic activity of phenolic additives determine by an in vitro yeast bioassay*. Environmental Health Perspectives, 109(2), 133 - 138.

Mogus J P, La Plante C D, Bansal R, Matouskova K, Schneider B R, Daniele E, Silva S J, Hagen M J, Dunphy K A, Jerry D J, Schnieder Sallie S and Vandenberg L N (2021). *Exposure to Propylparaben During Pregnancy and Lactation Induces Long-Term Alterations to the Mammary Gland in Mice*. Endocrinology, 162(6), 1-18.

Molins-Delgado D, Díaz-Cruz M S and Barceló D (2016). *Ecological risk assessment associated to the removal of endocrine-disrupting parabens and benzophenone-4 wastewater treatment*. Journal of Hazardous Metals, 310, 143 - 151.

Moos R K, Koch H M, Angerer J, Apel P, Schröter-Kermani C, Brüning T and Kolossa-Gehring M (2015). *Parabens in 24 h urine samples of the German Environmental Specimen Bank from 1995 to 2012*. Int. J. of Hyg. And Env. Health, 218(7), 666 - 674.

Myridakis A, Chalkiadaki G, Fotou M, Kogevinas M, Chatzi L, Stephanou E G (2016). *Exposure of preschool-age Greek children (RHEA cohort) to bisphenol A, parabens, phthalates, and organophosphates*. Environ. Sci. Technol., 50, 932 - 341.

Nagar Y, Thakur R S, Parveen T, Patel D K, Ram K R and Satish A (2020). *Toxicity assessment of parabens in Caenorhabditis elegans*. Chemosphere, 246, 125730.

Nam S H and An Y J. (2015). *An efficient and reproducible method for improving growth of a soil alga (Chlorococcum infusionum) for toxicity assays*. J. Microbiol. Methods, 119(10), 59 - 65.

Nam S H and An Y J (2016). *Paper-disc method: an efficient assay for evaluating metal toxicity to soil algae*. Environ. Pollut., 216, 1 - 8.

Nieto A, Borrull F, Marcé R M and Pocurull E (2009). *Determination of personal care products in sewage sludge by pressurized liquid extraction and ultra high performance liquid chromatography–tandem mass spectrometry*. Journal of Chromatography A, 1216, 5619 – 5625.

Nikitakis J M and McEwen G N (1990). CTFA Compendium of Cosmetic Ingredient Composition - Descriptions I and II.

Nishihara T, Nishikawa J-I, Kanayama T, Dakeyama F, Saito K, Imagawa M, Takatori S, Kitagawa Y, Hori S and Utsumic H (2000). *Estrogenic Activities of 517 Chemicals by Yeast Two-Hybrid Assay*. Journal of Health Science, 46(4), 282 - 298.

Nobile M, Arioli F, Pavlovic R, Ceriani F, Lin S_K, Panseri S, Villa R and Chiesa L M (2020). *Presence of emerging contaminants in baby food*. Food Additives & Contaminants: Part A, 37(1), 131 - 142.

Núñez L, Tadeo JL, García-Valcárcel AI, Turiel E. (2008). *Determination of parabens in environmental solid samples by ultrasonic-assisted extraction and liquid chromatography with triple quadrupole mass spectrometry.* J. Chromatogr. A, 1214, 178 – 82.

Odashima S (1976). *The cooperative development in Japan of methods for screening chemicals for carcinogenicity*. IARC Sci. Publ., 12, 61 - 79.

OECD Series on Testing and Assessment n°23: Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals. https://www.oecd-ilibrary.org/docserver/0ed2f88e-en.pdf?expires=1682328331&id=id&accname=guest&checksum=7106CA695A6A504FFC359FB6DA93539A

OECD Series on Testing and Assessment n°142: Report of the phase 2 of the validation of the fish sexual development test for the detection of endocrine substances, ENV/JM/MONO(2011)23/REV1, 1-95. . http://www.oecd.org/officialdocuments/displaydocument/?cote=ENV/JM/MONO(2011)23/REV1&doclanguage=en

OECD Guidance document on standardised test guidelines for evaluating chemicals for endocrine Disruption, Series on Testing and Assessment, No. 150, ENV/JM/MONO(2012)22, 1 - 524. https://www.oecd.org/chemicalsafety/guidance-document-on-standardised-test-guidelines-for-evaluating-chemicals-for-endocrine-disruption-2nd-edition-9789264304741-en.htm

Oishi S (2002). *Effects of propylparaben on the male reproductive system*. Food Chem. Tox., 40, 1807 - 1813.

Okubo T, Yokoyama Y, Kano K, Kano I (2001). *ER-dependent estrogenic activity of parabens assessed by proliferation of human breast cancer MCF-7 cells and expression of ERa and PR*. Food and Chemical Toxicology, 39, 1225 – 1232.

O'Neil M J (2001) The Merck Index - An Enzyclopedia of Chemicals, drugs, and Biologicals, 13th edition, 680.

Özdemir E, Barlas N and Çetinkaya M A (2018). *Assessing the antiandrogenic properties of propyl*
paraben using the Hershberger bioassay. Toxicol. Res., 7, 235.

Park N-Y, Cho Y H, Choi K, Lee E-H, Kim Y J, Kim J H and Kho Y (2019). *Parabens in breast milk and possible sources of exposure among lactating women in Korea*. Environmental Pollution, 255, 113142.

Park Y-D, Jang J-H, Park J-E, Kim J H, Kim E-C, Song Y-J and Kwon H-J (2014). *Analysis of parabens in dentifrices and the oral cavity*. Biomed. Chromatogr., 28, 1692 - 1700.

Pedersen K L, Pedersen S N, Christiansen L B Korsgaard B and Bjerregaard P (2000). *The preservatives Ethyl-, Propyl-and Butylparaben are Oestrogenic in an in vivo Fish Assay*. Pharmacology and Toxicology, 86, 110 - 113.

Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors T L, Jorens P G, Blust R and Vanparys C (2013). *Evaluation of a screening system for obesogenic compounds: Screening of endocrine disrupting compoundsand evaluation of the PPAR dependency of the effect*. PloS One 8, e77481.

Pérez Martín J M, Fernández Freire P, Daimiel L, Martínez-Botas J, Sánchez C M, Lasunción M Á, Peropadre A, Hazen M J (2014). *The antioxidant butylated hydroxyanisole potentiates the toxic effects of propylparaben in cultured mammalian cells*. Food Chem Toxicol., 72, 195 - 203.

Perugini M, Merola C, Amorena M, D'Angelo M, Cimini A, Benedetti E J (2020). *Sublethal exposure to propylparaben leads to lipid metabolism impairment in zebrafish early-life stages*. Appl. Toxicol., 40(4), 493 - 503.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat A M, Ye X, Silva M J, Brambilla C, Pin I, Charles M-A, Cordier S and Slama R (2012). *Exposure to phthalates and phenols during pregnancy and offspring size at birth*. Environmental Health Perspectives, 120(3), 464 - 470.

Philippat C, Botton J, Calafat A M, Ye X, Charles M-A, Slama R, and the EDEN study Group (2014). *Prenatal Exposure to Phenols and Growth in Boys*. Epidemiology, 25(5), 625 - 635.

Phillips J C, Topp C S and Gangolli S D (1978). *The metabolism of ethyl and n-propyl-p-hydroxybenzoate (« parabens ») in male cats*. Toxicology letters, 2, 237 - 242.

Pohl J (2015). Thyroid endocrine disruption of propylparaben assessed using an optimized individual Xenopus tropicalis metamorphosing tadpole exposure system. Degree project in the Master of science, Uppsala University.

Pollack AZ, Perkins N J, Sjaarda L, Mumford S L, Kannan K, Philippat C, Wactawski-Wende J and Schisterman E F (2016). Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women (2016). Environmental Research, 151, 513 – 520.

Potouridis T, Knauz A, Berger E and Pütmann W (2019). *Examination of paraben release from baby teethers through migration tests and GC–MS analysis using a stable isotope dilution assay*. BMC Chemistry, 13(70), 1 - 14.

Prusakiewicz J J, Harville H M, Zhang Y, Ackermann C and Voorman RL (2007). *Parabens inhibit human skin estrogen sulfontransferase activity: possible link to paraben estrogen effects*. Toxicology, 232, 248 - 256.

Pycke B F G, Geer L A, Dalloul M, Abulafia O and Halden R U (2015). *Maternal and fetal exposure to parabens in a multiethnic urban U.S. population*. Environment International, 84, 193 – 200.

Radwan E K, Ibrahim M B M, Adel A and Farouk M (2020). *The occurrence and risk assessment of phenolic endocrine-disrupting chemicals in Egypt's drinking and source water*. Environmental Science and Pollution Research, 27, 1776 – 1788.

Ramaswamy B R, Kim J W, Isobe T, Chang K H, Amano A, Miller T W, Siringan F P, Tanabe S (2011). *Determination of preservative and antimicrobial compounds in fish from Manila Bay, Philippines using ultra high performance liquid chromatography tandem*

mass spectrometry, and assessment of human dietary exposure. J. Hazard. Mater., 192(3), 1739 - 1745.

Ramberger M, Woldegiorgis A, Kaj L, Andersson J, Palm Cousins A, Dusan D, Ekheden Y, E. Brorström-Lundén (2006). *Results from the Swedish screening 2005, Sub-report 2 biocides*. Swedish Environmental Research Institute, http://www.ivl.se/rapporter/pdf/B1700.pdf

Reguerio J, Becerril E, Garcia-Jares C and Llompart M (2009). *Trace analysis of parabens, triclosan and related chlorophenols in water by headspace solid-phase microextraction with in situ derivatization and gas chromatography-tandem mass spectrometry*. Journal of Chromatography A, 1216, 4693 - 4702.

Rodríguez-Gómez R, Jiménez-Díaz J, Zafra-Gómez A, Ballesteros O and Navalón A (2014). *A multiresidue method for the determination of selected endocrine disrupting chemicals in human breast milk based on a simple extraction procedure*. Talanta, 130, 561 – 570.

Rolland M, Lyon-Caen S, Sakhi A K, Pin I, Sabaredzovic A, Thomsen C, Slamy R, Philipat C and the SEPAGES study group (2020). *Exposure of phenols during pregnancy and the first year of life in a new type of couple-child cohort relying on repeated urine biospecimens*. Environment International, 139, 105678.

Routledge E J, Parker J, Odum J, Ashby J and Sumpter J P (1998). *Some Alkyl Hydroxy Benzoate Preservatives (Parabens) Are Estrogenic*. Toxicology and applied pharmacology, 153, 12 – 19.

Sabalitschaka T and Neufled C R (1954). *Zum verhalten der p-oxybenzoesäureester im menschlichen körper*. Arzneimittelforschung, 4, 575 - 579.

Saito D and Tanaka M (2009). *Comparative aspects of gonadal sex differentiation in medaka: a conserved role of developing oocytes in sexual canalization*. Sex. Dev. 3, 99-107.

Samarasinghe S V A C, Kishnan K, Aitken R J, Naidu R and Megharaj M (2021). *Persistence of the parabens in soil and their potential toxicity in earthworms*. Environmental Toxicology and Pharmacology, 83, 103574

Satoh K, Nonaka R, Ohyama K-i, and Nagai Fumiko (2005). *Androgenic and antiandrogenic effects of alkylphenols and parabens assessed using the reporter gene assay with stably transfected CHO-K1 cells (AR-Ecoscreen system)*. Journal of Health Science, 51(5), 557 - 568.

SCCS (Scientific Committee on Consumer safety) (2011). *Clarification on opinion SCCS/1348/11 in the light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age*. 10 October 2011 (SCCS/1446/11).

SCCS (Scientific Committee on Consumer safety) (2013). *Opinion on parabens*, 3 May 2013 (SCCS/1514/13).

SCCS (Scientific Committee on Consumer safety) (2021). *Opinion on propylparaben (PP)*, 30-31 March 2021 (SCCS/1623/20).

Schlumpf M, Kypke K, Wittassek M, Angerer J, Mascher H, Mascher D, Vökt C, Birchler M and Lichtensteiger W (2010). *Exposure patterns of UV filters, fragrances, parabens, phthalates, organoclor pesticides, PBDEs and PCBs in human milk: correlation of UV filters with use of cosmetics*. Chemosphere, 81(10), 1171 - 1183.

Schübel K and Manger H (1929). *Ein beitrag zur pharmakologie einiger paraoxybenzoes âureester: das schicksal im organismus und die toxizität*. J. Arch. Exptl. Path. Pharmakol., 146, 209 - 222.

Scott P D, Coleman H M, Colville A, Lim R, Matthews B, McDonald J A, Miranda A, Neale P A, Nugegoda D, Tremblay L A, Leusch F D (2017). *Assessing the potential for trace organic contaminants commonly found in Australian rivers to induce vitellogenin in the native rainbowfish (Melanotaenia fluviatilis) and the introduced mosquitofish (Gambusia holbrooki)*. Aquat Toxicol., 185, 105 - 120.

Seki T, Mochida J, Hosoya O, June K and Morimoto K (2003). *Measurement of diffusion coefficients of parabens and steroids in water and 1-octanol*. Chem. Pharm. Bull., 51(6), 734 - 736.

Shaw J and deCatanzaro D (2009). *Estrogenicity of parabens revisited: impact of parabens on early pregnancy and an uterotrophic assay in mice*. Reproductive Toxicology, 28, 26 - 31.

Shi M and Faustman E (1989). *Development and characterization of amorphological scoring system for medaka (Oryzias latipes) embryo culture*. Aquat. Toxicolo. 15, 127-140.

Shibata M, Yamada M, Hirose M, Asakawa E, Tatematsu M and Ito N (1990). *Early proliferative responses of forestomach and glandular stomach of rats treated with five different phenolic antioxydants*. Carcinogenesis, 11(3), 425 - 429.

Shin M-Y, Shin C, Choia J W, Lee J, Lee S and Kim S (2019). *Pharmacokinetic profile of propyl paraben in humans after oral administration*. Environment International, 130, 104917.

Shoghi E, Fuguet E, Rafols C and Bosch E (2009). *Kinetic and thermodynamic solubility values of some bioactive compounds*. Chemistry and Biodiversity, 6, 1789 - 1795.

Sivaraman L, Pouliot L, Wang B, Brodie T, Graziano M, McNerney M E (2018). *Safety assessment of propylparaben in juvenile rats*. Regul Toxicol Pharmacol., 92, 370 - 381.

Smith K W, Souter I, Dimitriadis I, Ehrlich S, Williams P L, Calafat A M and Hauser R (2013). *Urinary paraben concentrations and ovarian aging among women from a fertility center*. Environmental Health Perspectives, 121(11-12), 1299 - 1305.

Song B L, Peng D R, Li H Y, Zhang G H, Zhang J and Li K L (1991). *Evaluation of the effects of butyl p-hydroxybenzoate on the proteolytic activity and membrane function of human spermatozoa*. Journal of Reproduction and Fertility, 91(2), 435 - 440.

Song H, Alfiya Y, Dubowski Y and Friedler E (2017). *Sorption and biodegradation of propylparaben in greywater by aerobic attached-growth biomass.* Science of the Total Environment, 598, 925 – 930.

Souza I D, Melo L P, Jardim I CS F, Monteiro J C S, Nakano A M S and Querioz M E C (2016). *Selective molecularly imprinted polymer combined with restricted access material for in-tube SPME/UHPLC-MS/MS of parabens in breast milk samples*. Analytica Chimica Acta, 932, 49 - 59.

Sprague B L, Trentham-Dietz A, Hedman C J, Wang J, Hemming J Dc, Hampton J M, Buist D Sm, Aiello Bowles E J, Sisney G S and Burnside E S (2013). *Circulating serum xenoestrogens and mammographic breast density*. Breast Cancer research, 15(3), 45.

Strømmen K, Lych J L, Moltu S J, Müller M H B, Blakstad E W, Almaas A N, Sakhi A K, Thomsen C, Nakstad B, Rønnestad A E, Drevon C A, and Iversen P O (2021). *High urinary concentrations of parabens and bisphenol A in very low birth weight infants*. Chemosphere, 271, 129570.

Sugimura T, Sato S, Nagao M, Yahagi T, Matsushima T, Seino Y, Takeuchi M and Kawachi T (1976). *Overlapping of carcinogens and mutagens*. Fund. Cancer. Prev., 6th Symp. Princess Takamatsu cancer res. Fund.

Sumpter J P and Jobling S (1995). *Vitellogenin as a biomarker for estrogenic contamination of the aquatic environment*. ENV. Health Perspect., 103, 173 - 178.

Terasaki M, Kamata R, Shiraishi F and Makino M (2009). *Evaluation of estrogenic activity of parabens and their chlorinated derivatives by using the yeast two-hybrid assay and the enzyme-linked immunosorbent assay*. Environmental Toxicology and Chemistry, 28(1), 204 – 208.

Terasaki M, Abe R, Makino M and Tatarazako N (2015). *Chronic Toxicity of Parabens and Their Chlorinated By-Products in Ceriodaphnia dubia*. Environmental Toxicology, 30 (6), 664-673.

Torres T, Cunha I, Marins R and Santos M M (2016). *Screening the Toxicity of Selected Personal Care Products Using Embryo Bioassays: 4-MBC, Propylparaben and Triclocarban*. International Journal of Molecular Sciences, 17, 1762.

Twist J.N and Zats J.L (1986). *Influence of solvents on parabens permeation through idealized skin model membranes*. J Soc Cosmet Chem, 37, 429 - 444.

Van Meeuwen J A, van Son O, Piersma A H, de Jong P C and van den Berg M (2008). *Aromatase inhibiting and combined estrogenic effects of parabens and estrogenic effects of other additives in cosmetics*. Toxicology and Applied Pharmacology, 230, 372 – 382.

Vela-Soria F, Iribarne-Durána L M, Mustieles V, Jiménez-Díaz I, Fernández M F and Olea N (2018). *QuEChERS and ultra-high performance liquid chromatography–tandem mass spectrometry method for the determination of parabens and ultraviolet filters in human milk samples*. Journal of Chromatography A, 1546, 1 – 9.

Vela-Soria F, Jiménez-Díaz I, Diaz C, Pérez J, Iribarne-Durán L M, Serrano-Lopéz L, Arrebola J P, Fatima Fernandez M F and Olea N (2016). *Determination of endocrine-disrupting chemicals in human milk by dispersive liquid-liquid microextraction*. Bioanalysis, 8, 1777 - 1791.

Vinggaard A M, Körner W, Lund K H, Bolz U and Petersen J H (2000). *Identification and Quantification of Estrogenic Compounds in Recycled and Virgin Paper for Household Use As Determined by an in Vitro Yeast Estrogen Screen and Chemical Analysis*. Chem. Res. Toxicol., 13, 1214 - 1222.

Vo T T B, Yoo Y-M, Choi K-C and Jeung E-B (2010). *Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model*. Reproductive Toxicology, 29, 306 – 316.

Vo T T B, Junga E-M, Choia K-C, Yub F H and Jeung E-B (2011). *Estrogen receptor is involved in the induction of Calbindin-D9k and progesterone receptor by parabens in GH3 cells: A biomarker gene for screening xenoestrogens*. Steroids, 76, 675 – 681.

Wang D, Li W, Yang C, Chen X, Liu X, He J, Tong C, Peng C, Ding Y, Geng Y, Cao X, Li F, Gao R and Wang Y (2021). *Exposure to ethylparaben and propylparaben interfere with embryo implantation by compromising endometrial decidualization in early pregnant mice*. Journal of Applied Toxicology, 41(11), 1732 - 1746.

Wang L, Liao C, Liu F, Wu Q, Guo Y, Moon H-B, Nakata H and Kannan K (2012). *Occurrence and Human Exposure of p-Hydroxybenzoic Acid Esters (Parabens), Bisphenol A Diglycidyl Ether (BADGE), and Their Hydrolysis Products in Indoor Dust from the United States and Three East Asian Countries*. Environ. Sci. Technol., 46, 11584 − 11593.

Wang P., Li J., Tian H and Ding X (2013). *Investigation of parabens in commercial cosmetics for children in Beijing, China*. J. Cosmet. Sci., 64, 67 - 72.

Watanabe Y, Kojima H, Takeuchi S, Uramaru N, Ohta S and Kitamura S (2013). *Comparative study on transcriptional activity of 17 parabens mediated by estrogen receptor α and β and androgen receptor*, Food and Chemical Toxicology, 57, 227 - 234.

WHO (2002): *Global assessment of the state-of-the-science of endocrine disruptors*. World Health Organization, WHO/PCS/EDC/02.2, 1 - 180.

Wróbel A and Gregoraszczuk E Ł (2013). *Effects of single and repeated in vitro exposure of three forms of parabens, methyl-, butyl- and propylparabens on the proliferation and estradiol secretion in MCF-7 and MCF-10A cells*. Pharmacological Reports, 65, 484 – 493.

Wróbel A M and Gregoraszczuk E Ł (2014). *Actions of methyl-, propyl- and butylparaben on estrogen receptor-α and -β and the progesterone receptor in MCF-7 cancer cells and non-cancerous MCF-10A cells*. Toxicol. Lett., 230(3), 375 - 81.

Wróbel A M and Gregoraszczuk E Ł (2015). *Action of methyl-, propyl- and butylparaben on GPR30 gene and protein expression, cAMP levels and activation of ERK1/2 and PI3K/Akt signaling pathways in MCF-7 breast cancer cells and MCF-10A non-transformed breast epithelial cells*. Toxicology Letters, 238, 110 - 116.

Wu C, Huo W, Li Y, Zhang B, Wan Y, Zheng T, Zhou A, Chen Z, Qian M, Zhu Y, Jiang Y, Liu H, Chen X, Xu B, Xia W and Xu S (2017a). *Maternal urinary paraben levels and offspring size at birth from a Chinese birth cohort*. Chemosphere, 172, 29 - 36.

Wu N-X, Deng L-J, Xiong F, Xie j-Y, Li X-J, Zeng Q, Sun J-C, Chen D and Yang P (2022). *Risk of thyroid cancer and benign nodules associated with exposure to parabens among Chinese adults in Wuhan, China*. Environmental Science and Pollution Research, 29, 70125 – 70134.

Wu Y, Sun Q, Wang Y-W Deng C-X and Yu C-P (2017b). *Comparative studies of aerobic and anaerobic biodegradation of methylparaben and propylparaben in activated sludge*. Ecotoxicology and Environmental Safety, 138, 25 – 31.

Yalkowsky S H and He Y (2003). *Handbook of Aqueous Solubility Data - An extensive compilation of aqueous solubility data for organic compounds extracted from AQUASOL database*. CRC press, 871.

Yamamoto H, Tamura I, Hirata Y, Kato J, Kagota K, Katsuki S, Yamamoto A, Kagami Y and Tatarazako N (2011). *Aquatic toxicity and ecological risk assessment of seven parabens: individual and additive approach*. Science of Total Environment, 410-411, 102 - 111.

Yan W, Li M, Guo Q, Li X, Zhou S, Dai J, Zhang J, Wu M, Tang W, Wen J, Xue L, Jin Y, Luo A and Wang S (2022). *Chronic exposure to propylparaben at the humanly relevant dose triggers ovarian aging in adult mice*. Ecotoxicology and Environmental Safety, 235, 113432.

Yazar K, Johnsson S, Lind M-L, Boman A and Lidén C (2010). *Preservatives and fragrances in selected consumer-available cosmetics and detergents*. Contact Dermatitis, 64, 265 - 272.

Ye X, Bishop A M, Reidy J A, Needham L L, Calafat A M (2006). *Parabens as urinary biomarkers of exposure in humans*. Environmental Health Perspectives, 114, 1843 – 1846.

Ye X, Bishop A M, Needham L L and Calafat AM (2008). *Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan,*

and other environmental phenols in human milk. Analytica chimica acta, 622, 150 – 156.

Zhang N-S, Liu Y-S, Van den Brink P J, Price O R and Ying G-G (2015). *Ecological risks of home and personal care products in the riverine environment of a rural region in South China without domestic wastewater treatment facilities*. Ecotoxicology and Environmental Safety, 122, 417 – 425.

Zhu H and Kannan K (2020). *Parabens in stretch mark creams: A source of exposure in pregnant and lactating women*. Science of the Total Environment, 744, 141016.

17.15 Abbreviations

*: p<0.5

**: p<0.05

***: p<0.1

****: p<0.01

*****: p<0.001

******: p<0.0001

Abs: absolute

AChE: Acetylcholinsterase

ADME: absorption, distribution, metabolism, excretion

AF: assessment factor

AFC: antral follicle count

AGD: ano-genital distance

ALH: amplitude of lateral head displacement

AMA: amphibian Metamorphosis Assay

aP2: adipocyte specific protein 2

Approx.: approximately

aPTT: activated partial thromboplastin time

AR: androgen receptor

AUC: area under the curve

BA: benzoic acid

BBN: N-(4-hydroxybutyl)nitrosamine

BCF: bioconcentration factor

BE CA: Belgian Competent Authority

BLQ: below limit of quantification

BPA: Bisphenol A

BPB: Butylparaben

BZPB: Benzylparaben

Bw: body weight

BWG: body weight gain

CaBP-9k: Calbindin-D9k

CALUX: Chemical Activated LUciferase gene eXpression

CAR: constitutive androstane receptor

CAS: Chemical Abstract Service

CAT: Chloramphenicol acetyltransferase

BBPI: Cytokinesis-Block Proliferation Index

CF: conceptual framework

CHG: choriogenin

CHO: Chinese hamster ovary

CI: confidence interval

CLH: harmonised classification

CLP: Classification and labelling

CMR: carcinogen, mutagen, reprotoxic

Conc.: concentration

CoRAP: Community Rolling Action Plan

Corresp.: corresponding

Crt: creatinine

CYP19a1: Cytochrome P450 Family 19 Subfamily a Member 1 (gonad)

CYP19b: Cytochrome P450 Family 19 Subfamily b (brain)

d: day

d50: median particle diameter

Da: dalton

Dam.: damage

DBP: di(n-butyl) phthalate

DEHP: di(2-ethylhexyl) phtalate

DES: diethylstilbestrol

DHEA: dehydroepiandrosterone

DHT: dihydrotestosterone

Dio: deiodinase

DIT: developmental immunotoxicity test

DLA: degree of lipid accumulation

DLAI: degree of lipid accumulation compared to insulin

DMEL: Derived minimal effect level

DMSO: dimethyl sulfoxide

DNA: deoxyribonucleic acid

DNEL: Derived no effect level

DNT: developmental neurotoxicity test

DPB: docecylparaben

Dpf: day post-fertilisation

Dph: day post hatch

DPP: day post-partum

DSP: daily sperm production

Dw: dry weight

E2: 17β-estradiol

EARTH: Environment and Reproductive health

EAS: estrogenic, androgenic, and steroidogenic

EC10: concentration producing effect in 10 % of the test organisms

EC50: concentration producing effect in 50 % of the test organisms

ECHA: European Chemicals Agency

ED: endocrine disruptor

ED50: effective dose for 50 % of the population

EDA: effect-directed analysis

EDEG: Endocrine Disruptor Expert Group

EDI: estimated daily intake

EE2: 17α-ethinylestradiol

EEF: estrogen equivalent factor

EEQ: estradiol equivalence quantities

ELISA: Enzyme-linked immune assay

eMS: evaluating member state

eMSCA: evaluating member state competent authority

ENV: environment

EOGRTS: Extended One-Generation Reproductive Toxicity Study

EPA: Environmental Protection Agency

EPB: Ethylparaben

ER: estrogen receptor

ErC50: concentration affecting growth rate in 50 % of the test organisms

ERE: estrogen-responsive element

EROD: ethoxyresorufin-O-deethylase

ERR: estrogen-related receptor

ESR1: estrogen receptor α

ESR2: estrogen receptor β

EU: European union

EVA: ethylene-vinyl acetate

Excl.: excluding

Exp: Experiment

F: female

FAI: free androgen index

FBW: final body weight

FET: Fish Embryo Acute Toxicity Test

FLU: flutamide

FM: fast movements

FR: fast rearing

FSDT: Fish Sexual Development Test

FSH: follicle stimulating hormone

GC-MS: Gas Chromatography coupled to Mass Spectrometry

GD: gestational day

Geom.: geometric

GLP: good laboratory practice

GPMT: Guinea Pig Maximisation Test

GPR: G-protein coupled receptor

GPx: Glutathione peroxidase activity

GR: glucocorticoid receptor

GSH: gluthatione

GST: Glutathione S-transferase

h: hour

HaCaT: immortalized human keratinocytes

hADSC: human adipose-derived stem cell

hAR: human androgen receptor

HBA: 4-hydroxybenzoic acid

HBM: human biomonitoring

HCD: historical control data

hCG: human chorionic gonadotropin

hER: human estrogen receptor

HF: hydroxyflutamide

Hg: hemoglobin

HH: human health

HLL: hind limb length

HPB: heptylparaben

Hpf: hours post-fertilisation

HPG: Hypothalamic-pituitary-gonadal

HPGL: Hypothalamic-pituitary-gonadal-liver

HPLC: high-performance liquid chromatography

hPXR: hypoxanthine phosphoribosyltransferase

HPLC-DAD: high-performance liquid chromatography with diode-array detection

Ht: hematocrit

IBPB: Isobutylparaben

IC50: concentration with 50 % inhibition

IC95: 95 % confidence interval

ICI 1 82,780: fulvestrant (CAS 129453-61-)

ICT : isothermal titration colorimetry

ID: intradermal

IPPB: Isopropylparaben

IPCS: International Programme on Chemical Safe y

IQR : interquartil range

Irrit.: irritation

IV: intravenous

IW: immature Wistar

JH: juvenile hormone

KD : dissociation constant

Kg: kilogram

KLH: keyhole limpet haemocyanin

Koc: organic carbon water partition co-efficient

Kow: octanol water partition co-efficient

L: litter

L.: left

LABC: levator ani plus-bulbocavernosus

LBD: ligand binding domain

LC10: Lethal concentration causing 10 % death

LC50: Lethal concentration causing 50 % death

LC-MS/MS: liquid chromatography – tandem mass spectrometry

LD50: Lethal doses causing 50 % death

LH: luteinising hormone

LIN: linearity (VSL/VCL *100)

LLNA: Local Lymph Node Assay

LOAEL: No observed adverse effect concentration

LOD: limit of detection

LOEC: Lowest observed effect concentration

LOEL: lowest observed effect level

M: male

MALDI-TOF-MS: Matrix assisted laser desorption ionization – Time of flight – mass spectrometry

Max.: maximum

MCF: Michigan Cancer Foundation

MCH: mean corpuscular haemoglobin

MCI: Molecular Connectivity Index

MCV: mean corpuscular volume

MDL: Method detection limits

Meas.: measured

MedER: medaka estrogen receptor

Met. act.: metabolic activation

Mg: milligram

Min: minute

Min.: minimum

ML: Milliliter

mM: millimolar

MMTV: murine mammalian tumor virus

MoA: mode of action

MPB: Methylparaben

MSC: Member State Committee

MSCA: member state competent authority

MSMS: tandem mass spectrometry

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MMTV: murine mammalian tumour virus

MS: Member State

MW: molecular weight

N: number

Na: not applicable

Nb: number

NC: not calculated

ND: not determined

Neg.: negative

NF: Nieuwkoop faber

NHANES: National Health and Nutrition Examination Survey

NHEK: normal human epidermal keratinocytes

NK: natural killer

NO: nitric oxide

NOAEL: No observed adverse effect concentration

NOEC: No observed adverse effect concentration

NOErC: No observed adverse effect concentration for growth rate

NOEL: no observed effect level

Nom.: nominal

NOS: non-organ-specific

NT: not tested

NZW: New Zealand White

OCSPP: Office of Chemical Safety and Pollution Prevention

OECD: Organisation for Economic Co-operation and Development

OMC: Octyl-methoxycinnamate

OPB: octylparaben

OPPTS: Office of Prevention, Pesticides and Toxic Substances

ORO: Oil Red O

OS: organ-specific

OV: ovarian volume

PBT: persistent, bioaccumulative and toxic

PCA: principal component assessment

PDII: primary dermal irritation index

Plt: platelet

PC: positive control

PC50: response that is 50% of the maximal positive control response

PCR: polymerase chain reaction

PEC: predicted environmental concentration

Pf: post fertilization

PG: prostaglandin

pHBA: p-hydroxybenzoic acid, 4-Hydroxybenzoic acid, CAS 99-96-7

pKa: acid dissolution constant

PLA2: phospholipase A2

Plt: platelet

PMD: post-mating day

PND: post-natal day

PNEC: predicted no effect concentration

PNPB: phenyl paraben

Pos.: positive

PPARγ: peroxisome proliferator-activated receptor gamma

PPB: Propyl 4-hydroxybenzoate, Propylparaben

PR: progesterone receptor

PRE: progesterone responsive element

pS2: estrogen related gene encoding the Tefoil 1 protein

PT: prothrombine time

PXR: Pregnane X receptor

QSAR: quantitative structure-activity relationship

R 1881: methyltrienolone

R.: right

RA: relative activity

RAC: Risk Assessment Committee

RBA: relative binding affinity

RBC: red blood cell

RCR: risk characterization ratio

REACH: Registration, Evaluation Authorisation, and restriction of Chemicals

REC10: 10 % relative effective concentration

REC20: 20 % relative effective concentration

REC50: 50 % relative effective concentration

REG: registrant

Rela: relative

Repr.: reproductive toxicity

Repro.: reproductive

Resp.: respectively

RFU: relative fluorescent units

RIC20: 20 % relative inhibitory concentration

RMOA: Regulatory Management Option Analysis

RNA: ribonucleic acid

ROS: Reactive oxygen species

rPXR: rat Pregnane X receptor

RSD: relative standard deviation

RT-PCR: reverse transcription-polymerase chain reaction test

RTA: relative transactivation activity

RU486: mifepristone

S.E.M: standard error of the mean

S. typh: *Salmonella typhimurium*

SAR: structure-activity relationship

SC: subcutaneous (in HH section)

SC: solvent control (in ENV section)

SCCS: Scientific Committee on Consumer Safety

SD: Sprague-Dawley

SE: standard error

Sed.: sediment

Sens.: sensibilisation

SEv: substance evaluation

SHBA: Sulfate of hydroixybenzoid

SHBG: sex-hormone–binding globulin

SI: simulation index

Sign: significantly

SM: slow movements

SOD: superoxide dismutases

SPE: solid phase extraction

SPP: Sulfate of propylparaben

SR: slox rearings

St. Dev.: standard deviation

Stat.: statistically

STOT RE: Specific Target Organ Toxicity – Repeated Exposure

STOT SE: Specific Target Organ Toxicity – Single Exposure

STP: sewage treatment plant

STR: straightness (VSL/VAP *100)

STTA: stably transfected transcriptional activation assay

SULT: sulfontransferase

SVHC: substance of very high concern

SVL: snout -vent length

T: testosterone

T1/2: half-life

T3: triiodothyronine

T4: thyroxine

TDM: tail distributed moment

TG: test guideline

TH: thyroid hormone

TK: toxicokinetic

Tot.: total

Tot. prot.: total protein

Tox.: toxicity

TP: testosterone propionate

TR: testosterone receptor

TSH: thyroid-stimulating hormone

Ttr: transthyretin

TWM: time weighted mean concentration

µg: microgram

UHPLC: ultra-high performance liquid chromatography

UHPLC-MS/MS: ultra-high performance liquid chromatography-tandem mass spectrometry

UV: ultraviolet

Undiff: undifferentiated

VAP: average path velocity

VCD: 4-vinylcyclohexen diepoxide

VCL: curvilinear velocity

Vmax: maximum rate of reaction

Vol.: volume

VPvB: very persistent very bioaccumulative

VSL: straight line velocity

VTG: vitellogenin

WBC: white blood cell

WDU: wide dispersive use

WHO: World Health Organisation

Wist.: Wistar

WoE: weight of evidence

WS: water solubility

WW: wet weight

Wt: weight

WWTP: wastewater treatment plant

YAAS: yeast anti-androgen screen

YAS: yeast androgen screen

YES: yeast estrogen screen