Scientific Committee on Consumer Safety

SCCS

OPINION ON
Propylparaben (PP)

The SCCS adopted this document
at its plenary meeting on 30-31 March 2021

ACKNOWLEDGMENTS
SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this Opinion.

For the preliminary Opinion

SCCS members
Dr U. Bernauer
Dr L. Bodin (Rapporteur)
Prof. Q. Chaudhry (SCCS Chair)
Prof. P.J. Coenraads (SCCS Vice-Chair, Chairperson of the WG)
Prof. M. Dusinska
Dr J. Ezendam
Dr E. Gaffet
Prof. C. L. Galli
Dr B. Granum
Prof. E. Panteri
Prof. V. Rogiers (SCCS Vice-Chair)
Dr Ch. Rousselle
Dr M. Stepnik
Prof. T. Vanhaecke
Dr S. Wijnhoven

SCCS external experts
Dr A. Koutsodimou
Dr A. Simonnard (Rapporteur until resign in July 2020)
Prof. W. Uter
Dr N. von Goetz

For the final Opinion

SCCS members
Dr U. Bernauer
Dr L. Bodin (Rapporteur)
Prof. Q. Chaudhry (SCCS Chair)
Prof. P.J. Coenraads (SCCS Vice-Chair, Chairperson of the WG)
Prof. M. Dusinska
Dr J. Ezendam
Dr E. Gaffet
Prof. C. L. Galli
Dr B. Granum
Prof. E. Panteri
Prof. V. Rogiers (SCCS Vice-Chair and Rapporteur)
Dr Ch. Rousselle
Dr M. Stepnik
Prof. T. Vanhaecke
Dr S. Wijnhoven

SCCS external experts
Dr A. Koutsodimou
Prof. W. Uter
Dr N. von Goetz

This Opinion has been subject to a commenting period of eight weeks (from 6 November until 4 January 2021). Comments received during this time period were considered by the SCCS. The following section has been slightly revised: physicochemical properties and the related discussion part. The final conclusions have not been modified.

All Declarations of Working Group members are available on the following webpage:
Register of Commission expert groups and other similar entities

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Propylparaben, does the SCCS consider Propylparaben safe when used as a preservative in cosmetic products up to a maximum concentration of 0.14 %?*

On the basis of the safety assessment of Propylparaben, and considering the concerns related to potential endocrine disrupting properties, the SCCS has concluded that propylparaben is safe when used as a preservative in cosmetic products up to a maximum concentration of 0.14 %.

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Propylparaben as a preservative in cosmetic products?*

/

3. *Does the SCCS have any further scientific concerns with regard to the use of Propylparaben in cosmetic products?*

The available data on Propylparaben provide some indications for potential endocrine effects. However, the current level of evidence is not sufficient to regard it as an endocrine disrupting substance, or to derive a toxicological point of departure based on endocrine disrupting properties for use in human health risk assessment.

The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of propylparaben for the environment.

Keywords: SCCS, scientific opinion, Propylparaben, preservative, Regulation 1223/2009, CAS No 94-13-3, EC No 202-307-7

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Propylparaben (CAS No 94-13-3, EC No 202-307-7), preliminary version of 27-28 October 2020, final version of 30-31 March 2021, SCCS/1623/20

About the Scientific Committees
Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and are made up of scientists appointed in their personal capacity.
In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease Prevention and Control (ECDC) and the European Chemicals Agency (ECHA).
SCCS
The Committee shall provide Opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Contact
European Commission
Health and Food Safety
Directorate C: Public Health
Unit C2: Health information and integration in all policies
L-2920 Luxembourg
SANTE-SCCS@ec.europa.eu

PDF ISSN 1831-4767 ISBN 978-92-76-54756-3 doi:10.2875/607198 EW-AQ-22-006-EN-N

TABLE OF CONTENTS

2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted a review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting properties. The review concluded that the Cosmetics Regulation provides the adequate tools to regulate the use of cosmetic substances that present a potential risk for human health, including when displaying ED properties.
The Cosmetics Regulation does not have specific provisions on EDs. However, it provides a regulatory framework with a view to ensuring a high level of protection of human health. Environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 ('REACH Regulation').
In the review, the Commission commits to establishing a priority list of potential EDs not already covered by bans or restrictions in the Cosmetics Regulation for their subsequent safety assessment. A priority list of 28 potential EDs in cosmetics was consolidated in early 2019 based on input provided through a stakeholder consultation. The Commission then organised a public call for data[2] from 16 May 2019 – 15 October 2019 on 14[3] of the 28 substances (to be treated with higher priority) in order to be able to prepare the safety assessment of these substances. Propylparaben is one of the above-mentioned 14 substances for which the call for data took place.

Existing information on Propylparaben

In cosmetic products, the ingredient Propylparaben (CAS No 94-13-3, EC No 202-307-7) with the chemical names Propyl 4-hydroxybenzoate and 4-Hydroxybenzoic acid propyl ester is currently regulated as a preservative in a concentration up to 0.14 % (as acid) (Annex V/12a). In addition, a safe concentration has been established for mixtures of parabens, where the sum of the individual concentrations should not exceed 0.8 % (as acid). However, in such mixtures the sum of the individual concentrations of butyl- and propylparaben and their salts should not exceed 0.14 %.
Propylparaben has been subject to different safety evaluations in 2005 (SCCP/0874/05), 2006 (SCCP/1017/06), 2008 (SCCP/1183/08), 2010 (SCCS/1348/10), 2011 (SCCS/1446/11) and 2013 (SCCS/1514/13). In particular, the last SCCS opinion from 2013 states that '*The additional submitted data does not remove the concern expressed in the previous opinions on the relevance of the rat model for the risk assessment of parabens. Although much toxicological data on parabens in rodents exists, adequate evidence has not been provided for the safe use of propyl- or butylparaben in cosmetics'*.
During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Propylparaben as a preservative in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Propylparaben in view of the information provided.

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Propylparaben, does the SCCS consider Propylparaben safe when used as a preservative in cosmetic products up to a maximum concentration of 0.14 %?*

[1] https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-739-F1-EN-MAIN-PART-1.PDF
[2] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic%20products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, Propylparaben, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, Propylparaben, benzyl salicylate, genistein and daidzein

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Propylparaben as a preservative in cosmetic products?*

3. *Does the SCCS have any further scientific concerns with regard to the use of Propylparaben in cosmetic products?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Propylparaben

3.1.1.2 Chemical names

Propyl 4-hydroxybenzoate
4-Hydroxybenzoic acid propyl ester
Propyl p-hydroxybenzoate
Propyl parahydroxybenzoate
n-Propyl 4-hydroxybenzoate
n-Propyl p-hydroxybenzoate
p-Hydroxypropyl benzoate
p-Hydroxybenzoic acid propyl ester
N-Propylparaben
p-Hydroxybenzoic propyl ester
Propyl-4-hydroxybenzoate
4-Hydroxybenzoic acid, propyl ester
Propyl-paraben
Benzoic acid, 4-hydroxy-, propyl ester
Benzoic acid, p-hydroxy-, propyl ester
p-Hydroxybenzoic acid, propyl ester
propyl 4-oxidanylbenzoate
p-Hydroxybenzoic acid n-propyl ester
n-propyl paraben
4-Hydroxybenzoic acid propylester
propyl para-hydroxybenzoate
n-propyl-p-hydroxy-benzoate
4-Hydroxybenzoic acid-propyl ester
4-hydroxybenzoic acid n-propyl ester

3.1.1.3 Trade names and abbreviations

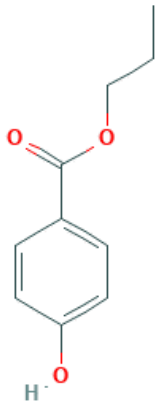
Nipasol
Nipazol
Nipasol P
Nipasol M
Propyl Parasept
Propagin
Chemacide pk
Chemocide pk
Aseptoform P
Solbrol P
Paseptol
Preserval P
Bonomold OP
Parasept
Propyl chemsept
Pulvis conservans
Lexgard P
Propyl Chemosept
Betacide P
Protaben P
Tegosept P
Propyl aseptoform
Nipagin P
Propyl Butex
Bayer D 206

3.1.1.4 CAS / EC number

CAS No 94-13-3, EC No 202-307-7

3.1.1.5 Structural formula

Formula: $C_{10}H_{12}O_3$



3.1.1.6 Empirical formula

Formula: $C_{10}H_{12}O_3$

3.1.2 Physical form

Colorless crystals or white powder or chunky white solid

3.1.3 Molecular weight

180.2 g/mol

3.1.4 Purity, composition and substance codes

3.1.5 Impurities / accompanying contaminants

The level of impurities varies according to the different batches of products used to perform the studies.

SCCS comment
A full report of the chemical characterisation of propylparaben in terms of purity, identity and impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown.

3.1.6 Solubility

In water, 463 mg/L at 20 °C (Source: PubChem)

3.1.7 Partition coefficient (Log Pow)

Log Pow = 3.04 (Experimental value) (Source: PubChem)

Property	Propylparaben
Molecular Formula	$C_{10}H_{12}O_3$
Molecular Weight (g/mol)	180.2 g/mol
Physical Form	Colorless crystals or white powder or chunky white solid
Stability	Stable in air and does not hydrolyse in hot or cold water or in acidic conditions. Above pH7, considerable hydrolysis occurs.
Boiling point (°C)	132°C at 1 mm Hg
Melting point (°C)	95-98°C
Solubility	less than 1 mg/mL at 12° C
Octanol:water partition coefficient ($logP_{o/w}$)	3.04
pH of saturated aqueous solution	pH: 6.5-7.0 (slightly acidic) in solution
Vapour pressure	$3.07x10^{-4}$ mm Hg at 25°C (estimated)
pKa	pKa = 8.5 (phenol) (estimated)

Table 1: Physico chemical properties of propylparaben (Source: PubChem)

3.1.8 Homogeneity and Stability

Stable in air and does not hydrolyse in hot or cold water, or in acidic conditions. Considerable hydrolysis occurs above pH 7 (Source: PubChem).

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

Dermal absorption *ex vivo*

In the previous safety evaluations of parabens by the SCCS/SCCP (SCCS/1348/10; SCCP/1017/06), a dermal absorption value of 3.7% (for parent paraben based on the available data for butyl paraben) was used. In this new submission, two recent studies with respect to dermal absorption of propyl paraben were included:

1) Geniès et al. (2019) conducted a study in which they compared the metabolism of propyl paraben in human skin versus pig skin *ex vivo*, taking account of the complete metabolic profile following application. For this study, human abdominal skin was obtained from six male and 34 female healthy donors (average age 41 ± 11 years), kept at 4°C and placed in culture within 3 hours of surgery.
The skin samples were dermatomed to a thickness of 450 ± 50 µm. Skin punches were seeded dermal side down in polycarbonate Transwell® inserts (4.16 cm^2 application area with an 8 µm pore size filter). Skin explants were incubated for 1 hour before application of the

formulation. The doses tested on pig and human skin were 2.4 nmol/cm^2 and 2.7 nmol/cm², respectively. The dosing volume was 10 µL/cm².
Analysis of the ring - labeled 14C radiolabeled chemicals and their metabolites in medium after 24 hours of incubation with pig and human skin was carried out by radio - high - performance liquid chromatography (LC) and a flow scintillating counting. The metabolites were identified by LC-electrospray ionization-HR mass spectrometry.
The results indicated that the parent compound plus metabolites detected in the receptor medium[4] after 24 hours following application of propylparaben to dermatomed pig and human skin respectively were 50.3 ± 1.1 and 56.0 ± 2.6 percent of the applied dose. Out of this, the total metabolites were 50.3 ± 1.1 and 55.8 ± 2.9 percent of the applied dose. This means that the vast majority of the applied dose was metabolised in human skin and only a small proportion (0.2 ± 0.2 % of the applied dose) remained in the form of propylparaben. In human skin, the predominant metabolite (42%) was p-hydroxybenzoic acid (pHBA) along with small amounts of propylparaben sulphate and unidentied metabolites, whereas in pig skin it was pHBA-glucuronides (12.9%) along with other unidentified metabolites. pHBA is not regarded as toxic and would, in humans, be rapidly cleared via urine by other conjugation pathways.

2) A cutaneous penetration study in which the absorption of 56 cosmetic ingredients, including propylparaben, was studied *ex vivo* in human skin was recently presented by Hewitt et al (2020).
In this study a set of seven in-line flow-through cells were used with a 1cm² application area. The receptor compartment of the diffusion cell was filled with 0.9% NaCl in water, supplemented with 1% [w/v] bovine serum albumin and 0.05% [v/v] gentamycin sulfate). Flow rate was adjusted to 1 mL/h.
The study was performed on frozen skin samples according to the OECD test guideline 428. All chemicals were exclusively tested as 14C-radiolabeled solutions. The radiolabeled chemicals were used as tracers and were mixed with label-free chemicals to achieve the final concentrations with a radiolabel concentration of 0.30 µCi/10 µL. The penetration of the applied dose (10 µL/ cm² or 0.30µCi/cm^2) was measured over 24 hours.
The overall recovery of propylparaben was of 95.04 ± to 97.1 ± 6.97 when applied in PBS and 85.03 ± 5.65 when applied in 100% ethanol. These data are in accordance with the acceptance criteria laid down in the SCCS Note of Guidance (SCCS/1602/18).
The dermal delivery (DD) (amount in the epidermis, dermis and receptor fluid [RF]) of propylparaben (parent and metabolites) ranged between 1.66 ± 0.23 and 1.83 ± 0.15 µg/cm2 when it was applied in phosphate buffered saline (PBS) and 0.33 ± 0.18 µg/cm2 when applied in 100% ethanol. These values correspond to 66.24 ± 10.31% to 75.66 ± 5.86 % of the applied dose, respectively in PBS and to 11.02 ± 5.97 % of applied dose in 100% ethanol.
Excluding propylparaben tested in ethanol, the cumulative amount detected in the RF for propylparaben applied in PBS resembled a hyperbolic profile, with lag times (t_{lag}) varying from 0.48 to 0.59. The cumulative amount reached a plateau at 8 hours, well before the end of the experiment (24h). For finite doses, the observation of a plateau indicates that a chemical cannot penetrate the skin. There maybe a couple of explanations for this: the chemical may have been entirely absorbed, or evaporated/precipated.

SCCS conclusion on dermal absorption of propylparaben
The Geniès et al. study (2019) is a dermal metabolism study, and as such is not a dermal absorption study as mentioned by the applicant. The conditions applied were not designed for determining dermal absorption. The skin samples were seeded, dermal side down, on inserts that were placed in multi-well plates containing 1.5ml culture medium per well, immersing the samples in culture medium. The data reported were **not** obtained after dermal absorption of the compound applied, but show that propyl paraben can be completely metabolised when staying long enough in contact with skin enzymes at a suitable pH and incubation

[4] Medium samples were taken from the medium samples after 24 hours incubation

temperature. Therefore, this study cannot be used to derive dermal absorption values for propylparaben.

In the Hewitt et al. study (2020), an amount of 10 µL/ cm² of propylparaben was applied either in PBS or in 100% ethanol. In the case of application in PBS, the cumulative graph of the amount of propylparaben absorbed versus time shows a hyperbolic form (in sharp contrast to the linear form after application in ethanol) pointing to the fact that after a limited time of 8 hours, the applied dose of propylparaben had already depleted. Therefore, it is not possible to derive a dermal absorption value from this study under the conditions indicated in the SCCS Notes of Guidance, 10th Revision.
In the absence of a dermal absorption study carried out in accordance with the SCCS Notes of Guidance (10th Revision), the SCCS will use the same dermal absorption value (3.7%) as used in the previous Opinion (SCCS/2013) for the calculation of MoS.

3.2.2 Other studies on toxicokinetics

Toxicokinetics studies on propylparaben have been extensively reviewed and evaluated in previous Opinions (SCCS/1348/10 and SCCS 2013).

From SCCS/1446/11 and SCCS (2013)
The ADME properties and kinetics of propylparaben in rats have been studied comprehensively, via oral and dermal routes by Aubert et al. (study report 2009; published in 2012) and via the oral route in humans (Ye et al., 2006). In rats it was found that parabens are metabolized in a fast and complete way into p-hydroxybenzoic acid (pHBA), which is considered to be the main inactive metabolite (SCCS/1446/11).
Total propylparaben appeared to be eliminated very rapidly following oral administration as suggested by the half-life values observed at 10 and 100 mg/kg which were 0.789 and 0.970 hours, respectively. The half-life for total parabens at the dose of 1000 mg/kg was estimated to be about 3.5 hours.
After dermal administration, it may be absorbed to a great extent, but the majority is available as inactive metabolite(s) and not as the active parent form, and this is rapidly eliminated. Blood analysis only showed the presence of pHBA.

In human
A research study was performed in human subjects by Unilever (2003) under the European guidelines of good clinical practice (GCP) and with appropriate ethical approval, propylparaben was used as a comparator compound in an oral toxicokinetic evaluation.
This study indicated that following oral exposure, propylparaben is very rapidly absorbed, metabolised and cleared within 4-6 hours via urinary excretion in humans. These data support the conclusion that rat and human oral toxicokinetics are similar in outcome, and even though there may be differences in quantitative levels of different Phase 2 metabolites (of PP and pHBA), the outcome of rapid and complete clearance within a few hours by multiple Phase 2 metabolism pathways is the same.

In a more recent study, Shin et al (2019) have investigated the pharmacokinetic profile of propylparaben in humans after oral administration. Deuterated d_4-propylparaben (98.8% purity) was used as the test material. Twelve healthy adult volunteers were recruited for this study in Korea. Only male volunteers were chosen to exclude potential variations according to sex. For dosage, isotope-labeled analog (d4-labeled) propylparaben was used due to the known background exposure to parabens. The administered oral dose in this study was 0.6 mg/kg/day. Propylparaben was completely eliminated from the blood 48 hours after oral dosing in humans, which is also consistent with findings in the rat (Aubert et al., 2009).

Conclusions on oral toxicokinetics and metabolism
According to the Applicant, propyl paraben is rapidly and completely absorbed, metabolised to a large extent, and cleared within 4-6 hours via urinary excretion in humans. Also, irrespective of the species studied, the metabolism of parabens results in rapid hydrolysis by carboxylesterases to the principal metabolite para-hydroxybenzoic acid (pHBA). pHBA may be further conjugated with glycine, glucuronic acid and/or sulphate, although there are quantitative differences in the degree of conjugation in rat and human.
Excretion of propylparaben and metabolites is principally through urine, and is fast with more than 90% of the propylparaben dose excreted within 24 h post-dosing in both rat and human. This may indicate that parabens do not accumulate in the body.

Overall SCCS conclusions on toxicokinetics of propylparaben following oral ingestion
The available information from a number of *ex-vivo*, *in vivo*, and human studies indicates that propylparaben is rapidly absorbed following oral ingestion, metabolised, and eliminated through unrinary route (terminal half-life: 2.9 h). This indicates that accumulation of propylparaben in the body may not be of a concern for consumer safety.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

According to the Applicant, propylparaben has been used widely and safely as a preservative (to prevent the growth of harmful microbes that could be a cause of adverse health effects) in cosmetic, food and pharmaceutical preparations around the world for more than 50 years.

3.3.1.1 Cosmetics Use

The use of propylparaben in cosmetics is regulated in Annex V (List of Preservatives Allowed) in the EC Cosmetic Products Regulation EC 1223/2009 of the European Union. The latest update to Annex V relating to parabens was published on 5 August 2019.
Propylparaben can maximally be used in any cosmetic product up to 0.14% (alone, as acid) or up to a max of 0.14% (as acid) as the sum of the individual concentrations of butyl paraben and propylparaben when used together as a binary mixture in the same product. The maximum concentration of propylparaben in the context of its use with methyl or ethyl paraben is 0.8% (as acid).

Given the concentration in the regulation is cited 'as acid', molecular weight conversion for propyl ester equivalent would be 0.183% use in cosmetic products.

3.3.1.2 Food use

According to the Applicant, under FDA regulation, propylparaben is generally recognized as safe (GRAS) when used as a chemical preservative in foods, with a use limit of 0.1%. Propylparaben is not approved for use as a preservative in EU foods (EFSA 2004; Directive 2006/52/EC). At the time of the last evaluation by EFSA, 15 years ago, there was insufficient data on propylparaben to allow an ADI to be set and there were concerns at the time over male reproductive effects from a limited study by Oishi (2002) that have since been robustly overturned. As a significant amount of new data has been generated since 2004, a review by EFSA for propylparaben would in principle be warranted for use in food (Snodin, 2015).

3.3.1.3 Pharmaceutical use

According to the Applicant, parabens are used widely as antioxidant excipients in pharmaceuticals and contribute to everyday exposure (Dodge et al 2015). Based on a 'reflection paper' from the European Medicines Agency 2015 (EMA, 2015), oral pharmaceutical formulations include combinations of methyl paraben and propylparaben with concentrations generally ranging from 0.015 to 0.2% for methylparaben and 0.02% to 0.06% for propylparaben. Based on current posology of medicines containing methyl paraben and propylparaben, concentrations of 0.2% and 0.06% would correspond to maximal intakes of approximately 140 mg/day and 50 mg/day, respectively.
It is to be noted that risk assessment and evaluation of endocrine disrupting properties for parabens in pharmaceuticals by the European Medicines Agency (EMA) has drawn upon all available *in vivo* data that has been generated for propylparaben.
The EMA concluded that there were no labelling requirements for parabens on pharmaceutical products 'due to the absence of sufficient clinical evidence of parabens-related effects in humans'. A permitted daily exposure level of 2 mg/kg/day was calculated as applicable to both adults and children

3.3.2 Calculation of SED

3.3.2.1 Tier 1 Exposure Assessment

SCCS 10th Notes of Guidance Approach – Deterministic Consumer Aggregate Exposure Assessment using Maximum % Levels

A value for systemic availability of parent propyl paraben after dermal administration of 3.7% has been used for all products in these calculations, except for oral care (mouthwash and toothpaste) where a SCCS default of 100% absorption is used. Until a properly conducted dermal absorption and toxicokinetic study in humans will allow the assignment of a more scientifically solid value, the SCCS will use a dermal absorption value of 3.7% in its MoS safety calculations. This enables a systemic exposure dose (SED) of parent propyl parabens via the dermal route to be calculated in mg/kg/day and the resulting SED can be used to calculate a Margin of Safety for each product.

Tier 1 approaches use a deterministic approach to aggregate exposure assessment which is a worst-case highly conservative approach that does not necessarily represent real life. Tier 2 approaches represent a more realistic approach, using probabilistic exposure models and the use of product occurrence data for the substance.

Two Tier 1 scenarios are presented below:
Tier 1 - Scenario 1A – Based on the entry in Annex V of the Cosmetics Regulation (see section 3.3.1.1), a maximum value of 0.183% propylparaben is allowed in any cosmetic product. This value is used for the maximum % level of propylparaben in each of the product types to calculate the total external exposure dose to propylparaben (in mg/kg/day) from each product for adults (see Table 2).
Tier 1 - Scenario 1B – In 2017, Cosmetics Europe commissioned Creme Global to assist in performing a large industry-wide survey on propylparaben use in cosmetic products across Europe (see Annex 1 by Creme Global, 2019). The product use concentration survey data from 90 companies in 28 EU member states (plus Norway, Iceland, Lichtenstein, Switzerland and Monaco) enables the refinement of the consumer exposure to be closer to illustrating current real life exposure but still be highly conservative. Values for the maximum % level of propylparaben in each of the product types from the survey are used to calculate the total dermal external exposure dose to propylparaben (in mg/kg/day) from each product for adults (see Table 3).

Table 2: Scenario 1A – Tier 1 - Systemic exposure dose (SED) calculation of propylparaben using regulatory maximum 0.183% inclusion level for all product types. Values are calculated according to the SCCS 10th notes of guidance approach to calculating worst-case aggregate exposure on the basis of deterministic additive methods assuming 100% occurrence and simultaneous exposure to all products.

Product	Maximum use (w/w%) in the finished product	Calculated relative daily exposure to product [1] (mg/kg bw/day)	Total dermal external exposure (mg/kg bw/day)	Calculated SED[2] (mg/kg bw/day)
Bathing and Showering				
Shower gel	0.183	2.79	0.005	0.000185
Hand wash	0.183	3.33	0.006	0.000222
Hair care				
Shampoo	0.183	1.51	0.003	0.000111
Hair conditioner	0.183	0.67	0.001	0.000037
Hair Styling	0.183	5.74	0.01	0.00037
Skin care				
Body lotion	0.183	123.2	0.226	0.008362
Face cream	0.183	24.14	0.044	0.001628
Hand cream	0.183	32.7	0.06	0.00222
Make-up				
Liquid foundation	0.183	7.9	0.015	0.000555
Lipstick, lip salve	0.183	0.9	0.002	0.000074
Make-up remover	0.183	8.33	0.015	0.000555
Eye shadow	0.183	0.33	0.001	0.000037
Mascara	0.183	0.42	0.001	0.000037
Eyeliner	0.183	0.08	0.0001	0.0000037
Deodorants				
Non-spray	0.183	22.08	0.04	0.00148
Oral care				
Toothpaste[3]	0.183	2.16	0.004	0.004
Mouthwash[3]	0.183	32.54	0.06	0.06
Aggregate			**0.492**	0.0798767

1. According to values in Table 2A and 2B on page 21-22 of the SCCS notes of guidance (10th revision) (2018)
2. Total dermal external exposure x 3.7% dermal penetration
3. 3.7% dermal penetration applied; SCCS default 100% absorption.

Table 3: Scenario 1B – Tier 1 - Systemic exposure dose (SED) calculation of propylparaben using 2017 Cosmetics Europe survey data maximum % levels for product types. Values are calculated according to the SCCS 10th notes of guidance approach to calculating worst-case aggregate exposure on the basis of deterministic additive methods assuming 100% occurrence and simultaneous exposure to all products.

Product	Maximum use (w/w%) in the finished product	Calculated relative daily exposure to product [1] (mg/kg bw/day)	Total dermal external exposure (mg/kg bw/day)	Calculated SED[2] (mg/kg bw/day)
Bathing and Showering				
Shower gel	0.175	2.79	0.005	0.000185
Hand wash	0.18	3.33	0.006	0.000222
Hair care				
Shampoo	0.18	1.51	0.003	0.000111
Hair conditioner	0.183	0.67	0.001	0.000037
Hair Styling	0.183	5.74	0.01	0.00037
Skin care				
Body lotion	0.183	123.2	0.226	0.008362
Face cream	0.183	24.14	0.044	0.001628
Hand cream	0.18	32.7	0.06	0.00222
Make-up				
Liquid foundation	0.183	7.9	0.015	0.000555
Lipstick, lip salve	0.18	0.9	0.002	0.000074
Make-up remover	0.18	8.33	0.015	0.000555
Eye shadow	0.183	0.33	0.001	0.000037
Mascara	0.183	0.42	0.001	0.000037
Eyeliner	0.183	0.08	0.0001	0.0000037
Deodorants				
Non-spray	0.15	22.08	0.033	0.001221
Oral care				
Toothpaste[3]	0.1	2.16	0.002	0.002
Mouthwash[3]	0.05	32.54	0.016	0.016
Aggregate			**0.44**	0.0336177

1. According to values in Table 2A and 2B on page 21-22 of the SCCS notes of guidance (10th revision) (2018)
2. Total dermal external exposure x 3.7% dermal penetration
3. 3.7% dermal penetration applied; SCCS default 100% absorption.

The deterministic approaches in Scenarios 1A and 1B assumes 100% occurrence of propylparaben in all cosmetics products used by an individual in a day, which of course is not a realistic scenario.
Considering children's exposure specifically, Gosens et al (2014) recently estimated children's external exposure dose to be 1.05 mg/kg/day, using a lower body weight than for an adult and different product use considerations than for a standard SCCS evaluation.

3.3.2.2 Tier 2 exposure assessment

Tier 1 approaches above use a deterministic approach to aggregate exposure assessment which, as we have highlighted, is a worst-case highly conservative approach that does not represent real life, but is a simple place to start. Tier 2 approaches represent a more realistic approach, using probabilistic exposure models and the use of product occurrence data for the substance.
According to the Applicant, a simultaneous analysis of probabilistic exposure modelling and analysis of occurrence data was being performed by Crème Global. These data are in Annex 1 to their report (Annex 1 Crème Global 2019 report for propyl paraben), and should be considered alongside this evaluation, as these exposure data build on and refine the risk assessment. The most appropriate technical approach to performing an exposure assessment are such Tier 2 assessments.

3.4 TOXICOLOGICAL EVALUATION

The toxicology evaluation is focused specifically on the data available for propylparaben. Where there are data gaps, and where scientifically appropriate, reference to other structurally related parabens e.g. methyl, ethyl, and butyl paraben were used.

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

From the Applicants dossier
In silico predictions in the OECD Toolbox v4.0 and DEREK nexus v.6.0.1 profiling gives no evidence for skin irritation of propylparaben or any of the n-alkyl parabens.

A study was performed according to OECD 404 (Unnamed, 1983 study as cited in ECHA REACH dossier). The test substance was applied (0.5 g test substance, moistened with water) on the clipped backs of three male New Zealand White rabbits and covered with a semi-occlusive dressing. After 4 h, the dressing was removed and the treated skin sites were cleaned with water. Erythema and oedema formation were assessed 1, 24, 48 and 72 h and 7 d after removal of the test substance using the Draize scoring system. Dermal application of the test substance did not result in erythema or oedema in any of the animals tested at any observation time point.

In another study, the skin irritation properties of propylparaben were investigated in an old study, which used a protocol similar to OECD 404 (Sokol, 1952 as summarised in CIR 1984). A 10% solution of propylparaben in hydrophilic ointment base was applied to the skin of rabbits. No noticeable skin irritation was detected after 48 h.

Two other studies were performed in 1979: one with a product containing 0.2% Propylparaben produced a minimal irritation with a PII of 0.5 (LEBERCO LABORATORIES, 1978) and the

second one with a product containing both 0.2% Methylparaben and 0.1% Propylparaben was minimally irritating with a PII of 0.5. (CTFA – April, 1979 – Code N° 2-7-15).

To strengthen the assessment of skin irritation, it is possible to consider the reliable data for methyl paraben and ethyl paraben which are structurally similar to propylparaben. Therefore, read-across was performed for this endpoint in REACH based on an analogue approach. The target substance and the source substances form a homologue series of esters of p-hydroxybenzoic acid and differ only in the length of the alkyl side chain, which contains 1, 2 or 3 carbon atoms for methyl paraben, ethyl paraben and propylparaben, respectively. Methyl paraben was investigated for skin irritation using a modified Draize test (Anonymous, 1976; ECHA REACH dossier). Methyl paraben (0.1 mL) was applied to the shaved skin sites of nine female rabbits and covered with an occlusive dressing for 24 h. The observation period after removal of the dressing was 72 h with reading time points at 24 and 72 h. Based on the primary dermal irritation index of 0.67, methyl paraben was found to be not irritating to the skin. The skin-irritating properties of ethyl paraben were examined in a manner similar to that used in OECD 404 (ECHA REACH dossier). The test substance was applied on the clipped backs of three male New Zealand White rabbits and covered with a semiocclusive dressing. After 4 h, the dressing was removed and the treated skin sites were cleaned with water. Erythema and edema formation were assessed 1, 24, 48 and 72 h and 7 d after removal of the test substance using the Draize scoring system. No erythema and edema formation were observed in any animal. Under the test conditions, ethylparaben was not irritating to the skin. Conclusion: Accoding to the applicant, the available data show that propylparaben is not or only minimally irritating.

SCCS comment
Based on the available information, the SCCS does not consider propylparaben as a skin irritant.

3.4.1.2 Mucous membrane irritation / eye irritation

In vivo
A study was performed according to OECD 405 (2012 study as cited in ECHA REACH dossier). The instillation of propylparaben into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae, sclerae and ocular discharge. These effects were reversible and were no longer evident 7 days after treatment, the end of the observation period for all animals. No abnormal findings were observed in the cornea or in the iris light reflex of any animals at any of the examinations. No corrosion was observed at any of the examinations. No staining of the treated eyes by the test item was observed. No test item remnants were observed in the treated eyes of any animal at any examination. No clinical signs were observed.

Thus, the test item did not induce significant or irreversible damage to the rabbit eye. The mean score was calculated separately for each animal across three scoring times (24, 48 and 72 hours after instillation) for corneal opacity, iris light reflex, redness and chemosis of the conjunctivae, respectively. The individual mean scores for corneal opacity and iris light reflex were 0.00 for all three animals. The individual mean scores for the conjunctivae were 1.00, 2.00 and 1.67 for reddening and 0.00 for chemosis for all animals, respectively.

In vitro
Eye corrosion properties of propylparaben were investigated in a Bovine Corneal Opacity and Permeability Test according to OECD 437 and GLP (Heppenheimer, 2012). A solution of 20% (v/v) propylparaben in physiological saline was applied to three bovine corneas for 240 minutes. After further incubation of the corneas with fluorescein for 90 minutes, the permeability of the corneas was determined spectrophotometrically. The mean *in vitro*

irritation score was determined to be 13.03. Under these *in vitro* test conditions, the test substance was not corrosive to the eyes.

According to the applicant, based upon the referred classification criteria (Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008), propylparaben is considered to be "not irritating" to the rabbit eye and not classified with respect to eye irritation.

SCCS comment
Based on the available information, the SCCS does not consider propylparaben as an eye irritant.

3.4.2 Skin sensitisation

Animal data
Propylparaben was examined in several reliable local lymph node assays (LLNA) in mice according to OECD 429 (Basketter et Scholes, 1992; Basketter et al., 1994). Groups of mice were treated by topical applications of 5, 10 or 25% propylparaben in acetone/olive oil (4:1, v/v). Stimulation indices for propylparaben were in all dose groups < 3. The positive controls were valid. Under the test conditions, propylparaben was not considered to be a skin sensitizer.
Moreover, propylparaben was examined in a Guinea Pig Maximization Test according to OECD 406 (Basketter et Scholes, 1992). After intradermal and epicutaneous induction treatment with 0.5% and 25% propylparaben and a challenge application of 10% propylparaben, no positive response was observed at the 24 and 48 h reading time point in any animal. The positive controls were valid. Under the test conditions, propylparaben was not considered to be a skin sensitizer.

Human data
Fransway (2019) reviewed a significant body of clinical evidence for parabens over the past 3 decades and demonstrated that in large populations, parabens have a very low incidence of reported skin sensitisation (<1%). Given the widespread use of parabens in consumer products, exposure does not often lead to skin skin allergy.

In line with this, complaint experience data reported by CIR (2019) showed that parabens can induce contact allergy, but this occurred mainly after application of paraben-containing medicaments to damaged or broken skin. Even when applied to patients with chronic dermatitis, parabens generally induce sensitisation in less than 3 percent of such individuals. Of 27,230 patients with chronic skin problems, 2.2 percent were sensitized by preparations of parabens at concentrations of 1 to 30 percent. Contact allergy is not elicited by cosmetics containing parabens in subjects that were sensitised due to the use of paraben-containing medications.
In a more recent study, the prevalence of allergic contact dermatitis assessed in patch tests with paraben mixture was analysed. The European Surveillance System on Contact Allergies (ESSCA) network collected patch test data from 12 European countries (Gimenez-Arnau AM et al, 2017). Of the 52,586 patch tests conducted during the study period, parabens yielded less than 1% positive reactions.
According to the applicant, based on animal and human data, parabens are not considered as a skin sensitisers.

SCCS comment
The human data show that parabens in general (patch-tested as a mixture containing propylparaben), despite their widespread use, rarely cause contact allergy. Contact allergy in humans is observed mostly in patients who apply medical creams on damaged or irritated

skin. The available animal data show that propylparaben is not a skin sensitiser. Therefore, the SCCS does not consider propylparaben as a skin sensitiser.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

An acute oral toxicity study with a (cited by ECHA REACH Dossier) single propylparaben dose of 5000 mg/kg bw by gavage was conducted in groups of 5 male and 5 female rats. During the observation period of 14 days after application, no mortality occurred and no clinical signs were noticed. Necropsy of animals chosen on a random basis revealed no test substance related findings. Therefore, the LD50 is >5000 mg/kg bw for male and female rats.

3.4.3.2 Acute dermal toxicity

There are no animal studies covering the acute dermal toxicity of propylparaben.

3.4.3.3 Acute inhalation toxicity

There are no animal studies covering the acute inhalation toxicity of propylparaben.

Applicant's conclusion on acute toxicity: propylparaben is not acutely toxic.

3.4.4 Repeated dose toxicity

3.4.4.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

A combined repeated dose toxicity study with the reproduction/developmental toxicity screening test was performed (OECD 422) (Harlan et al. 2012 - as reported in full detail in the REACH dossier for propylparaben).
Propylparaben was administered to 11 rats per sex and group in doses of up to and including 980 and 1076 mg/kg bw/d in the diet to males and females. Test item was administered to male rats for 28 days and to female rats for 14 days prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum.
No signs of adverse toxicity were observed in males or females at any dose level following repeat daily dosing. At the dose level of 1076 mg/kg/d ppm in males, reduced body weight gain was noted in the absence of statistically significant changes in absolute body weights and an increase in plasma triglicerydes concentration was noted in the absence of any histopathological or other changes related to the finding. No further test item-related effects were noted in males or females at any dose level.

SCCS comment
The SCCS concludes that respective NOAEL values for general toxicity and reproduction (fertility) of 980 mg/kg bw/d for males and 1076 mg/kg bw/d for females should be considered for propylparaben.

3.4.4.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral route

There is no pre-2013 90-day repeat-dose oral toxicity study available that can be used for the purposes of a cosmetics safety dossier ***according to the applicant***.

An oral study was carried out in 2018 and can be reported in this Opinion. In a 90-day OECD 408 compliant study (2018, ascited in the ECHA REACH dossier) the test item was administered daily in graduated doses to 3 groups of test animals, while control group received 1% aqueous hydroxyethyl-cellulose, the vehicle used in this study. The 4 groups were comprised of 10 male and 10 female Wistar rats. Control, low-, mid- and high-dose group rats received the dose at 0, 100, 300 and 1000 mg/kg/day respectively, as a repeated dose at the dose volume of 5 mL/kg. All animals in the recovery group were observed for a period of 28 days following the last administration.
There were no adverse effects or effect of toxicological relevance recorded in this study. A NOAEL of 1000 mg/kg body weight/day was derived from that study.

SCCS comment

This study was performed after the animal testing ban for cosmetics but was ***obtained and*** accepted by the SCCS because it was performed to comply with the requirements under REACH regulation. (https://echa.europa.eu/documents/10162/f8e15bd5-5139-0e1a-8b55-c7136afd4ccf).

Dermal route

A dermal repeated-dose toxicity study performed in 1981 is reported in the REACH dossier for propylparaben (in 2018 - cited in ECHA REACH dossier). A medicated lotion containing 0.3% propylparabens (12.4 mg/kg/day) was applied daily to the skin of male (n=10) and female (n=10) Sprague Dawley rats for 13 weeks. No mortality occurred during the study period. Sporadic minimal to moderate skin irritation at the treated skin sites and a brown discoloration of the fur immediately around the treated skin site was noted. Additionally, in a number of animals, significant thinning of the fur around the treatment site was observed. Hyperactivity just before and immediately after dosing was noted in a few animals and some problems regarding equilibrium were observed. Lower body weight and a significant decrease in body weight gain was observed for males. The body weight and body weight gain for females was consistent with the values of the concurrent control group.
The determined blood parameters are in the range of the values of the untreated control group, with exception of the mean corpuscular volume (MCV), which was slight but significantly elevated in females after 7 and 13 weeks.
Most of the tested serum chemistry values were comparable to that of the untreated control. After 7 weeks, a decreased glucose value in females was noticed, which was in the range of the untreated control after 13 weeks. At the end of the study period, increases were observed in male urea nitrogen values and in female alkaline phosphate values.
No treatment-related changes were noticed in the urinalysis after 7 and 13 weeks. In males, decreases in absolute brain, heart, liver, spleen and adrenal weights and increased relative brain, lung and testes weights were observed. In females, absolute liver and relative heart and liver weights were increased and absolute and relative spleen weight were decreased. No treatment-related abnormalities were noticed at necropsy.
Significant histopathological changes were limited to the treated skin site. All observations were regarded as non-adverse and not treatment related and the only dose tested (12.4 mg/kg/day) is stated as a NOAEL from this study.

SCCS comment

In the REACH dossier, the reliability of this study is considered as low because the test substance was one of the ingredients of a formulation (reliability 4 – not assignable). Therefore this study has not been used by the SCCS in the current safety assessment.

3.4.4.3 Chronic (> 12 months) toxicity

From the applicant dossier.
CIR 2008 described an old chronic toxicity study (Matthews, 1956) for propylparaben, in boths rats and dogs, as follows: a chronic oral toxicity study in which propylparaben was incorporated into the diets at 2% or 8% and the diets fed to groups of 24 rats for 96 weeks. Negative controls were included in the study. Rats, especially the males, fed the 8% propylparaben diet had decreased weight gain in the early part of the study. At 2% of the diet, propylparabens exerted no toxic effect. Rats killed for necropsy upon completion of the feeding test had no treatment related abnormalities.
These authors also dosed weanling dogs as follows: six dogs, 1000 mg/kg/day propylparaben for 378 to 422 days; and three dogs, 500 mg/kg/day propylparaben for 318 to 394 days. Two untreated dogs served as a control group. All dogs were killed for necropsy upon completion of the feeding. No toxicity to the parabens was observed. All animals were in excellent condition throughout the experiment. All tissues were normal.

SCCS comment
Although this study is old, and from a secondary report from the CIR 2008, it corroborates a NOAEL of 1000 mg/kg/day.

3.4.5 Reproductive toxicity

In its Opinion from 2013 (SCCS/1514/13), the SCCS derived a NOEL for butylparaben of 2 mg/kg/day in male juvenile rats from a study by Fisher (1999).

3.4.5.1 Fertility and reproduction toxicity

Fertility and reproduction toxicity studies have been extensively reviewed and evaluated in previous Opinions (SCCS/1348/10 and SCCS 2013). In order to confirm or challenge and further characterize the effects by Oishi 2002, *in vivo* GLP-compliant studies on the toxicokinetics and reproductive toxicity of propylparaben in male juvenile Wistar rats starting from PND 21 were conducted in 2010-2012 (Ricerca Biosciences 2011, 2012a, 2012b, 2012c, 2012d). The findings of these studies were published in Gazin et al (2013) and previously reviewed by SCCS (2013) and EMA (2015). The No-Observed-Adverse-Effect-Level (NOAEL) can be derived at 1,000 mg/kg/day from this study.

The SCCS obtained and reviewed the study by Sivaraman et al (2018).The study by Sivaraman et al (2018) was designed to meet the requirements of the European Medicines Agency (EMA), Committee for Human Medicinal Products (CHMP), the United States Food and Drug Administration (US FDA) Guidance Document and the ICH S3a guidelines. The studies were also conducted in compliance with GLP.
Two separate studies on the safety of propylparaben were conducted to assess the potential estrogen-mimetic effects on:
(1) reproductive development and function in male and female rats when administered on PNDs 4 to 90; and

(2) uterine weights in immature female rats when administered on PNDs 21 to 23.
A total of 34 time-mated Sprague-Dawley female rats (F0 generation) were received from a breeder. The dams were used to produce the F1-generation litters and for the cross-fostering/nursing of litters. The F1 generation was the experimental population. On PND 3, the litters of the dams were culled to 8 pups each (4/sex/litter, where possible), then assigned to cross-fostered litters (4/sex/litter; where possible) using a randomization procedure such

that no more than one sibling from a given sex was assigned to a cross-fostered litter and no pup was assigned to its biological mother.

Results:
No treatment-related clinical signs or treatment related effect on the body weight at 10 or 100 mg/kg/day.
No propylparaben-related changes/effects were observed in the age of vaginal patency in females or preputial separation in males at any dose, on preputial separation in males (day of separation ranged from PND 42–43), on mating or fertility indices, mean number of days to mating, or the conception rate of the treated females paired with untreated males, on estrous cyclicity, on length of gestation/gestation index, duration of parturition, sex ratio (% males), number of implant scars, or group mean numbers of live births. No malformed pups were observed at any dose. No clinical signs were observed in F2 generation pups, and no effects were recorded on viability index on PND 4 (98.6–100%), nor on mean litter weights on PND 0 or 3.
No effects were observed on mating or fertility indices, mean number of days to mating, or conception rate of naïve females paired with PP-treated males, on the numbers of corpora lutea, implantation sites, live embryos, dead embryos, early resorptions, or pre- and postimplantation losses in naïve females paired with PP-treated males. No propylparaben-related reproductive organ weight changes were noted at end-of-dose or end-of-recovery necropsies and no gross findings or microscopic findings were recorded at the end-of-dose or end-of-recovery necropsies.
No treatment-related changes in hematology, coagulation, serum chemistry, or urinalysis parameters were noted.
The No-Observed-Adverse-Effect-Level (NOAEL) can be derived at 1,000 mg/kg/day from this study.

An extended One-Generation Reproductive Toxicity Study was performed after oral administration in male and female Wistar Rats at dose levels of 100, 300, and 1000 mg/kg body weight day (day (Clariant GMBH; 2019)

General toxicity:
There were no mortalities/morbidities related to the propylparaben treatment.
There were no clinical signs of toxicological relevance observed in the treatment groups. Body weight and food consumption were not affected by treatment. At termination no effects on clinical pathology (hematology, blood coagulation, clinical biochemistry and urinalysis) were observed in parental generation or in the cohort 1A (F1 generation – Treatment from weaning to week 13 of age (10 weeks treatment)). Furthermore, no test-item related gross pathological findings, effect on organ weight or histopathological findings were observed in the study.

Developmental and Reproduction toxicity:
There were no considerable differences in the length or sequence of oestrous cycle stages, duration of precoital interval or the duration of gestation of the parental generation and cohort 1A between the treatment groups and the control group. There were no signs of abortion or premature delivery, in litter parameters, i.e. number of still births, runts, total number of pups, sex ratio, number of live pups, weight of pups or survival index. Corpora lutea, implantation sites, percent preimplantation loss and post implantation loss in parental generation and cohort 1B were unaffected by the treatment. There was no toxicological effect of the test item on reproductive indices (male mating index, female mating index, male fertility index, female fertility index, gestation index and live birth index) in parental generation and in cohort 1B (F1 generation – Treatment until weaning of the F2, or until termination of the study (week 20-25)). No test-item related external findings were observed in the pups of this study. There was no toxicological effect on anogenital distance and nipple

retention and sexual maturity parameters (i.e. vaginal opening and balano-preputial separation).
There were no effects on sperm motility and morphology or on the sperm head count of the parental generation and cohort 1A males. The test item had no effect on serum T4 and TSH levels in parental generation (males and females) or, in cohort 1A animals, on PND4, PND 21 and at adult age. There was no indication of endocrine disruptive properties of the test item in this study.

Neurotoxicity:
There was no test-item related effects on learning and memory, auditory startle response, clinical and functional observations and motor activity. Histopathologically, there were no indications of morphological abnormalities in the brain. No morphometric changes were observed in dose groups compared to controls.

Immunotoxicity:
There was no sign of immunotoxicity in this study.

According to the applicant, the NOAEL of the study for male reproductive endpoints is 1000 mg/kg bw/day for the treatment period of 8 weeks. The present study did not confirm the effects on the reproductive functions reported by Oishi (2002a) and is regarded of sufficient quality to overturn the findings by Oishi, which is a study that suffers from numerous limitations as mentioned above.

SCCS comment
The SCCS considers that the results of these two additional studies support the derivation of NOAEL for reproductive endpoints to be at 1000 mg/kg bw/day.

3.4.5.2 Developmental Toxicity

In a Prenatal Developmental Toxicity study according to OECD 414 and GLP, propylparaben was administrated by oral gavage to 52 males and 104 females (cited in ECHA REACH dossier 2018). The doses evaluated were 0, 100, 300 and 1000 mg/kg body weight/day. No mortality was observed in the study and there were no clinical signs of toxicological relevance observed in the treatment groups.
The body weight, food consumption, prenatal, litter data and gross pathology of terminally sacrificed females remained unaffected in the treatment groups when compared to the controls. Furthermore, no treatment-related and toxicologically relevant external, visceral or craniofacial findings were observed in the high dose group and other treated groups. Findings of reduced ossification of some bones and few other skeletal findings were well within the historical control data range for this strain of rats and not considered to be a substance related effect. Generally delayed ossification is not regarded to persist postnatally and not associated with long-term consequences on survival, general growth and development and therefore is not considered to be adverse.

No effects of propylparaben on females and foetuses were found at dose levels up to 1000 mg/kg body weight/day. The NOAEL for both maternal toxicity and foetal toxicity is considered to be 1000 mg/kg body weight/day in this study.

<u>Conclusions from reproductive and developmental studies:</u>
It can be concluded from the Gazin et al (2013) study and as supported by an OECD guideline 422 study (Harlan, 2012), an OECD guideline 414 (cited in ECHA REACH dossier 2018), an EMA (CHMP), US FDA and the ICH S3a guidelines (Sivaraman et al, 2018) and Clariant GMBH (2019), that the NOAEL for reproductive and developmental effects in males and females with

propylparaben is the top dose tested at 1000 mg/kg. Given there are no effects and no dose response, a BMD could not be calculated and therefore the NOAEL value can be used as the toxicological point of departure (POD).

SCCS conclusion
Based on the studies performed after the publication of previous Opinion (SCCS/1514/13), the SCCS agrees that reproductive toxicity, neurotoxicity and immunotoxicity data (in rats) suggest a NOAEL of 1000 mg/kg/day.

3.4.6 Mutagenicity / genotoxicity

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

From the applicant dossier:
Available *in vitro* data for mutagenicity and genotoxicity for propylparaben are presented in Table 4.

Table 4: *In vitro* assays for propylparaben

Methods	Test Article	Metabolic activation	Results	Reference
Bacteria				
S. typhimurium strains TA100, TA98, TA1535, and TA1537	10 to 2000 µg/plate	Aroclor 1254-induced rat liver microsomal enzymes (S9)	Non mutagenic, with or without metabolic activation	McCann et al, 1975
S. cerevisiae strain D-4 and in S. typhimurium strains TA1535, TA1537, and TA1538	In plate tests, 0.075% PP was added to cultures. In suspension tests, 0.025% to 0.15% PP was used.	Presence and absence of mouse, rat, and monkey liver, lung, and testes homogenates	Non mutagenic, with or without metabolic activation	Brusick (Litton Bionetics), 1975
S. typhimurium strains TA100 and TA98, as well as E. coli strain D-2	PP in dimethyl sulfoxide (DMSO)	PCB-induced rat liver microsomal enzymes	Non mutagenic, with or without metabolic activation	Sugimura et al, 1976
Salmonella typhimurium TA98, TA100, TA102, TA1535 and TA1537 according to OECD guideline 471	0.01, 0.03, 0.10, 0.32 and 1 mg PP/plate in DMSO	1 mL of rat liver S9 homogenate was mixed with 9 mL cofactor	Not mutagenic	EU REACH dossier (2018)
Mammalian				
CHO-K1 cells Non OECD guideline study.	0, 0.5, 1, 1.5, 2, or 2.5 mM	-	Statistically-significantly elevated a) indices of DNA fragments in	Tayama et al. (2008)

			cells 1h with PP (≥1 mM). b) SCEs/cell in cells incubated with PP (≥1.5 mM) for 3h. c) CAs/cell in cells incubated with PP (≥1 mM) for 3h.	
Vero cells (derived from African green monkey kidney) Non OECD guideline study.	0, 50, 200, 300, 400, or 500 µM PP	-	4-fold decrease in % of mitotic cells at 500 µM, compared with control. Statistically-significant induction of DNA DSBs (2-fold compared to control) at 500 µM	Perez Martin et al. (2010)
Human peripheral lymphocytes. Non OECD guideline study.	10, 25, 50, and 100 µg PP/mL for 24 and 48 h	-	PP increased MN and Chromosomal aberration frequency in a concentration-dependent manner at 24 and 48 h and did not induce Sister Chromatid Exchanges	Güzel Bayülken & Ayaz Tüylü (2018)
OECD Guideline 476 (In Vitro Mammalian Cell Gene Mutation Test) in Chinese hamster lung fibroblasts (V79)	7, 14, 28, 56, 93, 112, 224, 336, 448 µg/mL using DMSO as a vehicle	Phenobarbital/beta-naphthoflavone induced rat liver S9	Not mutagenic	EU REACH dossier (2012)
OECD Guideline 487 In vitro Mammalian Cell Micronucleus Test. Human lymphocytes.	125, 250, 500, 1000 and 2000 µg/mL using DMSO as a vehicle	1 mL of rat liver S9 homogenate was mixed with 9 mL cofactor	Non clastogenic and non aneugenic	EU REACH dossier (2018)

The overall conclusion from OECD guideline *in vitro* studies is that propylparaben is not mutagenic/genotoxic/clastogenic or aneugenic.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

There are no *in vivo* mutagenicity or genotoxicity assays for propylparaben. Propylparaben is negative in all OECD guideline *in vitro* assays (see above).

To provide additional information, the REACH dossier (2018) refers to the details of a study (Litton Bionetics. 1974) and states 'Methylparaben is considered to be non-mutagenic in rats in this dominant lethal assay when using dosages of 5, 50, 500 and 5000 mg/kg bw/d.

According to the Applicant, there are no *in vivo* mutagenicity or genotoxicity assays for propylparaben. The overall conclusion from OECD guideline *in vitro* studies is that propylparaben is not mutagenic/genotoxic/clastogenic or aneugenic.

SCCS overall comments on genotoxicity
Propylparaben has been tested in valid OECD guideline mutagenicity assays for bacterial and mammalian gene mutations assays, aneuploidy and clastogenicity endpoints with negative results. Although in some papers, available in open literature, PP has been tested positive for MN induction, the reports have several methodological limitations (e.g. quite high cytotoxicity, no S9 mix used, no data on historical controls, etc.). In conclusion, the SCCS is of the opinion that PP does not pose a genotoxic hazard.

3.4.7 Carcinogenicity

There are no OECD guideline carcinogenicity studies available for propylparaben.

Academic research raised suspicions in the previous decade about the presence of parabens in breast tissue and questioned whether parabens had a role in breast cancer (Darbre, 2004). Golden and Gandy (2005) effectively highlighted the limitations in the work. The SCCS (SCCP/0874/05 opinion) addressed parabens and breast cancer "*Extended Opinion on parabens, underarm cosmetics and breast cancer"* and concluded that '*according to the current knowledge, there is no evidence of a demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics*.' No further evidence exists that would warrant a change in this Opinion.

According to the Applicant, there is no evidence of propylparaben acting as a carcinogen.

SCCS overall comments
Based on all the available data, the SCCS considers propylparaben to have no carcinogenic potential.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

Photo-contact sensitisation and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methylparaben, Propylparaben, and/or Butylparaben gave no evidence for significant photoreactivity ((Elder R (ed), 1984).

According to the applicant, propylparaben is not phototoxic.

3.4.8.2 Photomutagenicity / photoclastogenicity

/

3.4.9 Human data

Taken from SCCS 2013 (SCCS/1514/13):
Information on exposure to parabens can be derived from human biomonitoring studies. Concentrations in human biological fluids (e.g. urine, blood) account for both dietary intake (e.g. from foods with paraben preservatives) and dermal application of products with parabens; according to Soni et al. (2005) the latter is considered to be the major contributor. Thus, such measurements are of interest as they provide information on the frequency and the magnitude of an overall exposure.
The results of these studies (see SCCS/1446/11, Annex 4 for details and references) indicate that the (average) systemic exposure dose is considerably lower than estimated in the previous paraben Opinion (SCCS/1348/10) for adults who use all types of cosmetic products with parabens at the authorized concentrations.
Exposure estimates based on biological monitoring data are considered by SCCS as useful additional information in their overall evaluation on the safety of parabens.

3.4.9.1 Biomonitoring studies

The Applicant provided Table A-1 in annex that presents information in the publically available literature on measured levels of parabens in humans.

There is a wealth of human biomonitoring data emerging for parabens, and the data specifically for propylparaben are presented above, indicating that systemic exposure is low. Recently, the Human Biomonitoring (HBM) Commission in Germany have defined 'reference values' for parabens (Apel et al 2017). These reference values are based on the 95% confidence interval of the 95th percentile of the concentration of a chemical substance in the matrix obtained from a reference population. Preferably, reference values are derived from data obtained from a representative population sample in the context of the German Environmental Survey (GerES). They allow a uniform assessment of the body burden at the German national level, and are indispensable to demonstrate whether a certain exposure level exceeds the background exposure level, e.g. accident-related exposures. Because of their statistical nature, reference values cannot serve to assess health risks. Reference values are checked continuously and are updated if new information becomes available. For propylparaben, the reference value set by the German HBM Commission is 100 µg/L for women and 50 µg/L for men (Apel et al 2017), reflecting the fact that women use more personal care products than men do..

3.4.9.2 Human evidence on the reproductive and developmental effects of propylparaben

The CIR 2019 Panel noted that "recent epidemiology studies suggested paraben exposure association with different types of health outcomes, such as lower mental developmental index in girls, adverse impacts on fetal and childhood growth, decreased diastolic blood pressure during pregnancy, increased risk for placental preterm birth, disturbance of reproductive hormone levels, and disomy of chromosome; although, these were not confirmed by subsequent or previous epidemiologic investigations. Sources of parabens exposure in these studies are broadly from the environment and not specified. More importantly, parabens exposures of the study population are always coupled with other preservatives and active ingredients that are used in a wide variety of consumer products, including phthalates, BPA, TCS, etc. Therefore, the currently available scientific evidence lacks the clarity regarding any cause-and-effect relationship between parabens and human health outcomes." It remains to be determined whether the costimulatory effects require multiple exposures.

According to the applicant, no definitive conclusions can be reached from these data and they are considered not to be relevant to performing a regulatory safety evaluation.

SCCS comments
Humans are exposed to several different parabens. Only few human studies have investigated possible endocrine disrupting effects of parabens. One study analysed the association between urinary concentrations of MP, PP and BP and the markers for male reproduction health (Meeker et al., 2011). No associations were observed between the three parabens and serum hormone levels or semen quality parameters. Another study found no association between the urinary concentrations of parabens (methylparaben, propylparaben and butylparaben) and pubertal stage breast development and pubarche in U.S. girls (Wolff et al., 2010). Poster abstracts from two unpublished studies (Sabatini et al., 2011 and Smith et al., 2011), have indicated that:

1) increased urinary MP and PP were associated with increased incidence of poor embryo quality, and
2) that there was suggestive evidence for an association between PP and higher serum follicle stimulating hormone and lower antral follicle count on day three of the menstrual cycle.

Over the past decade, more than 40 epidemiology studies have also been published to investigate whether there is a putative association between parabens' exposure and adverse outcomes in human populations. However, a full and systematic review of this new epidemiology literature has not been performed to establish whether these studies conform to Bradford-Hill criteria, for example. Some of the studies have been selected and reviewed in brief in CIR (2019).
In conclusion, a few human studies have indicated weak associations between increased paraben exposure and markers for human reproductive health. However, the knowledge in this area is still very limited.

3.4.10 Special investigations

3.4.10.1 Endocrine activity.

Non-test information, in silico, read across, in chemico.

A plethora of *in vitro* studies have been performed over the last decades to try to determine quantitatively the *relative* binding potencies of parabens vs natural substrates. These studies are listed below.

3.4.10.2 Endocrine activity. In vitro and other assays

According to the Applicant, propylparaben has been investigated for endocrine activity *in vitro* in numerous studies. Table 5 provides an overview of these studies.

Table 5: An Overview of all studies related to the endocrine activity of propylparaben *in vitro*

Test principle	Results and conclusion	Reference
Recombinant yeast assay screen	DNA sequence of the human estrogen receptor is integrated into the yeast genome. PP was compared with the potency of estrogen at its receptor. **Propylparabens 30,000-fold less potent than 17β-estradiol**.	Routledge et al. 1998 Miller et al 2001

	The metabolite p-HBA, was 2.5 million-fold less potent as is considered non-estrogenic.	
Estrogen-receptor competitive binding assay	Substance competes with estradiol in binding with the ER. IC_{50} for PrP 1.5 ± 0.1 x 10^{-4} M , compared with an IC_{50} for 17β-estradiol of 0.0009 µM.	Blair et al 2000
MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Assaying estrogen-receptor (ER)-dependent proliferation of MCF-7 cells PP stimulated the proliferation to about the same level as the maximal cell yield attained with 3x10(-11) M 17β-estradiol, but at a concentration in the order of 10^5 to 10^7 higher than 17β-estradiol. **PP: EC50 1.9 µM** **17β-estradiol : EC50 0.0016 nM**	Okubo et al 2001
MCF-7 cells	Competitive inhibition of [3H]oestradiol binding to MCF7 cell estrogen receptors could be detected at 1,000,000-fold molar excess of *n*-propylparaben (77%).	Byford et al 2002
Skin and liver cytosol and **human** epidermal keratinocytes	**PP elevates estrogen levels by inhibiting estrogen sulfotransferases** (SULT) in skin. SULT activity was inhibited in skin cytosol by PP but **not** by PHBA. Potency increased with chain length (IC_{50} BuPB = 37 µM). No inhibition of androgen sulfation. **No positive control was included.**	Prusakiewicz et al. 2007
A stably transfected **human** embryonic kidney cell line that lacks critical steroid metabolizing enzymes	Investigate **anti-androgenic activity** by measuring inhibition of 0.1 nM testosterone (T)-induced transcriptional activity. **PP inhibited 0.1 nM T-induced transcriptional activity at concentrations above 10 µM (max. 40% inhibition).** PHBA was negative. Pos. controls (flutamide and vinclozolin) inhibited 1nM T-induced signal at concentrations of 0.1 to 10 µM (11 to 90% inhibition).	Chen et al. 2007
MCF-7 cells	Investigate **estrogenic effects** of mixtures of parabens on cell proliferation; investigate anti-estrogenic effect through inhibition of aromatase, the enzyme that converts androgens into estrogens. **Induced cell proliferation with EC_{50} values 1 µM**. PHBA was negative. Potency of PP remains about 5 orders of magnitude below that of 17β-oestradiol. **Parabens inhibited aromatase with IC_{50} values of 3.5 µM.** **Authors note that typical human PB concentrations (10-80nM) are much lower than EC_{50} and IC_{50} values encountered here.**	van Meeuwen et al. 2008
Recombinant rat androgen receptor (rrAR) assay	Determine the ability of probable endocrine disruptors to compete with synthetic androgen methyltrienalone (R1881) for binding to recombinant rat AR. (Screening tool). **PrPB IC_{50} 9.7 x 10^{-4} M (Rank of Binbing Affinity (RBA) 0.0019)** **However di(n-butyl) phtalate (DNP) and di(2-ethylhexyl) phtalate (DHNP), known anti-androgenic chemicals, did not show any significant AR binding activity.**	Kim et al. 2010
Stably transfected human estrogen receptor-α transcriptional	STTA assay evaluates the ability of chemicals to function as an estrogen receptor alpha (ERα) ligand and activate an ERα agonistic responses. PC50, the concentration of chemical estimated to cause 50% of activity of the positive control (17β-oestradiol) response on a plate by plate basis	Kim et al. 2011

activation (STTA) assay (**OECD 455**)	**17β-estradiol PC50 (M): 2.88 × 10−^{11}; logRTA: 2** **propylparaben PC50 (M): 02.0 × 10−6 logRTA: −2.84164** (Relative transcriptional activation (RTA) is calculated as 100 × (PC50) of E2/(PC50) of test compound; a value of 100 indicates that the compound tested is a full agonist). **PP was shown weak estrogenic activity which was approximately 69,000-fold lower than E2**.	
GH3 rat pituitary cancer cell line	Induction of an estrogenic biomarker gene - Calbindin-D(9k) (CaBP-9k). CaBP-9k and PR are induced by PP via the ER pathway in GH3 cell line.	Vo et al 2011
MCF-12A and MCF-10A non-transformed immortalised breast epithelial cells	10μM PrPB in DMSO. ERα, ERβ and G-protein coupled estrogen receptor (GPER) competent. Cells cultured with PrP for 16 days at 37ºC. Under normal conditions, MCF-12A cells formed organised acini, with deposition of basement membrane and hollow lumen (tubular structure). Treatment with 17β-estradiol, and propylparaben resulted in deformed acini and filling of the acinar lumen. When these chemicals were combined with ER and GPER inhibitors the deformed acini recovered normal features, **suggesting a role for the ER and GPER in the estrogenic disruption of acinar formation.**	Marchese & Silva 2012
Mouse and Human adipocytes	1) Murine 3T3-L1 fibroblasts 2) hADSC (human adipogene -derived multipotent stromal cells) 3) GR-responsive luciferase reporter construct MMTV-Luc 4) PolarScreen GR competitor assay (1) PP promote adipocyte differentiation in murine 3T3-L1 cells, (2) parabens activate GR and/or PPARγ in 3T3-L1 preadipocytes; no direct binding to, or modulation of, the ligand binding domain of the glucocorticoid receptor by parabens was detected by glucocorticoid receptor competitor assays (3) PP promote adipose conversion of hADSC **Adipogenic effects of PP in both murine 3T3-L1 and hADSC**	Hu et al 2013
Protocol for obesogen screening based on the 3T3-L1 cell line Also PPARγ activation and antagonist experiments	Positive controls: acknowledged obesogens rosiglitazone and tributyltin. 0.39-200 μM test concentrations of PP. **LOECs (3T3-L1 cell line): Rosiglitazone 16nM, Tributyltin 6.25nM, PP 100μm** **LOECs (PPARγ): Rosiglitazone 30nM, Tributyltin 3nM, PrPB 10μm**	Pereira-Fernandes et al 2013
MCF-7 and MCF-10A cells	Analyzed the dose- (0.2, 2, 20, 200 nM or 2 μM) and time- (48, 96, 144 and 196 h) dependent activity of a single or repeated PP on the proliferation of MCF-7 human breast cancer cells and MCF-10A human breast epithelial cells. Additionally, the effect on estradiol secretion, gene and protein expression of aromatase (*CYP19A1)* was investigated. **Low doses of PP significantly increased 17*b*-estradiol (E2) secretion in MCF-7 cells but had the opposite effect on MCF-10A cells.** Different mechanisms of proliferative action of parabens in these two cell lines. **?**	Wróbel & Gregoraszczuk 2013
Human estrogen receptor α (hERα), hERβ and androgen receptor (hAR) models	Transcriptional activities mediated by human estrogen receptor α (hERα), hERβ and androgen receptor (hAR). Fourteen of 17 parabens exhibited hERα and/or hERβ agonistic activity at concentrations of ≤ $1\times10^{(-5)}$M, whereas no parabens showed AR agonistic or antagonistic activity. The results indicate that parabens are selective agonists for ERβ over ERα; their interactions with ERα/β are dependent on the size and bulkiness of	Watanabe et al 2013

	the alkyl groups; and they are metabolized by carboxylesterases, leading to attenuation of their estrogenic activity.	
In vitro nuclear receptor coactivator recruiting assay	Antagonist competitive binding on the human estrogen-related receptor γ (ERRγ). **All of the test parabens possessed clear inverse antagonist activities on ERRγ, with a lowest observed effect level (LOEL) of $10(^{-7})$M and the 50% relative effective concentrations (REC_{50}) varying from $3.09\times10(^{-7})$ to $5.88\times10(^{-7})$M**	Zhang et al 2013
MCF-7 and MCF-10A	Methyl, butyl- and propylparaben (20 nm) or 17β-estradiol (10 nm). Cell cycle and apoptotic gene expression were evaluated by real-time polymerase chain reaction and protein expression by Western blot. **Cyclins in MCF-7 cells were not** affected by any of the parabens. In MCF-10A, all parabens tested **increased the expression of G1 /S phase genes**, and **downregulated cell cycle inhibitors. Propylparaben upregulated both the extrinsic and intrinsic apoptotic pathways.** There are differences in cell cycle and apoptosis gene expression between parabens and17β-estradiol in MCF-7 cells	Wróbel & Gregoraszczuk 2014a
MCF-7 and MCF-10A	PP (20 nm) or 17β-estradiol (10 nm). Effects on mRNA and protein expression of estrogen receptor (ER)-α (ESR1) and -β (ESR2) and progesterone receptor(PGR). **In MCF-7 cells, PB stimulated Progesterone receptor (PGR) mRNA expression.** **In MCF-10A cells, PB increased only PGR mRNA expression.** **In MCF-7 and in MCF-10A cells, PP increased ESR1 gene and protein expression.** **In MCF-7 cells, PP increased ESR2 mRNA and ESR2 protein expression cells** **In MCF-10A cells, PP significantly increased only ESR2 protein expression**	Wróbel & Gregoraszczuk 2014b
Human MDA-kb2 breast carcinoma cells	0,10nm and 1 μM test substance in DMSO. Cells were incubated 24h with and without test compound and evaluated by means of a cell proliferation assay and an assay for glucocorticoid activity (luciferase reporter gene). **EC50 for glucocorticoid activity was 10.01 mM for PB; PB tested induced glucocorticoid-like activity at 1 μM, but not at 10 nM.**	Klopčič et al 2015
Human MDA-kb2 breast carcinoma cells	0 and 25 μM in DMSO. Cells were incubated 24h with and without test compound, and with or without the AR (androgenic receptor) agonist flutamide (5μM). **PP did not enhance the hydrocortisone-induced GR (glucocorticoid receptor) signal. GR activity was increased by 50% with PP in the presence of flutamide.**	Kolšek et al 2015
In vitro testing of PP for inhibition of 17β-HSD1 and 17 β-HSD2 activities.	Endogenous 17β-HSD1 activity assays were performed in intact COV434 cells. Lysates of HEK-293 cells expressing 17β-HSD1 or 17β-HSD2. **PP inhibited 17 β-HSD2 at 20μM** but p-hydroxybenzoic acid didn't. Regarding the very rapid metabolism to the inactive p-hydroxybenzoic acid by esterases, it needs to be determined **under which conditions low micromolar concentrations can occur in target cells to effectively disturb estrogen effects *in vivo*.**	Engeli et al 2017
Direct effects of propylparaben on the growth and	Antral follicles were isolated from the ovaries of Swiss mice (age: 32-42 days) and cultured in media with dimethylsulfoxide vehicle control or propylparaben (0.01-100 μg/mL) for 24-72 h. Follicle diameter was measured every 24 h to assess growth. Follicles and	Gal et al 2019

steroidogenic function of mouse antral follicles (ex vivo)	media were collected at 24 and 72 h for gene expression and hormone measurements. **Propylparaben (100 µg/mL) significantly inhibited follicle growth (48-72 h) and steroidogenic function by altering the cell-cycle, apoptosis, and steroidogenesis pathways.**	
Tox 21 Endocrine screening program assays	Estrogen receptor (ER) assays: **14/28 ER assays positive**, as an agonist for the ER. Androgen receptor (AR) assays : **3/17 AR assays positive at high dose** above a cytotoxis dose – not a substrate for the AR. Thyroid receptor (TR) assays : No assays positive for the TR Steroidogenesis assays : **9/26 assays positive; 7 only positive at high dose** above a cytotoxic dose.	US EPA Endocrine Screening program 2019* Ref DTXSID4022527

Estrogenic activity

A study by Routledge et al (1998) showed early indications that parabens could be weakly estrogenic, finding propylparaben to be 30,000-fold less potent than 17β-estradiol in interacting with the ER in an *in vitro* recombinant yeast assay incorporating human estrogen receptor (ER).

Subsequent *in vitro* studies suggested that propylparaben could be weakly estrogenic, because propylparaben:

• binds with estrogen receptors (rat and/or human ERα and ERβ) (Blair et al., 2000; Watanabe et al 2013; Zhang et al 2013);

• promotes human cancer cell proliferation *in vitro* (Okubo et al., 2001; Byford et al., 2002; Terasaka et al 2006; van Meeuwen et al 2008; Vo et al 2011; Marchese & Silva, 2012; Wrobel and Gregoraszczuk, 2013, 2014a, 2014b; Klopcic et al 2015; Kolsek et al 2015);

• induces ER reporter gene expression (Routledge et al., 1998; Miller et al., 2001; Watanabe et al., 2013; Bazin et al., 2013).

In estrogen receptor (ER) competitive binding assays using the human MCF-7 breast cancer cell line and isolated ER from rat uteri, propylparaben showed a weak ER binding affinity with IC_{50} values of 1.65 - 245 µM (Okubo et al., 2001; Byford et al., 2002; Blair et al., 2000; Vo et al., 2010). In comparison, for 17β-estradiol an IC_{50} value of 0.0009 µM was determined (Blair et al., 2000).

For cell proliferation in MCF-7 cells, an EC50 value of 1.9 µM propylparaben was determined (Okubo et al., 2001). In comparison, for 17β-estradiol, an EC50 value of 0.0000016 µM was determined for cell proliferation.

These literature reports demonstrate that the estrogenic activity of PP is extremely weak (approximately 20,000–700,000-fold lower at maximum concentrations) compared to 17β-estradiol (Routledge et al.,1998; Watanabe et al., 2013; Okubo et al., 2001). When potency is so weak, it raises questions on the biological relevance of these findings.

A definitive stably transfected human estrogen receptor-α transcriptional activation STTA assay (OECD TG 455) was performed by Kim et al (2011). This showed that propylparabens were weakly active *in vitro* with estrogen receptor-α at a 69,000-fold lower concentration than estradiol.

Anti-androgen activity of propylparaben has been investigated using stably transfected human embryonic kidney cell lines lacking steroid metabolising enzymes (Chen et al 2007). Propylparaben inhibited 0.1 nM Testosterone-induced transcriptional activity at concentrations above 10 µM (max. 40% inhibition). Propylparaben weakly inhibited rat androgen receptor; PrPB IC_{50} 9.7×10^{-4} M (RBA 0.0019) compared to Dihydrotestosterone IC_{50} 1.8×10^{-8} M (Kim et al 2010).

Investigations into effects of propylparaben on adipocytes have been performed (Hu et al 2013; Pereira-Fernandez et al 2013). Parabens activate GR and/or PPARγ in 3T3-L1 preadipocytes but there is no direct binding to, or modulation of, the ligand binding domain

of the glucocorticoid receptor by parabens as detected by glucocorticoid receptor competitor assays.
However, it remains difficult to extrapolate data from *in vitro* assays to humans. Due to a rapid metabolism of parabens *in vivo,* it is unlikely that estrogenic effects through direct estrogen/androgen receptor activation by parent parabens can cause harmful effects in humans (Engeli et al 2017).

3.4.10.3 Endocrine activity. In vivo and other assays

Animal data
Propylparaben has been investigated for endocrine activity *in vivo* in numerous studies. Table 6 provides an overview of these studies.

Table 6: An overview of all studies related to the endocrine activity of propylparaben in vivo

Test principle	Results and conclusion	Reference
OECD 440 Non GLP B6D2F1 mice Uterotrophic assay	Oral and SC. NOEL (mice) 100 mg/kg (top dose PrPB tested). **No change to uterine weights.**	Hossaini et al 2000
OECD 440 Non GLP CD1 mice Wistar rats Uterotrophic assay	In mice, ED50 of 17β-oestradiol (E_2) for increase in uterine weight was **7 µg/kg bw (Im or Ovx),** ED50 of PB were from **17 mg/kg bw (Im) or 43 mg/kg bw (Ovx).** In rats, ED50 of 17β-oestradiol (E_2) for increase in uterine weight was **10,3 µg/kg bw** ED50 of PB were from **33 mg/kg bw.** NOELs for uterotrophic activity of PBs in immature mice were **6.5**, in ovariectomized mice **7**, and in immature rats **20 mg/kg bw**, respectively. In the estrogen receptor binding assay, PP competed with E2 and Ki values correlated to their estrogenic activity. **Estrogenicity confirmed but at dose levels much higher than those of 17β-estradiol**	Lemini et al., 2003
Appears compliant with OECD 440 Non-GLP	Morphometric analysis of uteri in uterotrophic assay in adult ovariectomized (Ovx) CD1 mice. Subcutaneous route (sc) Treated daily for three days: PrPben (65 and 195 mg/kg), E_2 (10 mg/kg; 0.036 mmol/kg), **The highest parabens dose was able to produce uterotrophic effects (38 to 76%) compared to E_2 effects (100%)** in Luminal epithelium heights, glandular epithelium heights, and myometrium widths.	Lemini et al 2004
CF-1 and CD-1 female **mice** Non-GLP Non guideline No mention of group size	Evaluation of the effects of parabens on success of implantation in fertilised mice. Subcutaneous route i Doses : 0-949-1084 mg PrPB/kg bw/day on day 1 to 4 of gestation. **PP had no impact on the number of implantation sites observed and did not affect any of the measured parameters.** 17β-oestradiol terminated all pregnancies.	Shaw and deCatanzaro 2009
Sprague Dawley immature female rats Non GLP	Uterotrophic assay. Subcutaneous route Doses: 62.5-250-1000 mg/kg bw/day of paraben for 3 days. Investigation of Calbindin-D9-k (CaBP-9k), biomarker for estrogenic effects. **PP had no impact on the number of implantation sites observed and did not affect any of the measured parameters**, PB did not affect CaBP-9k gene expression.	Vo and Jeung 2009

Female Sprague–Dawley rat during the juvenile-peripubertal period. (8-week-old) Non GLP	n=10/group, Doses : 0, 62.5, 250 or 1000 mg/kg bw/day by gavage (day 21-40). Non guideline Investigation of Calbindin-D9-k biomarker for estrogenic effects. <u>Propylparaben at 1000 mg/kg/day:</u> **Myometrial hypertrophy** **Increased adrenal weight** IC50 value for binding ERα and ERβ receptors: 17β-estradiol: 3.10^{-9} M PrPB: 2.10^{-5} M No effects on Calbindin-D9-k NB. Animals were not necropsied at specific stages. It is very likely that a number of females were in proestrus or estrous, which could explain the unexpected observation of myometrial hypertrophy for propylparaben.	Vo et al. 2010
Neonatal Sprague Dawley female rats (n =5) - Non GLP Non guideline Klimisch 3	<u>Effects at 62.5 mg/kg/day and above:</u> MePB, PrPB: mRNA levels of StAR decreased **<u>Effects at 250 and 1000 mg/kg/day:</u>** CaBP-9k (dose-response relationship) Decreased numbers of early primary follicles (dose response relationship) mRNA levels of AMH (ovarian anti-Mullerian hormone) and FoxI2 (forkhead box protein I2 transcription factor) increased (both not affected by E2) (no dose response relationship) mRNA levels of CYP11a1, mid-dose increased, high dose decreased (no dose-response relationships) **<u>Effects only at 1000 mg/kg/day:</u>** increased numbers of primordial follicles **LO(A)EL (sc): 62.5 mg/kg bw/day** Not all data appear consistent.	Ahn et al. 2012
Mouse (C57BL/6J) Klimisch 1 OECD 440	**(Uterotrophic Bioassay in rodents).** Ovariectomised female mice 8 weeks of age (n=6/group, 11 groups) were dosed daily for 7 consecutive days by oral gavage and subcutaneous injection. 6 µg/kg bw/day estradiol (E2) was given orally as positive control for agonist and antagonist detection. Negative for estrogen agonism and antagonism **NOEL defined at a top dose of 1000 mg/kg/day**	Ohta et al 2012
Wistar **rat** Non GLP Non guideline Klimisch 3	Study of the effects on general function of the male rat reproductive system. Rats (19-21 days old) received PP through the oral route (diet) for 4 weeks at dosage levels of 12.4, 125 and 1290 mg/kg/day. At all three dosage levels, a decrease in cauda epididymal sperm reserve, sperm count and daily sperm production was observed and from 125 mg/kg/day serum testosterone concentration was decreased. **LOAEL: 12.4 mg/kg/day.** Recent toxicokinetic data indicate low systemic exposure to PrPB even at high doses and raise doubt on the relevance of the study for risk assessment. **Shortcomings:** • **control values outside normal range, not consistent with literature data and other Oishi studies,** • **absence of dose-response for Daily Sperm Production,** • **small group size, full study protocol and raw data not available**	Oishi 2002

Juvenile male rodent (Wistar) GLP compliant PK evaluation included Klimisch 1	PP orally administered by gavage to 20 Wistar male rats at doses of 3, 10, 100, or 1000 mg/kg/day for 8 weeks starting on PND21. A first subgroup of 10 males/dose was necropsied immediately after the 8-week exposure period; a second subgroup of 10 males/dose was necropsied after a 26-week washout period. GLP. **There was no evidence of an effect of PrP on the weight of the male reproductive organs, epididymal sperm parameters, hormone levels (LH, FSH or testosterone), or histopathology. The NOAEL was 1000 mg/kg/day**, corresponding to a maximum plasma concentration of **12,030 ng/ml** and exposure to 47 760 ng · h/ml (AUC0-8 h) at the end of the treatment.	Ricerca Biosciences 2012d (Written up in Gazin et al 2013)
Immature male Wistar rats Guideline: OPPTS 890.1400 Hershberger bioassay	PP was orally administered by gavage to immature Wistar male rats at doses of 10, 250, or 750 mg/kg/day for 10 days. The Hershberger bioassay serves as an *in vivo* screening method for androgen agonists or antagonists and other 5α-reductase inhibitors. The five androgen-dependent accessory reproductive tissues included in this assay include: the ventral prostate, seminal vesicles, levator plus-bulbocavernosus muscles, Cowper's gland, and glans penis. These tissues respond to antiandrogens with a difference in absolute tissue weight. **PP significantly decreased all the organ weights of accessory reproductive tissues at each dose of 250 and 750 mg kg−1 day.** **Antiandrogenic profile**	E. Özdemir et al, 2018

Routledge et al. (1998) reported weak estrogenic effects of parabens *in vivo* as well as *in vitro*. This work showed weak effects, primarily for butylparaben (approximately 100,000 times less potent than the positive control estradiol (0.4 mg/kg/day)), that then cast into doubt all of the parabens as potential endocrine active substances that warranted further study.

There are several other reports on *in vivo* estrogenic effects of propylparaben, which suggest that propylparaben:

- increased uterine weights in immature mice and rats following subcutaneous administration, as well as overiectomized mice (≥20 mg/kg in mice and ≥65 mg/kg in rat) (Lemini et al., 2003, 2004);
- increased myometrial hypertrophy and uterine weights when oral gavage to young (PNDs 21–40) female Sprague-Dawley (SD) rats (1,000 mg/kg/day) (Vo et al., 2010);
- inhibited folliculogenesis and steroidogenesis in the ovaries of neonatal (PNDs 1–7) SD rats following subcutaneous administration (≥250 mg/kg/day) (Ahn et al., 2012)

The main metabolite of propylparaben, pHBA was shown to be estrogen inactive with no effects on uterine weight in immature Wistar rats in an uterotrophic assay (Lemini et al., 2003). As with the *in vitro* reports, the *in vivo* estrogenic effects of propylparaben in these studies appear to be very weak compared to estradiol (E2). For example, in the Ahn study, primordial follicles were increased and early primary follicles decreased with both E2 (40 µg/kg/day) and propylparaben (1,000 mg/kg/day) i.e. at a 25,000-fold higher dose for propylparaben. E2 produced a 102% increase in myometrial thickness (relative to vehicle control) at 1 mg/kg/day, while propylparaben produced half the increase (57%) at a 1,000-fold higher dose (Vo *et al*., 2010). The route of administration, the oestrous stage of the animal at the time of uterine examination, or underpowered studies could have impacted on the conclusions in published studies. There are also reports that have shown propylparaben administration not to be associated with estrogenic effects *in vivo*:

- Propylparaben (up to 1,000 mg/kg/day) had no impact on implantation sites when administered subcutaneously to inseminated CF-1 mice on GDs 1–4 and sacrificed on GD 6 (Shaw and deCatanzaro, 2009).
- No effects were seen in two uterotrophic studies (Hossaini 2000; Vo & Jeung 2009).

- In Vo et al (2010), no significant effects were observed on vaginal opening, estrous cycle as well as body weight and uterus, pituitary, ovary, thyroid, kidney and liver weights of peripubertal rats treated by gavage with 62.5-1000 mg/kg bw/d propylparaben for 20 days.
- Crucially, an OECD TG455 Uterotrophic assay was negative (Ohta et al 2012), with a No observed effect level for females of 1000 mg/kg/day.

The first *in vivo* study on potential male reproductive effects (Oishi, 2002), indicated that propylparaben reduced testicular and epididymal sperm counts (average propylparaben intake of approximately 12.4 ± 3 mg/kg/day), as well as serum testosterone levels (approximate intake 125 ± 30 mg/kg/day) when administered in the diet to post-weaning (PNDs 19–21) male Crj:Wistar rats for 4 weeks. This sudy was non-guideline and was subsequently refuted by a higher quality study from Ricerca Biosciences (2012; written up in Gazin et al 2013).
An oral (gavage at a dose volume of 10 ml/kg) administration of propylparaben (0, 3, 10, 100, or 1000 mg/kg/day) performed on male Wistar rats for 8 weeks, initiating on PND 21, had no effects on reproductive organ weights, sperm count or motility, or hormone levels (LH, FSH and testosterone). Importantly, this study was robustly powered and GLP compliant, with confirmation of systemic propylparaben exposures (Gazin et al., 2013).

Gazin et al., 2013 determined a **No observed adverse effect level for males of 1000 mg/kg/day.**

SCCS comment
Hass et al. (2012), based on *in vitro and in vivo* studies, considered propylparaben as a suspected endocrine disrupter, as there is evidence of an estrogenic mode of action *in vivo* that is suspected to be linked to adverse effects *in vivo*.
Conversely, the additional studies requested in the context of the REACH registration process for propylparaben, and provided by Clariant, were analyzed for all measured parameters and endpoints that can be used for the evaluation of potential endocrine properties of propylparaben according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals [OECD 2018: Revised Guidance Document 150] did not show any findings and are summarized in the table hereafter.
These studies were as follows:

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2 REACH; test method: EU B. 26 / OECD TG 408) in rats
2. Prenatal developmental toxicity study (Annex IX, Section 8.7.2; test method: EU B. 31 / OECD TG 414) in a first species (rat or rabbit), oral route
3. Extended one-generation reproductive toxicity study (oral route, with rats, with the developmental neurotoxicity and immunotoxicity (DNT / DIT) cohorts and with the extension of cohort 1B to mate the F1 animals to produce an F2 generation); test method: EU B. 56 / OECD TG 443

Any effect that could indicate an endocrine disrupting effect was not noted in these studies. These studies have been discussed in more detail in section 3.4.5 above on reproductive and developmental toxicity studies.

3.4.10.4 Endocrine activity. Human data

See section 3.4.9.2

3.4.10.5 Conclusion on (potential) ED disruption in humans

According to the Applicant, based on the data from authoritative guideline studies for propylparaben and the definition from the European Commission, it is considered that the substance is not an endocrine disrupter based on current EU classification criteria (ECHA/EFSA, 2018).

Female endocrine effects: an uterotrophic study that followed OECD guidelines and was performed to GLP (Ohta et al 2012). *In vivo*, propylparaben is negative for estrogen agonism and antagonism and no estrogenicity was observed. Propylparaben did not modify levels of 17β-estradiol. Para-hydroxybenzoic acid, a non-estrogenic compound, was the predominant metabolite contributing to 95% of the total exposure at 1,000 mg/kg/day on PND 7**.**
The No-Observed-Adverse-Effect-Level (NOAEL) for females is a top dose at 1,000 mg/kg/day.

Male endocrine effects**:** the pivotal study covering the potential for adverse effects in the intact organism is by Gazin et al. (2013), which is the published write up of Ricerca Biosciences 2012 study. In this *in vivo* study there was no evidence of an effect of propylparaben on the weight of the male reproductive organs, epididymal sperm parameters, hormone levels (LH, FSH or testosterone), or histopathology.
The No-Observed-Adverse-Effect-Level (NOAEL) for males is a top dose of 1,000 mg/kg/day.

A risk assessment can be performed based on no adverse effects in males and females using the NOAEL of 1000 mg/kg/day from the pivotal studies in female and male animals. A BMD cannot be calculated from these studies as there were no quantifiable observed effects with a dose-response.

Overall SCCS conclusions on endocrine disruption properties of propylparaben
The SCCS has analysed all the relevant information provided in the dossier and available in the published literature for safety asesesment of propylparaben with an emphasis on its potential endcrine effects (CIR (2019), ECHA (Reach dossier, 2018), Centre for endocrine disruptors (May, 2012). The SCCS is of the view that, although the available data on propylparaben provide some indications for potential endocrine effects, the current level of evidence is not sufficient to conclusively regard it as an endocrine disrupting substance or to derive a specific endocrine-related toxicological point of departure for use in safety assessment.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

Toxicological Point of Departure from Animal Data

The point of departure for use in safety assessment is derived from reproductive effects of propylparaben, as described in section 3.4.5.

Pivotal study for calculating the oral POD

The finding of five good quality GLP studies (detailed in sections 3.4.4 and 3.4.5) support a NOAEL of 1000 mg/kg/day for propylparaben. These include Harlan (2012), Gazin et al (2013), Sivaraman et al. (2018), the OECD guideline 414 study cited in ECHA REACH dossier (2018) and Clariant GMBH study (2019).

The POD as an oral NOAEL for use in risk assessment is 1000 mg/kg/day

Safety Evaluation Outcome

Table 7: Tier 1 – Scenario 1A - Margin of Safety calculations for individual cosmetic product types and aggregate exposure to propylparaben.

Product	**Maximum use (w/w%) in the finished product**	**Calculated relative daily exposure to product [1]**	**Total dermal external exposure (mg/kg bw/day)**	**Calculated SED[2]**	**Margin of Safety**
		(mg/kg bw/day)		**(mg/kg bw/day)**	**(POD 1000 mg/kg/day divided by SED)**
Bathing and Showering					
Shower gel	0.183	2.79	0.005	0.000185	5 405 405
Hand wash	0.183	3.33	0.006	0.000222	4 504 505
Hair care					
Shampoo	0.183	1.51	0.003	0.000111	9 009 009
Hair conditioner	0.183	0.67	0.001	0.000037	2 702 7027
Hair Styling	0.183	5.74	0.01	0.00037	2 702 703
Skin care					
Body lotion	0.183	123.2	0.226	0.008362	119 589
Face cream	0.183	24.14	0.044	0.001628	614 251
Hand cream	0.183	32.7	0.06	0.00222	450 450
Make-up					
Liquid foundation	0.183	7.9	0.015	0.000555	1 801 802
Lipstick, lip salve	0.183	0.9	0.002	0.000074	13 513 514
Make-up remover	0.183	8.33	0.015	0.000555	1 801 802
Eye shadow	0.183	0.33	0.001	0.000037	27 027 027
Mascara	0.183	0.42	0.001	0.000037	27 027 027
Eyeliner	0.183	0.08	0.0001	0.0000037	270 270 270
Deodorants					
Non-spray	0.183	22.08	0.04	0.00148	675 676
Oral care					
Toothpaste[3]	0.183	2.16	0.004	0.004	250 000
Mouthwash[3]	0.183	32.54	0.06	0.06	16 667
Aggregate			**0.492**	0.0798767	12 519

Table 8: Tier 1 – Scenario 1B - Margin of Safety calculations for individual cosmetic product types and aggregate exposure to propylparaben

Product	Maximum use (w/w%) in the finished product	Calculated relative daily exposure to product [1] (mg/kg bw/day)	Total dermal external exposure (mg/kg bw/day)	Calculated SED[2] (mg/kg bw/day)	Margin of Safety (POD 1000 mg/kg/day divided by SED)
Bathing and Showering					
Shower gel	0.175	2.79	0.005	0.000185	5 405 405
Hand wash	0.18	3.33	0.006	0.000222	4 504 505
Hair care					
Shampoo	0.18	1.51	0.003	0.000111	9 009 009
Hair conditioner	0.183	0.67	0.001	0.000037	27 027 027
Hair Styling	0.183	5.74	0.01	0.00037	2 702 703
Skin care					
Body lotion	0.183	123.2	0.226	0.008362	119 589
Face cream	0.183	24.14	0.044	0.001628	614 251
Hand cream	0.18	32.7	0.06	0.00222	450 450
Make-up					
Liquid foundation	0.183	7.9	0.015	0.000555	1 801 802
Lipstick, lip salve	0.18	0.9	0.002	0.000074	13 513 514
Make-up remover	0.18	8.33	0.015	0.000555	1 801 802
Eye shadow	0.183	0.33	0.001	0.000037	27 027 027
Mascara	0.183	0.42	0.001	0.000037	27 027 027
Eyeliner	0.183	0.08	0.0001	0.0000037	270 270 270
Deodorants					
Non-spray	0.15	22.08	0.033	0.001221	819 001
Oral care					
Toothpaste[3]	0.1	2.16	0.002	0.002	500 000
Mouthwash[3]	0.05	32.54	0.016	0.016	62 500
Aggregate			**0.44**	0.0336177	29 746

3.6. DISCUSSION

Physicochemical properties
The data provided on physicochemical properties on propylparaben is not complete. A full report of the chemical characterisation of propylparaben in terms of purity, identity and impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown. The level of impurities varies according to the different batches of products used to perform the studies.

Toxicokinetics
In the absence of an acceptable dermal absorption study according to the SCCS Notes of Guidance (SCCS/2017), the SCCS will use the same dermal absorption value (3.7%) as used in the previous Opinion (SCCS/2013) for the calculation of the MoS.

Exposure
Propylparaben can maximally be used in any cosmetic product up to 0.14% (alone, as acid) or up to a maximum of 0.14% (as acid), as the sum of the individual concentrations of butyl paraben and propylparaben, when used together as a binary mixture in the same product. This would be equivalent to a maximum concentration of propylparaben of 0.183% in cosmetic products.

Toxicological Evaluation
Irritation and corrosivity
Propylparaben is not expected to be an eye or skin irritant.

Skin sensitisation
Propylparaben is not considered to be a skin sensitiser.
Acute toxicity
The LD50 is >5000 mg/kg bw for male and female rats.

Repeated dose toxicity
The newly submitted repeated dose toxicity studies provide a NOAEL of 980 mg/kg bw/d for males and 1076 mg/kg bw/d for females.

Reproductive toxicity
The newly submitted reproductive and devevelopmenral toxicity studies provide data that support a change of the NOEL of 2 mg/kg body weight per day described in the previous SCCS opinion. Additional studies support a new NOAEL derived from reproductive endpoints to be 1000 mg/kg bw/day.

Mutagenicity / genotoxicity
Propylparaben has been tested in valid OECD guideline mutagenicity assays for bacterial and mammalian gene mutations assays, aneuploidy and clastogenicity endpoints with negative results. The SCCS is of the opinion that propylparaben does not pose a genotoxic hazard.

Carcinogenicity
Based on all the available data, the SCCS considers propylparaben to have no carcinogenic potential.

Photo-induced toxicity
Propylparaben is considered to be not phototoxic.

Human data

Biomonitoring data
There is a wealth of human biomonitoring data emerging for parabens indicating that systemic exposure is low.

In urines:
There are many data from men, pregnant women and women who are not pregnant, adolescents and children of different races and from different continents. Considering all the results obtained, the urine concentrations range between around 0.5 to around 60 µg/L.

In plasma:
Data were obtained from 566 women pregnant, neonates and postmenopausal women. Concentrations were from about 2 to about 44 µg/L.

For propylparaben, the reference value in urine set by the German HBM Commission is 100 µg/L for women and 50 µg/L for men (Apel et al 2017), reflecting the general difference between men and women in that women generally use more personal care products than men.

Further work is needed to assess whether the available exposure models agree with the current measured values in blood and plasma from human biomonitoring in European populations.

Human evidence on the reproductive and developmental effects of propylparaben
Few human studies have indicated weak associations between increased paraben exposure and the markers for human reproductive health.

Special investigations

Endocrine activity
The SCCS is of the view that, although the available data on propylparaben provide some indications for potential endocrine effects, the current level of evidence is not sufficient to conclusively regard it as an endocrine disrupting substance, or to derive a specific endocrine-related toxicological point of departure for use in safety assessment. A NOAEL of 1000 mg/kg bw/day has been derived from the pivotal study covering the potential for adverse effects in the intact organism by Gazin et (2013) and is used for this safety assessment.

The SCCS mandates do not address environmental aspects. Therefore this assessment did not cover the safety of Propylparaben for the environment.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Propylparaben, does the SCCS consider Propylparaben safe when used as a preservative in cosmetic products up to a maximum concentration of 0.14 %?*

On the basis of the safety assessment of Propylparaben, and considering the concerns related to potential endocrine disrupting properties, the SCCS has concluded that propylparaben is safe when used as a preservative in cosmetic products up to a maximum concentration of 0.14 %.

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Propylparaben as a preservative in cosmetic products?*

/

3. *Does the SCCS have any further scientific concerns with regard to the use of Propylparaben in cosmetic products?*

The available data on Propylparaben provide some indications for potential endocrine effects. However, the current level of evidence is not sufficient to regard it as an endocrine disrupting substance, or to derive a toxicological point of departure based on endocrine disrupting properties for use in human health risk assessment.

The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of propylparaben for the environment.

5. MINORITY OPINION

/

6. REFERENCES

Abbas S, Greige-Gerges H, Karam N, Piet MH, Netter P, Magdalou J (2010). Metabolism of parabens (4-hydroxybenzoic acid esters) by hepatic esterases and UDP-glucuronosyltransferases in man. Drug Metab Pharmacokinet. 25(6):568-77. Epub 2010 Oct 1.

Ahn HJ, An BS, Jung EM, Yang H, Choi KC, Jeung EB (2012) Parabens inhibit the early phase of folliculogenesis and steroidogenesis in the ovaries of neonatal rats. Mol Reprod Dev 79(9):626-36. doi: 10.1002/mrd.22070. Epub 2012 Jul 26.

Apel P, Angerer J, Wilhelm M, Kolossa-Gehring M (2017) New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. Int J Hyg Environ Health. 2017 Mar; 220(2 Pt A):152-166. doi: 10.1016/j.ijheh.2016.09.007. Epub 2016 Sep 17. PMID:27914867

Artacho-Cordón F, Fernández MF, Frederiksen H, Iribarne-Durán LM, Jiménez-Díaz I, Vela-Soria F, Andersson AM, Martin-Olmedo P, Peinado FM, Olea N, Arrebola JP (2018) Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. Environ Int. 2018 Oct;119:203-211. doi: 10.1016/j.envint.2018.05.052. Epub 2018 Jul 4. PMID:29980043

Aubert N (2009). Blood Plasma Pharmacokinetics and Mass Balance of Total Radioactivity in Sprague-Dawley Rats Following Single Administration of Three Different Parabens (Methyl-, Butyl-, Propyl-) by Three Different Routes of Administration (Oral, Dermal, Sub-Cutaneous). CIT, Centre International de Toxicologie, Evreux, France. Study Report No. 34851 PAR, 26 November, 2009.

Aubert N, Ameller T, Legrand JJ. (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 Chem Toxicol. 2012 Mar;50(3-4):445-54. doi: 10.1016/j.fct.2011.12.045. Epub 2012 Jan 12.

Barr L, Metaxas G, Harbach CA, Savoy LA, and Darbre PD.(2012) Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum. *J.Appl.Toxicol.* 2012;32(3):219-232.

Basketter DA, Scholes EW. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol. 1992 Jan; 30(1):65-9. PubMed PMID: 1544608.

Basketter DA, Scholes EW, Kimber I. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Food Chem Toxicol. 1994 Jun; 32(6):543-7. PubMed PMID: 8045461.

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Tong W, Shi L, Perkins R, Sheehan DM (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. Toxicol Sci. 2000 Mar; 54(1):138-53.

Boberg J, Taxvig C, Christiansen S, Hass U (2010). Possible endocrine disrupting effects of parabens and their metabolites. Reproductive Toxicology 30: 301-312. PMID:20381602

Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R (2014) Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. J Expo Sci Environ Epidemiol. 2014 Sep-Oct;24(5):459-66. doi: 10.1038/jes.2013.69. Epub 2013 Oct 23.

Brusick (Litton Bionetics) (1975). Mutagenic Evaluation of Compound FDA 73—68, Propyl Paraben (USP). NTIS Report PB-245 498.

Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. (2002) Oestrogenic activity of parabens in MCF7 human breast cancer cells. J Steroid Biochem Mol Biol.;80(1):49-60.

Calafat Antonia M, Ye Xiaoyun, Wong Lee-Yang, Bishop Amber M, Needham Larry L (2010). Urinary Concentrations of Four Parabens in the U.S. Population: NHANES 2005-2006. Environ Health Perspect. 118(5):679-85.

Campbell JL, Yoon M, Clewell HJ.(2015) A case study on quantitative *in vitro* to *in vivo* extrapolation for environmental esters: Methyl-, propyl- and butylparaben. Toxicology. 2015 Jun 5; 332:67-76. doi: 10.1016/j.tox.2015.03.010. Epub 2015 Mar 31. PubMed PMID: 25839974.

Casas L, Fernández MF, Llop S, Guxens M, Ballester F, Olea N, Irurzun MB, Rodríguez LS, Riaño I, Tardón A, Vrijheid M, Calafat AM, Sunyer J; INMA Project.(2011) Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int. 2011 Jul; 37(5):858-66. doi: 10.1016/j.envint.2011.02.012. Epub 2011 Mar 25. PubMed PMID: 21440302.

Centers for Disesae Control and Prevention (CDC). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2017. 2017, www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf. Accessed 4/20/2018. Pages1-656.

Chen J, Ahn KC, Gee NA, Gee SJ, Hammock BD, Lasley BL (2007). Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. Toxicol Appl Pharmacol. 221(3):278-84.

Cosmetics Ingredient Review (CIR) (2008) by Andersen FA. Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products. International Journal of Toxicology. 2008; 27(Suppl. 4):1-82

Cosmetics Ingredient Review (CIR) (2019). Amended Safety Assessment of Parabens as Used in Cosmetics. Revised Tentative Amended Report for Public Comment, https://online.personalcarecouncil.org/jsp/WhatsNewPage.jsp

Cowan-Ellsberry CE and Robison SH (2009). Refining aggregate exposure: example using parabens. Regul Toxicol Pharmacol. 55(3):321-9

CTFA. (April, 1979). Submission of unpublished data. CIR safety data test summary: oral, dermal, and ocular testing of product containing Methylparaben and Propylparaben. (CTFA Code No. 2-7-15).

Hass, U., Christiansen, S., Petersen, M. A., Boberg, J., Andersson, A-M., Skakkebæk, N. E., Bjerregaard, P. (2012). Evaluation of 22 SIN List 2.0 substances according to the Danish proposal on criteria for endocrine disrupters. DTU Food.

Darbre P.D., Aljarrah A., Miller W.R., Coldham N.G., Sauer M.J., Pope G.S. Concentrations of parabens in human breast tumours. J Applied Toxicology 24(1), 5-13 (2004).
Dewalque L, Pirard C, Charlier C (2014) Measurement of urinary biomarkers of parabens, benzophenone-3, and phthalates in a Belgian population, Biomed Res Int. 2014:649314. doi: 10.1155/2014/649314. Epub 2014 Feb 25.

Ding K, Kong X, Wang J, Lu L, Zhou W, Zhan T, Zhang C, Zhuang S (2017) Side Chains of Parabens Modulate Antiandrogenic Activity: *In Vitro* and Molecular Docking Studies. Environ Sci Technol. 2017 Jun 6; 51(11):6452-6460. doi: 10.1021/acs.est.7b00951. Epub 2017 May 9.

Directive 2006/52/EC OF THE European Paeliament and of the council of 5 July 2006 amending Directive 95/2/EC on food additives other than colours and sweeteners and Directive 94/35/EC on sweeteners for use in foodstuffs.

Dodge LE, Kelley KE, Williams PL, Williams MA, Hernández-Díaz S, Missmer SA, Hauser R. (2015) Medications as a source of paraben exposure. Reprod Toxicol. Apr; 52:93-100. doi: 10.1016/j.reprotox.2015.02.002. Epub 2015 Feb 26. PubMed PMID: 25728410; PubMed Central PMCID: PMC4426043.

EC Cosmetic Products Regulation EC 1223/2009 of the European Union ECHA/EFSA (2018). Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018; 16(6):5311

EFSA (2004) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a Request from the Commission related to para hydroxybenzoates (E 214-219). The EFSA Journal (2004) 83, 1-26.

Elder RL. Final report on the safety assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. Journal of the American College of Toxicology. 1984; 3(5):147-209.

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

Engeli RT, Rohrer SR, Vuorinen A, Herdlinger S, Kaserer T, Leugger S, Schuster D, Odermatt A (2017) Interference of Paraben Compounds with Estrogen Metabolism by Inhibition of 17β-Hydroxysteroid Dehydrogenases. Int J Mol Sci. 2017 Sep 19;18(9). pii: E2007. doi: 10.3390/ijms18092007.

European Chemicals Agency (ECHA). Propyl 4-hydroxybenzoate (Propylparaben) CAS No. 94-13-3. https://echa.europa.eu/registration-dossier/-/registered-dossier/13890

Fasano W.J. Butylparaben: *In Vitro* Dermal Penetration and Metabolism Using Full Thickness Human Skin. E.I. du Pont de Nemours and Company, HaskellSM Laboratory for Health and Environmental Sciences, Report November 17, 2004a

Fasano W.J. Methylparaben and Butylparaben: *In Vitro* Dermal Penetration and Metabolism in Rat and Human Skin. E.I. du Pont de Nemours and Company, Haskell SM Laboratory for Health and Environmental Sciences, Report November 22, 2004b

Fasano W.J. Butylparaben: *In Vitro* Kinetics and Metabolism Using Full Thickness Human Skin. E.I. du Pont de Nemours and Company, HaskellSM Laboratory for Health and Environmental Sciences, Report August 29, 2005

FDA (2006) Guidance for industry: nonclinical safety evaluation of pediatric drug products, February 2006.

Ferguson Kelly K, Colacino Justin A, Lewis Ryan C, Meeker John D. Personal Care Product Use Among Adults in NHANES: Associations Between Urinary Phthalate Metabolites and Phenols and Use of Mouthwash and Sunscreen. J Expo Sci Environ Epidemiol. 2017 May; 27(3):326-332.

Fourth National Report on Human Exposure to Environmental Chemicals – USA (2019) https://online.personalcarecouncil.org/jsp/WhatsNewPage.jsphttps://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf

Fransway AF, Fransway PJ, Belsito DV, Yiannias JA. Paraben Toxicology. *Dermatitis* 2019; 30(1):32-45.

Fransway AF, Fransway PJ, Belsito DV, et al. Parabens. *Dermatitis* 2018; 30(1):3-31.

Frederiksen H, Jørgensen N, Andersson AM. (2011) Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). J Expo Sci Environ Epidemiol. 2011 May-Jun; 21(3):262-71. doi: 10.1038/jes.2010.6. Epub 2010 Mar 10

Frederiksen H, Nielsen JK, Mørck TA, Hansen PW, Jensen JF, Nielsen O, Andersson AM, Knudsen LE.(2013) Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs. Int J Hyg Environ Health. 216(6):772-83. doi: 10.1016/j.ijheh.2013.02.006. Epub 2013 Mar 13. PubMed PMID: 23528233.

Frederiksen H, Jensen TK, Jørgensen N, Kyhl HB, Husby S, Skakkebæk NE, Main KM, Juul A, Andersson AM. (2014) Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. Reproduction. 2014 Mar 4; 147(4):555-65. doi: 10.1530/REP-13-0522. Print 2014.

Gal A, Gedye K, Craig ZR, Ziv-Gal A (2019) Propylparaben inhibits mouse cultured antral follicle growth, alters steroidogenesis, and upregulates levels of cell-cycle and apoptosis regulators. Reprod Toxicol. 2019 Jul 12; 89:100-106. doi: 10.1016/j.reprotox.2019.07.009. [Epub ahead of print]

Gazin V, Marsden E, Marguerite F. (Sept 2013) Oral propylparaben administration to juvenile male Wistar rats did not induce toxicity in reproductive organs. Toxicol Sci. 2013 Dec; 136(2):392-401. doi: 10.1093/toxsci/kft211.

Géniès C, Jamin EL, Debrauwer L, Zalko D, Person EN, Eilstein J, Grégoire S, Schepky A, Lange D, Ellison C, Roe A, Salhi S, Cubberley R, Hewitt NJ, Rothe H, Klaric M, Duplan H, Jacques-Jamin C (2019) Comparison of the metabolism of 10 chemicals in human and pig skin explants. J Appl Toxicol. 39(2):385-397. doi: 10.1002/jat.3730. Epub 2018 Oct 21.

Genuis SJ, Birkholz D, Curtis L, Sandau C (2013) Paraben levels in an urban community of Western Canada. ISRN Toxicol. 2013 Dec 17; 2013:507897. doi: 10.1155/2013/507897. eCollection 2013 Dec 17.

Gimenez-Arnau AM, Deza G, Bauer A, et al. Contact allergy to preservatives: ESSCA* results with the baseline series, 2009-2012. *J Eur Acad Dermatol Venereol* 2017;31(4):664-671.

Golden R, Gandy J (2004) Letters to the Editor. J Appl Toxicol 24: 297-306

Gosens I, Delmaar CJ, Ter Burg W, de Heer C, Schuur AG (2014) Aggregate exposure approaches for parabens in personal care products: a case assessment for children between 0 and 3 years old. J Expo Sci Environ Epidemiol. 2014 Mar-Apr;24(2):208-14. doi: 10.1038/jes.2013.33. Epub 2013 Jun 26. RIVM study.

Güzel Bayülken D, Ayaz Tüylü B.(2019) *In vitro* genotoxic and cytotoxic effects of some paraben esters on human peripheral lymphocytes. Drug Chem Toxicol. 2019 Jul;42(4):386-393. doi: 10.1080/01480545.2018.1457049. Epub 2018 Apr 23.

Harlan (2012) 28 day repeat dosing study. Study owned by Clariant and review in EU REACH dossier.

Harley KG, Kogut K, Madrigal DS, Cardenas M, Vera IA, Meza-Alfaro G, She J, Gavin Q, Zahedi R, Bradman A, Eskenazi B, Parra KL. (2016). Reducing phthalate, paraben, and phenol exposure from personal care products in adolescent girls: findings from the HERMOSA Intervention Study. Environ Health Perspect 124:1600-1607; http://dx.doi.org/10.1289/ehp.1510514.

Harvey PW, Everett DJ. Parabens detection in different zones of the human breast: consideration of source and implications of findings. J Appl Toxicol. 2012 May;32(5):305-9. doi: 10.1002/jat.2743. Epub 2012 Mar 7. PubMed PMID:22408000.

Heppenheimer (2012). Bovine cornea study owned by Clariant and reviewed in EU REACH dossier.

Hewitt NJ, Grégoire S, Cubberley R, et al. Measurement of the penetration of 56 cosmetic relevant chemicals into and through human skin using a standardized protocol. *J Appl Toxicol*. 2020; 40:403–415.

Hossaini A, Larsen JJ, Larsen JC. (2000) Lack of oestrogenic effects of food preservatives (parabens) in uterotrophic assays. Food Chem Toxicol.; 38(4):319-23.

Hu P, Chen X, Whitener RJ, Boder ET, Jones JO, Porollo A, Chen J, Zhao L. (2013) Effects of parabens on adipocyte differentiation. Toxicol Sci. 2013 Jan; 131(1):56-70. doi: 10.1093/toxsci/kfs262. Epub 2012 Sep 5.

Jain L, Po LW. (1993) Kinetic evaluation of the ciliotoxicity of methyl- and propyl-p-hydroxybenzoate using factorial experiments. J Pharm Phurmacol45:98-101.

Kim TS, Yoon CY, Jung KK, Kim SS, Kang IH, Baek JH, Jo MS, Kim HS, Kang TS. (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. 2010 Apr; 35(2):239-43.

Kim TS, Kim CY, Lee HK, Kang IH, Kim MG, Jung KK, Kwon YK, Nam HS, Hong SK, Kim HS, Yoon HJ, Rhee GS. (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. 2011 Sep; 27(3):181-4. doi: 10.5487/TR.2011.27.3.181.

Klopčič I, Kolšek K, Dolenc MS (2015) Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. Toxicol Lett. 2015 Jan 22; 232(2):376-83. doi: 10.1016/j.toxlet.2014.11.019. Epub 2014 Nov 21.

Kolšek K, Gobec M, Mlinarič Raščan I, Sollner Dolenc M (2015) Screening of bisphenol A, triclosan and paraben analogues as modulators of the glucocorticoid and androgen receptor activities. Toxicol *In Vitro*. 2015 Feb; 29(1):8-15. doi: 10.1016/j.tiv.2014.08.009. Epub 2014 Sep 2.

LEBERCO LABORATORIES (Sept. 8, 1978). Submission of unpublished data by CTFA. Primary skin irritation test of protective face cream containing Propylparaben (CTFA Code N° 2-7-66). Lemini C, Jaimez R, Avila ME, Franco Y, Larrea F, Lemus AE (2003) *In vivo* and *in vitro* estrogen bioactivities of alkyl parabens. Toxicol Ind Health 19(2-6):69-79.

Lemini C, Hernández A, Jaimez R, Franco Y, Avila ME, Castell A. (2004) Morphometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben. Toxicol Ind Health. 2004 Sep; 20(6-10):123-32.

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

Matthews, C., J. Davidson, E. Bauer, et al. (1956). p-hydroxybenzoic acid esters as preservatives. II. Acute and chronic toxicity in dogs, rats, and mice. J. Am. Pharm. Assoc. Sci. Ed. 45:260—267.

McCann, J E. Choi., E. Yamasaki, and B. N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test; Assay of 300 chemicals. Proc. Nat. Acad. Sci. U.S.A. 72:5135—5139.

Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Raaij MV, Vickers C (2011) Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regulatory Toxicology and Pharmacology 60 (2011) S1–S14.

Meeker JD, Cantonwine DE, Rivera-González LO, Ferguson KK, Mukherjee B, Calafat AM, Ye X, Anzalota Del Toro LV, Crespo-Hernández N, Jiménez-Vélez B, Alshawabkeh AN, Cordero JF.(2013)Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. Environ Sci Technol. 2013 Apr 2;47(7):3439-47. doi: 10.1021/es400510g. Epub 2013 Mar 19. PubMed PMID: 23469879; PubMed Central PMCID: PMC3638245.

Miller D, Brian B, Wheals BB, Beresford N, Sumpter JP. (2001). Estrogenic activity of phenolic additives determined by an *in vitro* yeast bioassay. Environ Health Perspect 109:133–138.
Moos RK, Angerer J, Wittsiepe J, Wilhelm M, Brüning T, Koch HM (2014) Rapid determination of nine parabens and seven other environmental phenols in urine samples of German children and adults. Int J Hyg Environ Health. 2014 Nov; 217(8):845-53. doi: 10.1016/j.ijheh.2014.06.003. Epub 2014 Jun 20.

Moos RK, Koch HM, Angerer J, Apel P, Schröter-Kermani C, Brüning T, Kolossa-Gehring M. (2015) Parabens in 24 h urine samples of the German Environmental Specimen Bank from 1995 to 2012. Int J Hyg Environ Health. 2015 Oct; 218(7):666-74. doi: 10.1016/j.ijheh.2015.07.005. Epub 2015 Jul 29.

Mulla H, Yakkundi S, McElnay J, Lutsar I, Metsvaht T, Varendi H, Nellis G, Nunn A, Duncan J, Pandya H, Turner M.(2015) An observational study of blood concentrations and kinetics of methyl- and propyl-parabens in neonates. Pharm Res. 2015 Mar; 32(3):1084-93. doi: 10.1007/s11095-014-1520-2. Epub 2014 Sep 19. PubMed PMID: 25236342.

Nassan FL, Coull BA, Gaskins AJ, Williams MA, Skakkebaek NE, Ford JB, Ye X, Calafat AM, Braun JM, Hauser R (2017) Personal Care Product Use in Men and Urinary Concentrations of Select Phthalate Metabolites and Parabens: Results from the Environment And Reproductive Health (EARTH) Study. Environ Health Perspect. 2017 Aug 18; 125(8):087012. doi: 10.1289/EHP1374.

Ohta R., Takagi A, Ohmukai H, Marumo H, Ono A, Matsushima Y, Inoue T, Ono H, and Kanno J. (2012) Ovariectomized mouse uterotrophic assay of 36 chemicals. *J.Toxicol.Sci.* 2012; 37(5):879-889.

Oishi S (2002) Effects of propyl paraben on the male reproductive system. Food Chem Toxicol 2002: 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 ERalpha and PR. Food Chem Toxicol. 2001 Dec; 39(12):1225-32.

Ozaki H, Sugihara K, Watanabe Y, Ohta S, Kitamura S (2016) Cytochrome P450-inhibitory activity of parabens and phthalates used in consumer products. J Toxicol Sci. 2016; 41(4):551-60. doi: 10.2131/jts.41.551.

Özdemir E et al (2018). Assessing the antiandrogenic properties of propyl paraben using the Hershberger bioassay. Toxicol. Res. 7, 235-243

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 & Toxicology 86, 110-113.

Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TL, Jorens PG, Blust R, Vanparys C. (2013) Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PLoS One. 2013 Oct 14;8(10):e77481. doi: 10.1371/journal.pone.0077481. eCollection 2013.

Perez Martin JM, Peropadre A, Herrero O, Fernandez FP, Labrador V, and Hazen MJ.(2010) Oxidative DNA damage contributes to the toxic activity of propylparaben in mammalian cells. *Mutat.Res.* 9-30-2010; 702(1):86-91.

Philippat C, Wolff MS, Calafat AM, Ye X, Bausell R, Meadows M, Stone J, Slama R, Engel SM.(2013) Prenatal exposure to environmental phenols: concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. Environ Health Perspect. 2013 Oct; 121(10):1225-31. doi: 10.1289/ehp.1206335. Epub 2013 Aug 13

Prusakiewicz JJ, Harville HM, Zhang Y, Ackermann C, Voorman RL (2007). Parabens inhibit human skin estrogen sulfotransferase activity: possible link to paraben estrogenic effects. Toxicology. 232(3):248-56.

Ricerca Biosciences SAS (2011). Propylparaben – Pilot toxicokinetic study by the oral route (gavage) in the juvenile rat. Study No. AA88670.

Ricerca Biosciences SAS (2012a). Propylparaben – Toxicokinetic study by the oral route 54 (gavage) in the juvenile rat. Study No. AA85354.

Ricerca Biosciences SAS (2012b). Propylparaben – Analytical method validation to determine test item concentrations in formulations. Study No. AA85355.

Ricerca Biosciences SAS (2012c). Propylparaben – Validation of a LC-MS/MS bioanalytical method in rat plasma. Study No. AA85356.

Ricerca Biosciences SAS (2012d). Propylparaben – 8-week post-weaning juvenile toxicity study with 26-week treatment-free period in the male Wistar rat by the oral route (gavage). Study No. AA85148.

Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP (1998). Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicol Appl Pharmacol. 153(1):12-9.

Sabatini ME, Smith KW, Ford J, Ehrlich SR, Toth TL, Hauser R (2011) Urinary paraben concentrations and *in vitro* ferterlization (IVF) outcomes. Fertility and Sterility 96 (3), Suppl.: 1, S154-S154.

Sandanger TM, Huber S, Moe MK, Braathen T, Leknes H, Lund E. (2011) Plasma concentrations of parabens in postmenopausal women and self-reported use of personal care products: the NOWAC postgenome study. J Expo Sci Environ Epidemiol. 2011 Nov-Dec; 21(6):595-600. doi: 10.1038/jes.2011.22. Epub 2011 May 25.

Seoul National University Institutional Review Board (IRB No. 27-2013-0520 and 1508/001-007)

SCCP/0873/05 - The Scientific Committee on Consumer Products (SCCP) Extended Opinion on the Safety Evaluation of Parabens, adopted by the SCCP by written procedure on 28 January 2005
https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_019.pdf

SCCP/0874/05 - The Scientific Committee on Consumer Products (SCCP) Extended Opinion on Parabens, underarm cosmetics and breast cancer, adopted by the SCCP by written procedure on 28 January 2005
https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_00d.pdf

SCCP/1017/06 - The Scientific Committee on Consumer Products (SCCP) Opinion on Parabens (Colipa n° P82), adopted during the 9th plenary meeting of 10 October 2006. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_074.pdf

SCCP/1183/08 - The Scientific Committee on Consumer Products (SCCP) Opinion on Parabens (Colipa n° P82), adopted during the 16th plenary meeting of 24 June 2008. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_138.pdf

SCCS/1348/10 Scientific Committee on Consumer Safety (SCCS) (2011). Opinion on parabens. 14 December 2010, revision of 22 March 2011, https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_041.pdf

SCCS/1446/11 Scientific Committee on Consumer Safety (SCCS) (2011). Clarification on Opinion SCCS/1348/10 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, https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_069.pdf

SCCS/1514/13 Scientific Committee on Consumer Safety (SCCS) (2013). Opinion on parabens; Updated request for a scientific opinion on propyl- and butylparaben, COLIPA No P82. European Commission. 2013. Report No. SCCS/1514/13. pp. 1-50. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_132.pdf

SCCS/1602/18 Scientific Committee on Consumer Safety (SCCS) (2018). The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation 10th revision. https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_224.pdf

SCCS/1358/10 Scientific Committee on Consumer Safety (SCCS) (2010). Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_002.pdf

Shaw J, deCatanzaro D (2009) Estrogenicity of parabens revisited: impact of parabens on early pregnancy and an uterotrophic assay in mice. Reprod Toxicol 28(1):26D31.

Shekhar S, Sood S, Showkat S, Lite C, Chandrasekhar A, Vairamani M, Barathi S, Santosh W (2017) Detection of phenolic endocrine disrupting chemicals (EDCs) from maternal blood plasma and amniotic fluid in Indian population. Gen Comp Endocrinol. 2017 Jan 15; 241:100-107. doi: 10.1016/j.ygcen.2016.05.025. Epub 2016 May 25.

Shin MY, Shin C, Choi JW, Lee J, Lee S, Kim S (2019) Pharmacokinetic profile of propyl paraben in humans after oral administration. Environ Int. Jun 21; 130:104917. doi: 10.1016/j.envint.2019.104917.

Shirai S, Suzuki Y, Yoshinaga J, Shiraishi H, Mizumoto Y.(2013) Urinary excretion of parabens in pregnant Japanese women. Reprod Toxicol. 2013 Jan; 35:96-101. doi: 10.1016/j.reprotox.2012.07.004. Epub 2012 Jul 10. PubMed PMID: 22789395.

Sivaraman L, Pouliot L, Wang B, Brodie B, Graziano M, McNerney M.E. (2018) Safety assessment of propylparaben in juvenile rats. Regul Toxicol Pharmacol. 2018 Feb; 92:370-381 doi: 10.1016/j.yrtph.2017.12.009. Epub 2017 Dec 15

Smith KW, Dimitriadis I, Ehrlich S, Ford J, Berry KF, Souter I (2011) The association of urinary paraben concentrations with measures of ovarian reserve among patients from a fertility center. Fertility and Sterility. 96 (3), Suppl.: 1, S197-S197

Snodin, D., Regulatory risk assessments: Is there a need to reduce uncertainty and enhance robustness? Human and Experimental Toxicology 2015, Vol. 34(12) 1258–1271

Snodin, D., Regulatory risk assessments: Is there a need to reduce uncer-tainty and enhance robustness? Update on propylparaben in relation to its EU regulatory status. Hum Exp Toxicol, 2017. 36(10): pp. 1007–14.

Sokol. H. 1952. Recent developments in the preservation of pharmaceuticals. Drug Standards 20:89—106.

Soni MG, Carabin IG, Burdock GA (2005). Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food and Chemical Toxicology. 43: 985-1015.

Sugimura, T., S. Sato, M. Nagao, et al. (1976). Overlapping of carcinogens and mutagens. In: Fundamentals in Cancer Prevention (Magee, P. N., T. Mutsushima, T. Sugimura, and S. Takayama, eds.). University of Tokyo Press, Tokyo, and University Park Press, Baltimore, Maryland, pp. 191—215.

Tayama S, Nakagawa Y, Tayama K.(2008) Genotoxic effects of environmental estrogen-like compounds in CHO-K1 cells. Mutat Res. 2008 Jan 8; 649(1-2):114-25. Epub 2007 Aug 19.

Terasaka S, Inoue A, Tanji M, Kiyama R (2006). Expression profiling of estrogen-responsive

genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity. Toxicol. Letters 163:130–141.

Tonning K, Jacobsen E, Pedersen E, Strange M, Brunn Poulsen P, Moller L et al. Survey and Health Assessment of the exposure of 2 year-olds to chemical substances in Consumer Products. Danish Ministry of the Environment, Copenhagen, Denmark, 2009, pp 1–327. https://www2.mst.dk/udgiv/publications/2009/978-87-92548-81-8/pdf/978-87-92548-82-5.pdf

US EPA Endocrine Screening program 2019. Ref DTXSID4022527

US NHANES program. National Health & Nutrition Examination Survey (NHANES) (the Fourth National Report)

Van Meeuwen JA, van Son O, Piersma AH, de Jong PC, van den Berg M (2008). Aromatase inhibiting and combined estrogenic effects of parabens and estrogenic effects of other additives in cosmetics. Toxicol Appl Pharmacol. 2008 Aug 1; 230(3):372-82.

Vo TTB and Jeung EB (2009) An evaluation of estrogenic activity of parabens using uterine calbindin-D9k gene in an immature rat model. Tox Sci 112(1), 68–77

Vo TTB, Yoo YM, Choi KC, Jeung EB (2010) Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reproductive Toxicology 29:306-316.

Walters RM, Khanna P, Hamilton M, Mays DA, Telofski L. Human Cumulative Irritation Tests of Common Preservatives Used in Personal Care Products: A Retrospective Analysis of Over 45 000 Subjects. *Toxicol Sci* 2015; 148(1):101-107.

Watanabe Y, Kojima H, Takeuchi S, Uramaru N, Ohta S, Kitamura S. (2013) Comparative study on transcriptional activity of 17 parabens mediated by estrogen receptor α and β and androgen receptor. Food Chem Toxicol. 2013 Jul; 57:227-34. doi: 10.1016/j.fct.2013.03.036. Epub 2013 Apr 6.

Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F et al. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. Environ Health Perspect 2010 July; 118(7):1039-46.

Wróbel A & Gregoraszczuk EŁ (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. Pharmacol Rep. 2013; 65(2):484-93.

Wróbel AM, Gregoraszczuk EŁ (2014a) Differential effect of methyl-, butyl- and propylparaben and 17β-estradiol on selected cell cycle and apoptosis gene and protein expression in MCF-7 breast cancer cells and MCF-10A non-malignant cells. J Appl Toxicol. 2014 Sep; 34(9):1041-50. doi: 10.1002/jat.2978. Epub 2014 Jan 30.

Wróbel AM, Gregoraszczuk EŁ (2014b) 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. 2014 Nov 4; 230(3):375-81. doi: 10.1016/j.toxlet.2014.08.012. Epub 2014 Aug 13.

Yakkundi S, Mulla H, Pandya H, Turner MA, McElnay J. (2016) Quantitative analysis of methyl and propyl parabens in neonatal DBS using LC-MS/MS. Bioanalysis. 2016 Jun;8(11):1173-82. doi: 10.4155/bio-2016-0029. Epub 2016 May 23. PubMed PMID: 27212021.

Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM (2006). Parabens as urinary biomarkers 25 of exposure in humans. Environ Health Perspect. 114(12): 1843-6.

Zhang Z, Sun L, Hu Y, Jiao J, Hu J. (2013) Inverse antagonist activities of parabens on human oestrogen-related receptor γ (ERRγ): *in vitro* and in silico studies. Toxicol Appl Pharmacol. 2013 Jul 1; 270(1):16-22. doi: 10.101

7. ANNEX

Table A-1: Biomonitoring studies

Study details	Observations	Reference
Urine measurements of parent propylparaben		
100 Latina adolescent girls who reported i) using makeup every day vs. ii) rarely/never (**USA**)	PP i) **60.4 µg/L** vs. ii) 2.9 µg/L; P-value <0.01	Berger et al 2019
Total population (**USA**) 2005-2006 (n = 2548) 2007-2008 (n = 2604) 2009-2010 (n = 2749) 2011-2012 (n = 2489) 2013-2104 (n = 2686) Children (age 6-11 years) 2005-2006 (n = 356) 2007-2008 (n = 389) 2009-2010 (n = 415) 2011-2012 (n = 396) 2013-2104 (n = 409) Males 2005-2006 (n = 1270) 2007-2008 (n = 1294) 2009-2010 (n = 1399) 2011-2012 (n = 1259) 2013-2104 (n = 1285) Females 2005-2006 (n = 1278) 2007-2008 (n = 1310) 2009-2010 (n = 1350) 2011-2012 (n = 1230) 2013-2104 (n = 1401)	**Geometric Mean** µg/L (95% conf. interval) Total population 2005-2006 7.91 (6.41-9.77) 2007-2008 7.59 (6.22-9.26) 2009-2010 6.97 (5.96-8.16) 2011-2012 5.39 (4.69-6.19) 2013-2104 5.74 (5.06-6.50) Children (age 6-11 years) 2005-2006 3.41 (2.43-4.77) 2007-2008 3.61 (2.39-5.44) 2009-2010 3.28 (2.58-4.17) 2011-2012 2.20 (1.81-2.68) 2013-2104 2.96 (2.20-3.99) Males 2005-2006 2.96 (2.33-3.77) 2007-2008 3.02 (2.47-3.71) 2009-2010 2.77 (2.26-3.40) 2011-2012 2.44 (1.99-2.98) 2013-2104 2.61 (2.20-3.09) Females 2005-2006 20.4 (16.0-25.9) 2007-2008 18.4 (15.7-21.5) 2009-2010 16.9 (14.6-19.4) 2011-2012 11.6 (10.0-13.4) 2013-2104 12.2 (10.6-14.1)	Fourth report - USA NHANES updated urine exposure data from 2019.
Four hundred men contributed 1,037 urine samples (mean of 3/man) (**USA**)	Median **2.3 µg/L**	Nassan et al 2017
100 Latina girls asked to choose to use paraben free cosmetics for 3 days. HERMOSA Intervention Study (**USA**)	Urine levels of PP dropped by 45.4%	Harley et al 2016
Neonates (196) dried blood spot (DBS) samples (n=927 measurements) from **4 sites in the UK and 1 in Estonia**.	621/927 - below the LOD (10 ng/ml) 178/927 – between 10-19 ng/ml 128/927 above LOQ (20 ng/ml) (7/83 in Estonia; 121/844 UK)	Yakkundi et al 2016
660 24h urine samples from the German Environmental Specimen Bank (ESB) (**Germany 1995 to 2012**)	Short chain parabens detected in 79-99% of samples median **4.8 µg/L** 95th percentile **74.0 µg/L**	Moos et al 2015

Self reported personal care product use in 24h before urine analysis of propylparaben in 177 pregnant women from a fertility clinic (**Boston, USA**)	Most women provided 2 (41%) or 3 (40%) urine samples. LOD (~0.1 to ~1 µg/L). Median values **31.8-39.9 µg/L** (99% detect rate)	Braun et al 2014 (data in supplemental material)
123 males (age 1-75); 138 females (age 2-85) and 48 children (age 1-11). (**Belgium 2013**)	Males – **0.5 µg/L** (median) Female – **3.3 µg/L** (median) Males – **20.2 µg/L** (95th%ile) Female – **116.5 µg/L** (95th%ile)	Dewalque et al 2014
Mother-child pairs: Children (age 6-11 yrs) n = 143; Mothers (age 31-52) n = 145. Pregnant women. (**Denmark 2011**)	Children - **1.7 µg/L** (median) Mothers - <LOD Pregnant women - **2 µg/L** (median)	Frederiksen et al 2013; Frederiksen etal 2014
98 Mother and child (age 6-11) couples (**Sweden**)	Mothers – **13.9 µg/L** Children – **2.1 µg/L**	Larsson et al 2014
157 spot urine samples general population (59 females, 39 males and 59 children) (**Germany**)	Median **1.2 µg/L** 95th%ile **68.1 µg/L**	Moos et al 2014
39 consecutive patients in an Alberta primary care clinic (Canada)	28 female patients (including 9 pregnant women) – median **2.8 µg/L** 11 male patients – median **3.09 µg/L** 1 person reported at 612 µg/L	Genuis et al 2013
Pregnant women n=105 (Puerto Rico 2010-2012)	**36.7 µg/L** (median)	Meeker et al 2013
SARAEH study Pregnant women (n =71) (**France 2005-2008**)	Median Urine **28.7 µg/L** (1st trimester) **45.6 µg/L** (2nd trimester) **36.5 µg/L** (3rd trimester) 95th%ile urine – **424 µg/L** (1st trimester) **531 µg/L** (2nd trimester) **589 µg/L** (3rd trimester) Amniotic fluid – median **0.3 µg/L**; 95th%ile **1.4 µg/L**	Philippat et al 2013
Pregnant women – average age 32.6 yrs n= 111 (**Japan 2007-2010**)	Median **20.2 µg/L**	**Shirai et al 2013**
One spot urine sample was taken during the third trimester of pregnancy from 120 pregnant women and from 30 4-year old boys belonging to 5 birth cohorts (**Spain 2004 – 2008**)	4 year old boys – **21.5 µg/L** (median) Pregnant women – **29.8 µg/L** (median)	**Casas et al 2011**
Males (age 18-26) n=60 (**Denmark 2006**)	**3.6 µg/L** (median)	Fredericksen et al 2011
Propylparaben measured in 100 urine samples collected between 2003 and 2005 from 100 adult anonymous volunteers with no known occupational exposure to parabens	PP (free) <LOD 0.18 µg/L PP total 9.1 µg/L (median) PP (glucuronide) 3.2 µg/L (median) PP (sulfate) 5.2 µg/L (median) **PP (free) was only 2% of all PP-related measures**. Urinary excretion of conjugated species reduces the bioavailable concentration of the parent PP for effecting target organ toxicity.	Ye et al 2006

Plasma concentrations of parent propylparaben		
Maternal blood and amniotic fluid were collected from 53 pregnant women at full term (**India**)	Maternal blood - **19.22 µg/L** Amniotic fluid- **18.82 µg/L**	Shekhar et al 2017
841 blood concentration data were available for evaluation from 181 pre- and term-neonates	Quantifiable blood concentrations of PP were observed in 49% of patients, and 25% of all concentrations were above limit of detection (**10 µg/L**).	Mulla et al 2015
332 postmenopausal women (**Norway**)	Plasma level; median < **2 µg/L** in 29% of subjects. Maximum level measured: **43.9 µg/L**	Sandanger et al 2011
Adipose tissue		
144 participants GraMo cohort, (**Southern Spain**)	n-PrP (54.2% detection, 0.06 ng/g tissue)	Artacho-Cordón et al 2018
Breast milk		
80 pregnant women (Plastics and Personal-Care Product Use in Pregnancy (P4) Study). Subset (n = 31) provided multiple spot urine samples (n = 542) collected over two 24-h periods. Breast milk samples collected at approximately 3 months postpartum (n = 56 women) (**Canada 2009-2010**)	Breast milk samples had >50% detection for MP, PP, and EP.	Fisher et al 2017
Breast tissue presence of propylparaben		
Human breast tissue collected from 40 mastectomies for primary breast cancer (**England 2005 to 2008**)	The overall median value for total paraben was **85.5 ng/g** tissue (range 0–5134.5). Median PP - **16.8 ng/g** (range 0–2052.7 ng/g)	Barr et al 2012 (plus Harvey & Everett 2012 commentary)

8. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181

9. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181