Scientific Committee on Consumer Safety

SCCS

OPINION on
Methylparaben
(CAS No. 99-76-3, EC No. 202-785-7)

The SCCS adopted this document
by written procedure on 14 December 2023

CORRIGENDUM adopted by written procedure on 28 February 2024

ACKNOWLEDGMENTS

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

For the preliminary and for the final version of the Opinion

SCCS members

Dr U. Bernauer	(Rapporteur)
Dr L. Bodin	
Prof. Q. Chaudhry	(SCCS Chair)
Prof. P.J. Coenraads	(SCCS Vice-Chair, Chairperson of the WG)
Prof. M. Dusinska	
Dr J. Ezendam	(Rapporteur)
Dr E. Gaffet	
Prof. C. L. Galli	
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. E. Benfenati
Dr N. Cabaton
Prof. E. Corsini
Dr A. Koutsodimou
Dr. H. Louro
Prof. W. Uter
Dr N. von Goetz

This Opinion has been subject to a commenting period of min eight weeks after its initial publication (from 8 June to 21 August 2023). Comments received during this period were considered by the SCCS. For this Opinion, main changes occurred in the following sections: 3.4.5. Reproductive Toxicity, 3.4.10.1.3. *In vivo* assays providing data on adverse effects on endocrine relevant endpoints (level 4), 3.5. Safety evaluation and 3.6. Discussion.

A **corrigendum clarifying SCCS conclusion under question 1** was adopted by written procedure on 28 February 2024

All Declarations of Working Group members are available on the following webpage:
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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 Methylparaben, does the SCCS consider Methylparaben safe when used as a preservative in cosmetic products up to a maximum concentration of 0.4% (as acid) when used on its own and up to 0.8% (as acid) for mixtures of esters as indicated in entry 12 of Annex V to the Cosmetics Regulation?*

   On the basis of the safety assessment considering all available data and the concerns related to endocrine activity, the SCCS is of the opinion that the use of Methylparaben as a preservative in cosmetic products at concentrations of up to 0.4% (expressed as acid) is safe. It is also safe when used up to 0.4% in a mixture of esters for which the total concentration of all esters does not exceed 0.8% (as acid), as indicated in entry 12 of Annex V to the Cosmetics Regulation.

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

   /

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

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

Keywords: SCCS, scientific opinion, methylparaben, preservative, Regulation 1223/2009, CAS No. 99-76-3, EC No. 202-785-7

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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.

These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they 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 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.).

Scientific Committee members

Ulrike Bernauer, Laurent Bodin, Qasim Chaudhry, Pieter Jan Coenraads, Maria Dusinska, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara Vanhaecke, Susan Wijnhoven

Contact

European Commission
Health and Food Safety
Directorate B: Public Health, Cancer and Health Security
Unit B3: Health monitoring and cooperation, Health networks
L-2920 Luxembourg
SANTE-SCCS@ec.europa.eu

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted the review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting (ED) 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 explicit 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 carried out a public call for data in 2019[2] for 14 substances (Group A)[3] and a second call in 2021[4] for 10 substances (Group B)[5] in preparation of the safety assessment of these substances. Methylparaben is one of the above-mentioned substances for which the call for data took place.

Background on Methylparaben

Methylparaben (CAS No. 99-76-3, EC No. 202-785-7) with the chemical name 'Methyl 4-hydroxybenzoate' is currently regulated as a preservative (Annex V, entry 12) in a concentration up to 0.4 % (as acid) when used on its own or up to 0.8% for mixtures of esters (Annex V, entry 12, column g).

Methylparaben is produced naturally in a variety of plants, but it is also synthesised for use in a range of products including, but not limited to cosmetics, food products and pharmaceuticals, since it has a wide spectrum of antimicrobial activity and is also effective against yeasts and moulds.

Methylparaben has been subject to different safety evaluations by the SCCP in 2005 (SCCP/0874/05)[6] and (SCCP/0874/05)[7], 2006 (SCCP/1017/06)[8], 2008 (SCCP/1183/08)[9] and by the SCCS in 2010 (SCCS/1348/10)[10] and 2011 (SCCS/1446/11)[11].

[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, Homosalate, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein
[4] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic-products-0_en
[5] Butylparaben, Methylparaben, Ethylhexyl Methoxycinnamate (EHMC)/Octylmethoxycinnamate (OMC)/Octinoxate, Benzophenone-1 (BP-1), Benzophenone-2 (BP-2), Benzophenone-4 (BP-4), Benzophenone-5 (BP-5), BHA/Butylated hydroxyanisole/tert-butyl-4-hydroxyanisole, Triphenyl Phosphate and Salicylic Acid
[6] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_019.pdf
[7] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_00d.pdf
[8] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_074.pdf
[9] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_138.pdf
[10] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_041.pdf
[11] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_069.pdf

During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Methylparaben as a preservative in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Methylparaben 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 Methylparaben, does the SCCS consider Methylparaben safe when used as a preservative in cosmetic products up to a maximum concentration of 0.4% (as acid) when used on its own and up to 0.8% for mixtures of esters as indicated in entry 12 of Annex V to the Cosmetics Regulation?

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

3. Does the SCCS have any further scientific concerns with regard to the use of Methylparaben 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

Methylparaben (INCI)
Methyl 4-hydroxybenzoate (IUPAC)

3.1.1.2 Chemical names

Benzoic acid, 4-hydroxy-, methyl ester
Methyl p-hydroxybenzoate
p-Hydroxybenzoate ester
4-Hydroxybenzoic acid methyl ester
p-Hydroxybenzoic acid methyl ester
4-(Carbomethoxy)phenol
p-Carbomethoxyphenol
4-(Methoxycarbonyl)phenol
p-Methoxycarbonylphenol

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
and safety dossier Cosmetics Europe

3.1.1.3 Trade names and abbreviations

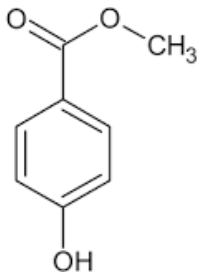
Faracide M	Tegosept M	Metagin
Microcare MHB	Methyl Chemosept	Killitol
Paratexin M	Methyl Parasept	Mekkings M
Solbrol M	Uniphen p-23	E218
Nipagin M	CoSept M	Aseptoform M
Nipagin		

Ref.: https://echa.europa.eu/el/registration-dossier/-/registered-dossier/14310
The Merck index, 12th edition
http://www.chemspider.com/Chemical-Structure.7176.html?rid=be164c5b-cf87-4bbe-84e0-0a405ed30085
Haley 2009

3.1.1.4 CAS / EC number

CAS No: 99-76-3
EC No: 202-785-7

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_8H_8O_3$

3.1.2 Physical form

Colourless crystals or white crystalline powder

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
safety dossier Cosmetics Europe, Soni 2002, Haley 2009

3.1.3 Molecular weight

Molecular weight: 152.15 g/mol

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
PubChem and safety dossier Cosmetics Europe, Haley 2009

3.1.4 Purity, composition and substance codes

>99%

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
PubChem and safety dossier Cosmetics Europe

SSCS comment
Details of the analytical methods used for the determination of purity of the test substance should be provided.

3.1.5 Impurities / accompanying contaminants

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SCCS comment
No data on impurities of the test substance were provided by the Applicant. Details of the analytical methods used for the determination of impurities along with the results of these studies should be provided.

3.1.6 Solubility

Water (at 20°C, pH 5.72)	1.88 g/L (OECD 105)
Water (at 25°C)	2.5 g/L
Water (at 80°C)	20 g/L
Methanol (at 25°C)	59 g/100 g
Ethanol (at 25°C)	52 g/100 g
Propylene glycol (at 25°C)	22 g/100 g
Peanut oil (at 25°C)	0.5 g/100 g
Acetone (at 25°C)	64 g/100 g
Benzene (at 25°C)	0.7 g/100 g
Diethyl ether (at 25°C)	23 g/100 g
Carbon tetrachloride (at 25°C)	0.1 g/100 g
Warm oil	1 g/40 ml
Warm glycerol	1 g/40 ml
Trifluoroacetic acid	soluble

Ref.: ECHA, https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
Matwiejczuk *et al.* 2020

3.1.7 Partition coefficient (Log Pow)

1.98 at 20°C

Ref.: ECHA

3.1.8 Additional physical and chemical specifications

Organoleptic properties:	Odourless or with faint characteristic odour Slight burning taste
Melting point:	125°C (OECD 102)
Boiling point:	Decomposes between 270 and 280°C before boiling
Vapour pressure:	2.8 x10^{-4} Pa at 20°C (OECD 104)
Density:	1.3775 g/cm³ (OECD 109)
pKa at 20°C	8.4 (OECD Guideline No. 112)
pH (1.88 g/L at 20°C)	5.72 (Sigma-Aldrich SDS)
pH (2.5 g/L at 20°C)	5.8 (Fischer Scientific SDS)
Refractive index	1.5250
UV spectrum in ethanol:	λ_{max}: 258 nm; log E = 4.22
Particle size distribution:	Median particle diameter (d50): 141.7 ± 18.4 µm

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
PubChem and safety dossier Cosmetics Europe, CIR 1984, Haley 2009

3.1.9 Homogeneity and Stability

Stable under recommended storage conditions (well-closed container in a cool, dry place); stable in air and resistant to hydrolysis in hot and cold water, as well as in acidic solutions. Aqueous solutions at pH 3–6 were found to be stable (less than 10% decomposition) for up

to about 4 years at room temperature (Kamada 1973, Soni 2002). It hydrolyses in alkaline solutions producing p-hydroxybenzoic acid and methanol. In strongly alkaline solutions it hydrolyses to the corresponding carboxylic acid which then becomes ionized. The rate of hydrolysis is pH dependent. Stable against hydrolysis under usual conditions of sterilisation (heating at temperatures up to 150°C) and also resistant to saponification. When heated at 200°C, Methylparaben first degraded into p-hydroxybenzoic acid through hydrolysis reaction and then further into phenol after decarboxylation. (Kapalavavi *et al.* 2015)
Methylparaben was stable in 67 mM phosphate buffer (pH 7.4) and remained stable after 24 h of incubation at 37°C (Abbas *et al.* 2010).
Sunderland and Watts (1984) reported that the time taken for a 10% loss of the initial methyl ester concentration at 130.5°C and pHs of 10.59, 8.9 and 6.58 are approximately 4 s, 3 min and 40 min, respectively. The authors concluded that Methylparaben is therefore unable to adequately withstand a normal sterilisation procedure unless the solution is within a pH range of 3–6 at the sterilisation temperature.
Methylparaben is stable in common organic solvents.

Ref.: https://pubchem.ncbi.nlm.nih.gov/compound/Methylparaben
PubChem and safety dossier Cosmetics Europe, ECHA, CIR 1984, CIR 2008, Aalto 1953, Abbas 2010, Kamada 1973, Kapalavavi 2015, Haley 2009, Soni 2002, Sunderland 1984, Raval 1967, McCarthy 1970

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

According to the Applicant

***In vitro* studies**

Table 1: Summary of observations for Methylparaben (MP) from *in vitro* skin penetration studies using animal skin

Species/ number/sex	Exposure concentration	Application site details	Observations	Reference
Rat	0.8%	n=10 replicates of dermatomed rat skin (to 450 µM); flowthrough diffusions cells. No occlusion. Oil in water emulsion applied 10µl/cm^2. MP and pHBA samples from receptor fluid were analysed by HPLC-MS.	Receptor fluid 54.94 ± 5.92% Receptor wash 0.43 ± 0.20% Skin 12.23 ± 5.57% Total % applied dose absorbable = 67.61 ± 6.06% (total radioactivity) Skin wash 17.81 ± 2.82% Donor chamber 0.03 ± 0.01% Tape strips 5.65 ± 1.12% Total unabsorbed dose = 2*et al.*9 ± 2.40% Total recovery = 91.09 ± 5.66%	Fasano, 2004

Rabbit	0.23-0.32%	Rabbit ear skin, 6-month-old. 2 mg/cm^2 in a cream base.	After 8h application, total penetration was 60% of the applied dose.	Pedersen *et al.*, 2007
Pig (Yucatan micropig)	1%	10µl of an aqueous solution. At 15, 60 and 120 mins the skin samples were removed from the diffusion cell and wiped. MP was analysed by HPLC.	MP increased in the stratum corneum with time. MP peaked in the epidermis at 60 min.	Ishiwatari *et al.*, 2007
Pig	25 µg	Dorsal male minipig skin (n=3), previously frozen at -70°C. Dermatomed to 350	Total absorption: At 6h: 2.84±0.48% in receptor fluid	Jewell *et al.*, 2007
		µM. 25 µg in DMSO 10µl/cm^2. Skin 1cm^2 application area, held in a glass ring sat in a 12 well plate. Bespoke method of skin absorption to assess potential of fresh skin to metabolise MP.	At 24h: 35.76±7.04% in receptor fluid 23.96±8.44% in skin However, a complete mass balance was not performed.	
Pig	0.1% w/w	Frozen full-thickness pig ear skin. Franz cell design. MP was applied in either 20% or 50% ethanol. 2ml/6h exposure.	No quantitative information could be derived when MP was applied in ethanol due to the likely transesterification effects with alcoholic vehicles.	Caon *et al.*, 2010
Pig	0.1% w/w	Full-thickness skin (FTS). Franz cell design. Frozen (intact and stripped) and fresh ear from 6-month-old domestic pigs. Various formulations tested (see Table 3 below) with and without penetration	See Tables 4 and 5 below After 4-h: <LOQ-2.3% applied dose (AD)(fresh, intact) and 2.3-3.3%AD (frozen, intact) unmetabolised MP penetrated into receptor fluid. The total recovery for previously frozen intact and stripped FTS ranged from 84.8 to 91.5% and from 88.2 to 98.8%, respectively, which are	Pažoureková *et al.*, 2013

		enhancer. 10 ± 0.05 mg/cm^2/24 h	percentages within the required range of 85–115%. With fresh skin recoveries were 79% in one experiment and ranged from 85-101% in the other three.	
Pig	0.2%	Applied to pig ear skin in an antiperspirant formulation in a Franz diffusion cell	Total penetration of 32%; however, there was no correlation between antiperspirant use and paraben serum concentration in the volunteers.	Martins *et al.*, 2019

A detailed skin absorption study was performed by Pažoureková *et al.* (2013) using 0.1% w/w Methylparaben as applied to pig ear skin, in a range of vehicles that were designed to represent a range of cosmetic product formulations. These experiments were performed according to the Guideline for the *in vitro* assessment of dermal absorption of cosmetic ingredients SCCS (2010).

SCCS comment
The study by Pažoureková *et al.* (2013), which was considered by the Applicant as key study to derive a value for dermal absorption of Methylparaben was - contrary to what is stated by the Applicant – not performed according to the SCCS Notes of Guidance. The study is from open literature, the original study report was not available. It is unclear, how many individual donors were used. A concentration of 0.1 % Methylparaben was used in the study whereas the intended maximum product concentration as given in the mandate is 0.4%. In addition, full thickness skin was used.
The Applicant selected a percentage of 14.2 % for dermal penetration of unmetabolised Methylparaben which was obtained from a 4 h experiment using fresh skin, where 2.3 % Methylparaben was detected in receptor fluid and 11.9% was detected in skin (which had not been separated in epidermis and dermis). The SCCS considers a 4 h exposure period too short and notes that in frozen intact skin, unmetabolised Methylparaben amounted to 27.4 % (sum of amount in skin and receptor fluid). In stripped frozen skin, unmetabolised Methylparaben amounted to 18.7 % (sum of amount in skin and receptor fluid). In 24 h experiments, only receptor fluid was investigated where unmetabolised Methyplaraben ranged between 2.0 and 5.8% in frozen intact skin and between 2.9 and 7.6 % in stripped and frozen skin.

Overall, the SCCS is of the opinion that the study did not comply with the basic criteria for dermal absorption studies described in the 11th Revision of the SCCS Notes of Guidance and the SCCS has identified several shortcomings in the study. Therefore, the study cannot be used to estimate dermal penetration of Methylparaben. Furthermore, the study indicates that under different conditions, the dermal absorption value is higher than the value of 3.7%. In previous SCCS opinions, this value was considered as a conservative estimate for dermal absorption of non-metabolised (parent) parabens, but according to the literature available and presented here, this value may not be accurate for Methylparaben.

In vivo animal skin absorption studies
<u>According to the Applicant</u>
Aubert *et al.* (2009; published in 2012) estimated toxicokinetics of Methylparaben in rat after dermal exposure. A total of 24 Sprague-Dawley rats (12 males and 12 females) were allocated to two groups: one group of nine males and nine females (group 1 in the study report) for pharmacokinetic (PK) investigations, and one group of three males and three females (group 4) for mass balance (MB) investigations. The animals were treated with radiolabelled Methylparaben at a dose-level of 100 mg/kg. The test item in 60% ethanol:water vehicle was applied over 10% of the body surface area for a 6-hour period

(during which the site was left uncovered but the animals wore an Elizabethan collar). After exposure, the skin application site and the walls of the upper part of the metabolism cages were washed with swabs impregnated with soap and water, which were pooled and frozen at -20°C until analysis for total radioactivity. Blood samples were taken from animals allocated to PK investigations as follows: pre-dose and 0.5, 1, 2, 4, 8, 12, 22 and 24 hours after the beginning of dermal application.
Following dermal application for 6 hours at a dose of 100 mg/kg of [^{14}C]-Methylparaben to rats, the total mass balance (urine, faeces, cage wash, rinsing swabs and strips, tissues and carcasses) over the 168-h collection period was complete and amounted to 114% and 115% relative to the administered dose for males and females, respectively. Most of the dose (55.9/46.4% for males/females) was unabsorbed and recovered in the swabs used for cleaning of the application site at the end of the exposure period. Only 14.5% or 25.8% of the applied radioactivity was found in the urine of males or females, respectively. Urinary excretion was the main route of elimination.

SCCS comment
The study was already considered in previous SCCS Opinions. However, as metabolites were not identified and only total radioactivity was determined, no conclusions on dermal penetration of non-metabolised Methylparaben can be drawn from this study.
The Applicant provided further *in vitro* studies using human skin and human *in vivo* data which do not allow to estimate dermal availability of intact (parent) Methylparaben after dermal application in humans; the Applicant also provided information on skin metabolism (see Annex I).

Applicant's overall conclusion of systemic bioavailability after dermal administration:
The following conclusions can be drawn from the body of evidence on skin penetration:

- The major penetrant into the systemic circulation following skin exposure to Methylparaben is its principal non-toxic metabolite p-hydroxybenzoic acid (pHBA), due to the action of esterases in the skin (Williams 1985).
- Low levels of unmetabolised Methylparaben can penetrate mammalian skin, and the absolute level varies with the vehicle in which it is applied; ranging from 0.057% (human skin – MP as applied in a commercial body lotion) (El Hussein *et al.* 2007) to 2.3% (fresh pig skin – oil in water emulsion with Transcutol penetration enhancer) (Pažoureková *et al.*, 2013).
- Even after repeat dosing of 0.1% Methylparaben in a commercial body lotion (3 times in 24 hours), human skin penetration of Methylparaben was low at 0.6% (El Hussein *et al.* 2007).
- Measures of Methylparaben within fresh pig skin ranged from 9.8-11.9% of the applied dose at 4h (Pažoureková *et al.*, 2013). It is expected that by 24h much of this would be converted to pHBA, as evidenced by the 24h data in receptor fluid.
- The use of ethanol vehicles in in vitro skin absorption studies may lead to transesterification of Methylparaben to Ethylparaben which can confound the experiment (Lakeram *et al.* 2006; Oh *et al.* 2002; Seo *et al.* 2016; Fujji *et al.* 2017).
- Rat skin appeared to metabolise Methylparaben more extensively than human skin (Fasano 2004).
- The data from pig skin and human skin should be given the most weight in deriving a value for skin absorption in risk assessment.

The most conservative study to use to estimate a value for risk assessment is that performed by Pažoureková *et al.*, (2013). This study partly followed SCCS criteria for *in vitro* dermal absorption studies. A worst-case value as a basis for estimating skin absorption of unmetabolised Methylparaben in fresh pig skin (as is the preferred model) is 2.3% of the

applied dose (AD) (receptor fluid) + 11.9%AD (as measured within the skin), at 4 hours (SD was not reported). Overall, a conservative and expectedly worst-case estimate of 15% skin absorption of unmetabolised Methylparaben can be used in the risk assessment for the following reasons:

- this value is from an experiment using a penetration enhancing vehicle,
- the extent of metabolism is expected to increase beyond 4h following application to the skin,
- following dermal application of Methylparaben for 6 hours to rats only up to 25.8% of the applied radioactivity was found in the urine which may be far less in humans taking into account that the skin of rats may be up to 10 times more permeable compared to human skin (van Ravenzwaay & Leibold, 2004),
- the SCCS considered a dermal absorption rate of 3.7% for the close analogue Propylparaben (SCCS/1623/20).

Further information (not provided by the Applicant)
A human study investigating the pharmacokinetics of deuterated, dermally applied methyl-, ethyl- and propylparaben was published in 2023. In that study, 5 male volunteers applied 24 g of a cream containing 0.8 % of a paraben mixture (0.26% Methylparaben, 0.26% ethylparaben and 0.28% propylparaben) on the whole arm for 30 min. Blood and urine were collected before and at different time points up to 48h after administration. Free (after enzymatic cleavage) and total parabens were quantified by HPLC-MS/MS analysis. The following results were obtained for Methylparaben:

Parameter	**Total Methylparaben**	**Free Methylparaben**
Maximum plasma concentration [nM] C_{max}	57 ± 33	37 ± 52
Time to reach maximum plasma concentration [h] T_{max}	7.8 ± 4.3	4.9 ± 2.8
Terminal half-life [h] $T_{1/2}$	12.2 ± 1.6	7.2 ± 1.9
Area under the curve extrapolated from time zero to infinity [µM·hour] $AUC_{(0-\infty)}$	1.4 ± 0.9	0.3 ± 0.5
Area under the moment curve extrapolated from time zero to infinity [µM·hour^2] $AUMC_{(0-\infty)}$	25.6 ± 17.8	3.1 ± 4.2
Mean residence time [h]	17.5 ± 2.3	10.4 ± 2.7

As information on oral absorption was lacking, a dermal absorption percentage could not be derived from that study.

Ref.: Shin *et al.*, 2023

SCCS overall comment on *in vitro* and *in vivo* skin absorption studies
As pHBA is considered as the common inactive metabolite of parabens, it is the systemic availability of intact (parent) compound that may be of concern for systemic adverse effects. Valid dermal penetration studies to estimate systemic availability of parent (intact) Methylparaben after dermal application in humans are not available. There are indications in the literature that there are differences in metabolism between animals and humans. *In vivo* pharmacokinetic data in humans are therefore required and have been requested from the

Applicants in the past. Up to now, this data has not been provided (see SCCP/1348/10 rev. 1) and *in vitro* dermal penetration studies using human skin that comply with the SCCS requirements have not been performed. A human pharmacokinetic study published in 2023 by Shin *et al.* (2023) does not inform on a dermal absorption percentage (due to the lack of oral data for comparison), however it informs about important toxicokinetic parameters for Methylparaben.
The key study presented by the Applicants suffers from several shortcomings; however, it indicates that a value of 3.7%, which was used in previous SCCS Opinions for dermal absorption of non-metabolised (parent) paraben, might not be protective in the case of Methylparaben. Therefore, in the absence of a proper dermal penetration study using human skin, a default value of 50% for non-metabolised Methylparaben will be used by the SCCS in the MoS calculation.

3.2.2 Other studies on toxicokinetics

According to the Applicant

Oral ADME/kinetic data in animals
Aubert *et al.* (2012) investigated the oral kinetics of ^{14}C-Methylparaben in rats looking at total radioactivity. A group of (n=3 male, n=3 female) Sprague-Dawley rats were dosed for mass balance analysis and another group for physiologically based kinetic (PBK) modelling. The mass balance data following a single oral dose of 100 mg/kg is shown below in Table 2.

Table 2: Mass balance parameters, total radioactivity recovered (as % of dose) in rat for Methylparaben, 100 mg/kg dosed orally, over a 168-hour total dosing period

Route	Gender	Urine	Faeces	Cage wash	Swabs	Strips/ biopsies	Tissues	Carcass	Total Recovery
Oral	Male	82.8 ± 3.5	0.92 ± 0.28	11.3 ± 3.0			0.04 ± 0.04	<LOQ	95.1 ± 1.1
Oral	Female	78.7 ± 6.6	0.90 ± 0.67	14.1 ± 4.7			0.03 ± 0.02	<LOQ	93.8 ± 1.7

n.c values below LOD and hence not calculated

From these data there is evidence for high oral bioavailability in rats. A significant amount of the applied dose is excreted within 8 hours. The mean maximum recovery levels of radioactivity in blood (C_{max}) were 26592 and 38664 ng eq/g for males (T_{max} 1h) and females (T_{max} 30 mins), respectively. The area under the curve (AUC) was 143630 (females) and 82153 ng eq h/g (Aubert *et al.* 2012).

Comparisons were made between Methylparaben dosed orally and dermally (see section 3.2.1 for the dermal data). Plasma levels of total radioactivity were measured at 30 mins, 1, 2, 4, 8, 12 and 22 hours after dosing (see Figure 3 below). Significantly more of the applied dose (parent plus metabolites) entered the systemic circulation from oral exposure than from dermal exposure.

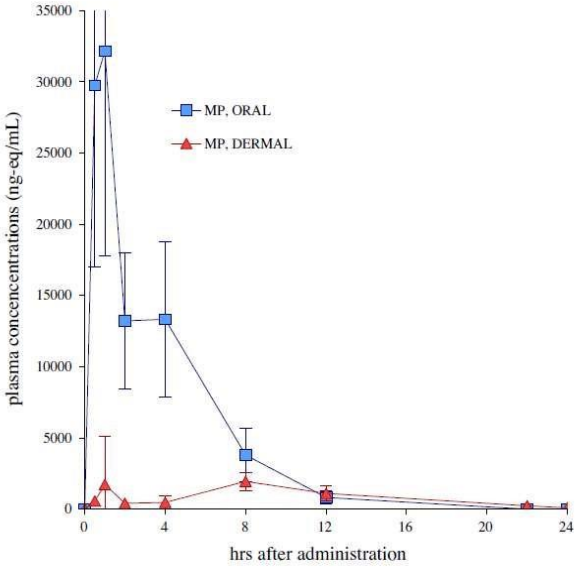

Figure 1. Plasma concentrations (ng [^{14}C]-eq/mL) in male and female Sprague-Dawley rats after single oral and dermal doses of 100 mg/kg ^{14}C-Methylparaben (MP). The minor peak at 1h after dermal treatment resulted from a single male animal and is most likely a result of oral uptake secondary to cage contamination from open treatment sites.

In rabbit urine, metabolites of Methylparaben have been described following gastric intubation (Tsukamoto and Terada, 1960, 1962, reviewed in CIR 2012). pHBA, pHHA (p-hydroxyhippuric acid), p-carboxyphenyl glucuronide, p-hydroxybenzoyl glucuronide, and p-carboxyphenyl sulphate were identified. It was reported that 0.2–0.9% of unchanged ester was excreted, and that the urinary excretion of pHBA was slower with increasing carbon chain length of the paraben alkyl group. Tsukamoto and Terada (1964) compared the metabolism of pHBA and parabens in rabbits and found that the urinary excretion of free pHBA is less after paraben exposure than after pHBA exposure, and that urinary excretion of free pHBA was lower with longer chain lengths, although some variation applies to these data. These authors postulated that any differences in toxicity of the different parabens is possibly related to differences in metabolism and clearance.

In 2015, Campbell *et al.* built a PBK model for parabens and modelled the Aubert *et al.* 2012 kinetic data. The resulting output is shown in Figure 2.

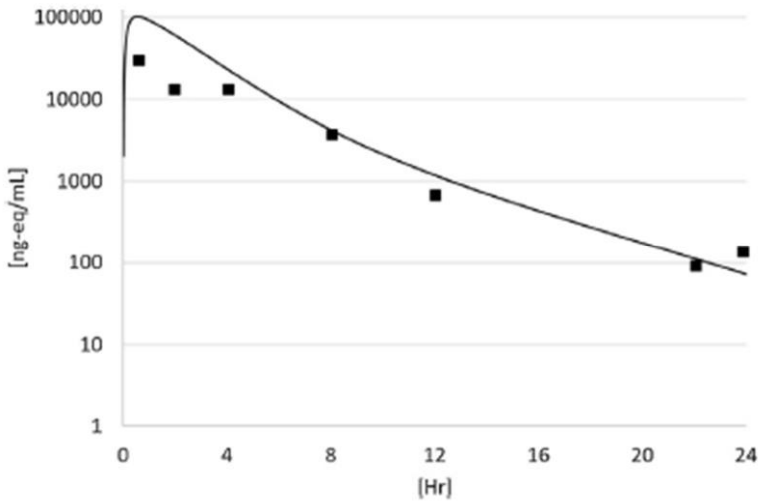

Figure 2. Simulation of the total radioactivity in plasma after a single oral bolus of Methylparaben at 100 mg/kg administered to adult Sprague-Dawley rats. (Data from Aubert *et al.* 2012; Figure taken from Campbell *et al.* 2015).

Oral ADME/kinetic data in humans

Ye *et al.* (2006) measured parabens in human urinary samples collected between 2003 and 2005 from 100 adult anonymous volunteers with no known occupational exposure to these compounds. They found 95% of Methylparaben and 98% of propylparaben in a conjugated form (sulphated and glucuronidated).

Distributional data for total (free plus conjugated) and free Methylparaben is shown below in Table 3.

Table 3: Total (free plus conjugated) and free urinary concentration of Methylparaben (ng/mL) at selected percentiles, and frequency of detection in adults (n=100) (Ye *et al.* (2006)).

		Percentile					
Compound	**Frequency of detection (%)**	**5th**	**25th**	**50th**	**75th**	**90th**	**95th**
Methyl paraben, total	99	4.2	14.6	43.9	180	412	680
Methyl paraben, free	75	< LOD	0.1	0.8	4.7	15.0	27.8

Further information (not presented in the Applicants dossier)

The oral toxicokinetics of ring-deuterated Methylparaben (D4-ring labelled) was investigated in three healthy volunteers (31 years old, one woman, 2 men). The volunteers ingested 10.07 mg Methylparaben which had been added to coffee or tea, resulting in doses between 0.12 and 0.19 mg/kg bw/d. Urine was collected from prior to dosage until 48 hr after dosage and analysed for parent Methylparaben and hypothesized metabolites after enzymatic hydrolysis with glucuronidase/sulfatase. Methylparaben was excreted with a halftime of 6.9 h, while PHBA (5.8 h), PHHA (5.7 h) and rOH-Methylparaben (2.5 h) were excreted slightly faster. Within two days, 84.4% of the Methylparaben dose was excreted via urine. Within 48 hr, the predominant metabolite excreted via urine was p-hHHA (63.8%, range 60.3-68.2%), followed

by Methylparaben (as the sum of free Methylparaben and glucuronide and sulfate conjugates; 17.4 % (range 15.5-19.2%) and p-hydroxybenzoic acid (PHBA (3%, range 2.7-3.2%)). Methyl 3,4-dihydroxybenzoate, a metabolite with an oxidative modification at the aromatic ring, accounted for 0.1% of the applied dose (range: 0.1 – 0.25).

Table 4: Mean values and ranges of urinary excretion factors of the three volunteers (in % of the dose, on a molar basis) of Methylparaben

Dosage	**Biomarker**	**Percentage of applied dose between 0 and 24 h (%)**	**Percentage of applied dose between 24 and 48 h (%)**	**Percentage of applied dose between 0 and 48 h (%)**
Methylparaben	Methylparaben	16.8 (15.3-18.3)	0.6 (0.3-0.9)	17.4 (15.5-19.2)
	rOH-Methylparaben	0.1 (0.1-0.25)	0.0 (-)	0.1 (0.1-0.25)
	PHHA	63.5 (59.8-68.1)	0.3 (0.1-0.5)	63.8 (60.3-68.2)
	PHBA	3.0 (2.7-3.2)	0.0 (-)	3.0 (2.7-3.2)
	Overall ∑	8*et al.* (81.2-86.8)	0.9 (0.4-1.4)	84.4 (82.6-87.2)

Ref.: Moos *et al.* 2016

SCCS comment on other studies on toxicokinetics
Apart from Campbell 2015 study, the studies presented by the Applicant were already considered in the previous SCCS/SCCP evaluation and therefore do not lead to a change in the conclusion drawn in SCCP/1348/19_rev 1: "The toxicokinetic study confirms that, in rats, short-, mid- and long-chain parabens are rapidly absorbed and eliminated after single oral or subcutaneous administration. After dermal administration, they are partly (15 to 27%) absorbed and rapidly eliminated. Blood analysis only showed the presence of PHBA." Based on the study by Moos *et al.*, 2016 (Table 2) using 3 male volunteers, 17.4% of dermally applied Methylparaben was excreted as parent (as the sum of free Methylparaben and glucuronide and sulfate conjugates) compound, 63.8 % % as PHHA, 3.0 % as PHBA and 0.1 % as ring hydroxylated Methylparaben.
In vivo animal studies point to high oral absorption (clearly above 50%). Therefore, adjustment for oral absorption is not necessary when MoS calculation is based on an oral study. 100 % oral absorption will be used for MoS calculation (i.e. no adjustment of PoD from oral study).

3.3. EXPOSURE ASSESSMENT

3.3.1 Function and uses

According to the Applicant
Methylparaben and its salts are widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used either alone or in combination with other parabens or with other antimicrobial agents. In cosmetics, Methylparaben is the most frequently used antimicrobial preservative.

In entry number 12 of Annex V of the Cosmetic Products Regulation EC 1223/2009 of the European Union and as amended on 18 September 2014 in Commission Regulation (EU) No 1004/2014, the maximum regulatory % use level for Methylparaben ester of 0.4% is cited (as acid), as Methylparaben and ethylparaben are discussed within entry 12 as '4-hydroxybenzoic acid and its methyl- and ethyl-esters, and their salts'. According to the Applicant, taking into account the conversion of molecular weights, the inclusion level of 0.441% (as ester) can be used in any cosmetic product.

According to Cosmetics Europe % use survey data *(i.e.* use by member companies) in the year 2016 (Cosmetics Europe 2017 report), the observed minimum, 50th percentile, mean, 95th percentile and maximum % use levels of Methylparaben in the product types are as shown below in Table 5.

Table 5: Observed levels using Cosmetics Europe % use survey data from use in the year 2016 (Cosmetics Europe 2017 report).

Product Type	Minimum	P50	Mean	P95	Maximum
Body lotion	0.0001	0.2000	0.2267	0.3000	0.4000
Deo nonspray	0.0001	0.0002	0.0422	0.2500	0.2700
Eye shadow	0.0002	0.2325	0.2113	0.2380	0.4000
Eyeliner	0.0005	0.2500	0.2458	0.3000	0.3791
Face cream	0.0001	0.2000	0.1945	0.3023	0.4000
Hair styling	0.0001	0.1625	0.1748	0.3000	0.4000
Hand cream	0.0001	0.2500	0.2480	0.3500	0.4000
Lipstick	0.0001	0.1400	0.1089	0.1400	0.4000
Hand soap	0.0001	0.0150	0.0187	0.0750	0.3500
Liquid make-up foundation	0.0001	0.3000	0.2235	0.4000	0.4000
Make-up remover	0.0002	0.2277	0.2042	0.3465	0.4000
Mascara	0.0001	0.0155	0.0763	0.3701	0.4300
Mouthwash	0.0200	0.1000	0.0800	0.1500	0.1500
Rinse-off conditioner	0.0001	0.2556	0.1942	0.3000	0.4000
Shampoo	0.0001	0.1500	0.1064	0.2000	0.4000
Shower gel	0.0001	0.0025	0.0778	0.3465	0.3500
Toothpaste	0.0001	0.1000	0.0864	0.1995	0.2000

Data were also available from Mintel on the occurrence of Methylparaben in these 17 product types. A yearly occurrence figure was derived for Methylparaben for the years 2008 to 2017 as shown in Table 6. The trend of the occurrence over time shows an overall decrease over the time period considered (2008 to 2017) for Methylparaben.

Table 6: Methylparaben occurrence from Cosmetics Europe % use survey data in the year 2016 (Cosmetics Europe 2017 report) (% by tonnage), and historical trends data (% number of formulations) from the Mintel database.

Product type	Survey (2016)	Historical trend (Mintel)									
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Body Lotion	6.899	27.11	29.58	25.97	25.06	16.48	16.06	11.72	14.32	14.01	10.3
Deodorant non-spray	0.827	2.58	5.29	1.98	2.88	2.59	1.20	1.61	1.37	1.96	0.9
Eyeliner	41.208	10.93	6.26	7.89	7.36	6.60	4.34	3.02	1.67	2.05	1.82
Eyeshadow	4.799	10.23	9.34	6.61	6.47	4.55	3.53	2.31	2.17	2.73	2.75
Face Moisturiser	13.375	28.29	26.27	22.16	18.69	14.05	11.50	8.92	8.79	8.79	7.06
Hair Styling	16.604	18.78	18.86	14.15	16.23	16.84	12.32	11.19	11.63	10.09	10.91
Hand Cream	9.36	34.96	37.73	32.66	36.72	23.71	22.86	17.21	19.01	15.47	10.95
Liquid Hand Soap	3.437	6.71	8.61	8.04	7.50	4.67	1.86	2.15	2.18	1.48	0.73
Lipstick	8.947	2.00	1.17	1.02	1.20	0.71	0.71	0.45	0.44	0.41	0.27
Liquid make-up foundation	24.516	10.53	9.39	6.35	6.59	4.64	3.83	2.97	2.29	2.51	1.94
Make-up remover	9.402	44.64	25.00	26.47	23.81	26.60	15.69	18.18	11.81	10.79	8.73
Mascara	26.847	20.98	21.11	18.79	17.89	13.26	9.08	10.53	8.60	7.97	6.19
Mouthwash	9.656	17.44	12.12	18.42	20.55	21.53	21.50	17.33	19.68	16.46	11.88
Rinse-off conditioner	17.554	36.64	41.38	26.51	23.17	22.78	19.07	23.35	17.04	15.10	14.8
Shampoo	17.243	24.03	17.52	14.55	12.75	10.03	6.90	9.04	5.17	5.24	3.38
Shower gel	10.787	14.91	15.36	15.75	13.86	7.71	4.31	3.72	2.90	2.51	1.76
Toothpaste	2.562	17.09	19.62	27.73	24.47	18.66	15.38	15.43	11.69	9.15	9.88

Methylparaben is also used as a food preservative as E number E218. Under EC Directive 95/2/EC, Annex III, Methylparaben is allowed for use as a 'conditionally permitted preservative and antioxidant', in a limited number of foods.
Methylparaben is used in certain natural health products, including anti-diarrheal medication, heartburn medication and radiological contrast media (Health Canada, 2020).

Ref.: CE file, Soni 2002, Haley 2009

SCCS comment
The SCCS assumes that the values presented in Table 6 relate to the ester and not to the acid form, and that therefore the level in mascara does not exceed the level permitted under the regulation.

3.3.2 Calculation of SED/LED

<u>According to the Applicant</u>

Exposure assessment is, by necessity, an iterative process that begins as simple as possible and moves to more complexity, bringing in more data as and when available to refine the assessment (Meek *et al.*, 2011). Deterministic additive methods for calculating aggregate exposure assume that everybody in the population uses all the products each day, and that all of the products contain the chemical of interest at a fixed concentration, which is not a realistic scenario but is a simple place to start. This technique is the basis of the current SCCS 11th Notes of Guidance (2021) approach to Tier 1 aggregate exposure assessment. However, as this approach grossly exaggerates realistic aggregate exposure, a more realistic and refined risk assessment should be used for aggregate exposures, where data allow. With good data on habits and practices of cosmetic product use and distributions of concentration use

data in products, a probabilistic approach to estimating exposure can be performed and so where data exist, further refinements of the risk assessment can be performed.

3.2.2.1 Scenarios and populations

According to the Applicant
Scenario A is typically the maximum allowable % use levels cited in regulation; Scenario B is a choice of % use levels, typically from a survey of cosmetics use in products, that could be a 95th percentile value, a maximum observed % use level, or the application of the whole survey distribution of use levels. Whatever % use level is selected for scenario B should be specified.
Accordingly, the consumer exposure assessments (external dermal exposure) contained within this dossier use the tiers and scenarios as follows:
Tier 1 Scenario A - Deterministic consumer aggregate exposure assessment (Table X below) using the maximum % allowable use level of 0.4% (as acid; 0.441% as ester), based on regulatory levels
Tier 1 Scenario B - Deterministic Consumer Aggregate Exposure Assessment using Maximum % Observed Use Levels (Cosmetics Europe % use survey data in the year 2016 (Cosmetics Europe 2017 report)).
Tier 1 represents deterministic exposure modelling; Tier 2 represents probabilistic exposure modelling. As Tier 1 models led to a clearly favourable outcome, Tier 2 modelling was not performed.
In addition, the Applicant provided calculations of the Systemic Exposure Dose (SED) following oral ingestion of toothpaste.

3.2.2.2. Parameters for adults

According to the Applicant, in the SCCS Notes of Guidance 11th revision (SCCS/1628/21), values are provided for the amount of product exposure an individual consumer could experience in gram product per day, for 17 different cosmetic products, and as calculated in mg/kg bw/day. These values were used in all scenario modelling. Values for the % level of Methylparaben in each of the 17 product types are then used to calculate the total dermal exposure to Methylparaben (in mg/kg/day) from each product for adults (see Table 7).

A generic maximal value for skin penetration of Methylparaben of 15% has been used for products applied on skin/hair (lipstick and oral care excluded) in these calculations. This is a conservative value that is supported by experimental data with vehicles known to maximise skin penetration. This enables a systemic exposure dose (SED) 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 (see Table 7).

All of the scenarios in this dossier have assumed 100% occurrence of Methylparaben in all cosmetics products used by an individual in a day. It was not necessary to progress further with any more complex probabilistic tiers for SED calculation, as a favourable outcome was obtained in Tier 1 Scenario A.

SCCS comment
SCCS will use a default value of 50% for dermal uptake (see section 3.2.1).

3.2.2.3 Exposure results

Results for the deterministic consumer aggregate exposure assessment with Scenario A using the maximum % allowable use level, based on regulatory level in Annex V of the Cosmetic Product Regulation (1223/2009) are shown in Table 7. According to the Applicant, this Table presents the results for a worst case deterministic aggregate assessment for Methylparaben, with theoretical use in 17 cosmetic products, using an approach for aggregate assessment as defined in the SCCS NoG (2021) and maximum potential % inclusion level as per Annex V of the EU Cosmetic Products Regulation. Calculation of Systemic Exposure Dose (SED) is also illustrated based on a dermal absorption value 15%. 100% occurrence in all products used daily is assumed. A calculation of Margin of Safety is also provided (based on the POD resulting in section *et al.*). The calculations with Scenario B are presented in Table 8.

Table 7: Results for scenario A – maximum use levels

Product	Calculated relative daily exposure to product [1] (mg/kg bw/day)	Maximum allowable % use levels	Total dermal exposure to methyl paraben with maximum allowable % levels (mg/kg bw/day)	Calculated SED[2] for methyl paraben (mg/kg bw/day)	Margin of safety[4]
Shower gel	2.79	0.441	0.0123	0.0018	541834
Hand wash	3.33	0.441	0.0146	0.0022	453969
Shampoo	1.51	0.441	0.0066	0.0010	1001136
Hair conditioner	0.67	0.441	0.0029	0.0004	2256292
Hair Styling	5.74	0.441	0.0253	0.0038	263365
Body lotion	123.20	0.441	0.5433	0.0815	12270
Face cream	24.14	0.441	0.1064	0.0159	62623
Hand cream	32.70	0.441	0.1442	0.0216	46230
Liquid foundation	7.90	0.441	0.0348	0.0052	191356
Lipstick/salve[3]	0.90	0.441	0.0039	0.0039	251953
Make-up remover	8.33	0.441	0.0367	0.0055	181478
Eye shadow	0.33	0.441	0.0014	0.0002	4580957
Mascara	0.42	0.441	0.0018	0.0003	3599323
Eyeliner	0.08	0.441	0.0003	5.29E-05	18896447
Non-spray Deodorant	22.08	0.441	0.0973	0.0146	68465
Toothpaste[3]	2.16	0.441	0.0095	0.0095	104980
Mouthwash[3]	32.54	0.441	0.1435	0.1435	6969
Aggregate			1.19	**0.31**	**3213**

1. According to values as derived in Tables 3A and 3B in the SCCS Notes of Guidance (11th revision) (2021). These are common values for all product types, as set by the SCCS in this model.
2. Total dermal external exposure x 15% dermal penetration.
3. No dermal penetration applied to lipstick, toothpaste and mouthwash; SCCS default 100% absorption used.
4. MOS = POD (1000 mg/kg/day)/SED.

Table 8: Deterministic Consumer Aggregate Exposure Assessment according to Scenario B using Maximum % Observed Use Levels (Cosmetics Europe % use survey data in the year 2016 (Cosmetics Europe 2017 report).

Product	Calculated relative daily exposure to product [1] (mg/kg bw/day)	Maximum observed % use levels	Total dermal exposure to methyl paraben with maximum observed % levels (mg/kg bw/day)	Calculated SED[2] for methyl paraben (mg/kg bw/day)	Margin of safety[4]
Shower gel	2.79	0.35	0.0098	0.0014	682710
Hand wash	3.33	0.35	0.0117	0.0017	572001
Shampoo	1.51	0.4	0.0060	0.0009	1103753
Hair conditioner	0.67	0.4	0.0027	0.0004	2487562
Hair Styling	5.74	0.4	0.0229	0.0034	290360
Body lotion	123.20	0.4	0.4928	0.0739	13528
Face cream	24.14	0.4	0.0965	0.0145	69042
Hand cream	32.70	0.4	0.1308	0.0196	50968
Liquid foundation	7.90	0.4	0.0316	0.0047	210970
Lipstick/salve[3]	0.90	0.4	0.0036	0.0036	277778
Make-up remover	8.33	0.4	0.0333	0.0050	200080
Eye shadow	0.33	0.4	0.0013	0.0002	5050505
Mascara	0.42	0.43	0.0018	0.0003	3691399
Eyeliner	0.08	0.3791	0.0003	0.00004	21981887
Non-spray Deodorant	22.08	0.27	0.0596	0.0089	111827
Toothpaste[3]	2.16	0.2	0.0043	0.0043	231481
Mouthwash[3]	32.54	0.15	0.0488	0.0488	20488
Aggregate			0.96	**0.19**	**5211**

[1] According to values as derived in Tables 3A and 3B in the SCCS Notes of Guidance (11th revision) (2021). These are common values for all product types, as set by the SCCS in this model.
[2] Total dermal external exposure x 15% dermal penetration
[3] No dermal penetration applied to lipstick, toothpaste and mouthwash; SCCS default 100% absorption used.
[4] MOS = POD (1000 mg/kg/day)/SED

SCCS comment

Footnote 1 of Tables 7 and 8 mentions that the product exposure values have been retrieved from the NoG Tables 3A and 3B. However, values for handwash and hair conditioners have been (correctly) retrieved from Table 5 in the NoG.

The SCCS accepts Scenario A that uses maximum allowed concentrations according to regulation. The Applicant has used a dermal uptake of 15%, but for the reasons explained in section 3.2.1 the SCCS will use a default value of 50%. The SCCS has recalculated the adjusted aggregate SED by using this default value, except for lipstick, toothpaste and mouthwash, for which a dermal absorption of 100% is used (Table 9). After recalculation, the adjusted aggregate SED for Methylparaben exposure of adults is 0.671 mg Methylparaben per kg bw/day.

Table 9: Recalculation of the aggregate SED for Methylparaben using a worst-case deterministic aggregate scenario as used in Table 7 (for adults).

Product	Calculated relative daily exposure to product (mg/kg bw/day)	Max allowable use level (%)	Total dermal exposure (mg/kg bw/day)	Calculated SED (mg/kg bw/day)
Shower gel	2.79	0.441	0.0123	0.006152
Hand wash	3.33	0.441	0.0147	0.007343
Shampoo	1.51	0.441	0.00666	0.00333
Hair conditioner	0.67	0.441	0.00296	0.001477
Hair styling	5.74	0.441	0.0253	0.01266
Body lotion	123.2	0.441	0.5433	0.27166
Face cream	24.14	0.441	0.1065	0.05323
Hand cream	32.7	0.441	0.1442	0.07210
Liquid foundation	7.9	0.441	0.03484	0.01742
Lipstick/salve	0.9	0.441	0.003969	0.0039
Make-up remover	8.33	0.441	0.03674	0.01837
Eye shadow	0.33	0.441	0.001455	0.000728
Mascara	0.42	0.441	0.001852	0.000926
Eyeliner	0.08	0.441	0.000353	0.000176
Non-spray deodorant	22.08	0.441	0.09737	0.04869
Toothpaste	2.16	0.441	0.009526	0.0095
Mouth wash	32.54	0.441	0.1435	0.1435

Aggregate			**1.185**	**0.671**

3.2.2.4 Parameters for children

From the Applicants Dossier

Assessment and results for children
In the risk assessment of oral ingestion for children, firstly intake values based on typical usage are calculated for 1-6 years and 7-18 years. The body weight data as per the EFSA values (Table 10), is then used to perform risk assessments for 1-3, 3-10, 10-14 and 14-18 years categories for European consumers.

Bodyweight values for European children in the assessment
EFSA (2012b) provide default values for use in risk assessment where there are no specific measured data. In this risk assessment of oral ingestion below, firstly intake values based on typical usage are calculated for 1-6 years and 7-18 years. The body weight data as per the EFSA values (Table 150), is then used to perform risk assessments for 1-3, 3-10, 10-14 and 14-18 years categories for European consumers.

Table 10: Body weight (kg) statistics for infants, children and adolescents in all surveys of the EFSA Comprehensive database (EFSA 2012b).

Age (years)	Gender	N	Mean	StdDev	Median	P5	P95
Infants [0-3 months[	♀+♂	205	4.8	1.4	4.8	3.2	6.4
Infants [3-6 months[	♀+♂	231	6.7	1.0	6.7	5.1	8.5
Infants [6-12 months[	♀+♂	441	8.8	1.2	8.7	7.0	11.0
Toddlers [1-3 years[	♀+♂	1679	11.9	2.2	11.6	8.7	15.9
Other children [3-10 years[	♀+♂	8902	23.1	7.1	21.7	14.0	37.0
Adolescents [10-14 years[	♀+♂	3222	43.4	10.6	42.0	29.4	62.0
Adolescents [14-18 years[	♀+♂	3996	61.3	11.9	60.0	45.0	83.0

Abbreviations: see table 1's footnotes
[6-12 months[means "from 6 to 12 months old, infants of exactly 12 months being excluded from this age category"

Intakes for 1-6 years of age: toothpaste
The use of toothpaste starts with first erupted teeth and occurs with a high percentage of dentifrice ingestion. Therefore, the amount of toothpaste to be used by children age 6 and under, as implemented for fluoride toothpastes, is generally set at a pea-size amount. The SCCNFP (2003) defined this as 0.25 grams when assessing the safety of fluoridated oral care products for children. Furthermore, a retention factor of 40% for children 7 months-8 years of age was explicitly stated to be "already an overestimate" when these exposure calculations were revisited (SCCP 2005).
Therefore, it was considered to be appropriately conservative to assume that children of this age use a pea-sized amount (0.25 g) of toothpaste twice a day with a retention factor (RF) of 40% (SCCP, 2005). Oral retention factors are needed to take into account that only a fraction of the orally applied products will be ingested. An industry-wide usage survey was conducted (Cosmetics Europe % use survey data in the year 2016 (Cosmetics Europe 2017 report)), and it was determined that currently marketed toothpaste contains up to 0.2% methyl paraben ester. Nevertheless, for conservatism, a value of the maximum allowable concentration of 0.441% (as ester) is applied.

The following intake levels can therefore be calculated:

1-6 years of age: Toothpaste			
Max Allowable Methyl paraben Concentration	IC	0.441	%
Amount used	A	0.25	g/use
Frequency	FQ	2	uses/day
Retention Factor	RF	40	%
Conversion Factor	CF	1000	mg/g
Systemic Exposure (mg/person/day) =		(IC)(A)(FQ)(RF)(CF)	
Systemic Exposure (mg/person/day) =		0.441/100 (0.25 g/use) (2 uses/day) (40)/100 (1000 mg/g)	
Intake (mg/person/day) =		**0.88**	

Intakes 7-18 years of age: toothpaste

For this age group, ingestion of toothpaste is lower primarily as the spitting reflex develops. It is assumed that 2.75 g of toothpaste is used per day for adolescents and adults, with a RF of 5%. According to the EU Cosmetic Products regulation, toothpaste can contain up to 0.441% methyl paraben ester (0.4% as acid).

The following intake levels can therefore be calculated:

7-18 years of age: Toothpaste			
Max Allowable Methyl paraben Concentration	IC	0.441	%
Amount used	A	2.75	g/day
Retention Factor	RF	5	%
Conversion Factor	CF	1000	mg/g
Systemic Exposure (mg/person/day) =		(IC)(A)(RF)(CF)	
Systemic Exposure (mg/person/day) =		0.441/100 (2.75 g/day) (5)/100 (1000 mg/g)	
Intake (mg/person/day) =		**0.61**	

Intake from Mouthwash 6-18 years

The use of mouthwash starts at age 6 (it is generally recommended that children under 6 should not use mouthwash). The usage volume of 21.62 ml/day and retention factor of 10% is used. This is appropriate, considering published literature on the ingestion of mouthwash by children age 6, with a reported 8% retention (Zuanon, 2005). An industry-wide usage survey was conducted (Cosmetics Europe % use survey data in the year 2016 (Cosmetics Europe 2017 report)), and it was determined that currently marketed mouthwash contains up to 0.15% Methylparaben, and assuming roughly 1 ml mouthwash is equivalent to 1g. To be conservative, a value of the maximum allowable % use level 0.441% is applied.

The following calculation can therefore be made:

6 - 18 years of age: Mouthwash			
Max Allowable Methyl paraben Concentration	IC	0.441	%
Amount used	A	21.62	g/day
Retention Factor	RF	10	%
Conversion Factor	CF	1000	mg/g
Systemic Exposure (mg/person/day) =		(IC)(A)(RF)(CF)	
Systemic Exposure (mg/person/day) =		0.441/100 (21.62 g/day) (10)/100 (1000 mg/g)	
Intake (mg/person/day) =		**9.5**	

Calculation of intakes in mg/kg bw/day

Taking the above intake values from product use scenarios and dividing by the EFSA default body weights (EFSA 2012b) (P5; lowest 5th percentile body weight) for specific age ranges, the following conservative intakes in mg/kg/day are calculated in Tables 11 and 12.

Table 11: Intake in mg/kg/day of Methylparaben in toothpaste

Age range (y)	Estimated Intake of product (mg/person/day)	P5 body weight (kg)	Mean body weight (kg)	Intake at P5 bw (mg/kg/day)	Intake at mean bw (mg/kg/day)
1-3	0.88	8.7	11.6	0.101	0.076
3-10	0.88	14	21.7	0.063	0.041
10-14	0.61	29.4	42	0.021	0.015
14-18	0.61	45	60	0.014	0.010

Mouthwash is not generally recommended for use for children under 6 years of age due to evidence of high levels of unintended ingestion of mouthwash in pre-school children (Zuanon (2005); www.ada.org). Because of this recommendation, the safety of Methylparaben in mouthwash for children below 6 years of age was not included in this safety assessment.

The target child population is aged 6-18. The EFSA data on body weights was only generated for a broader 3–10-year age range (at the lower end of this range) and this includes very conservative low-end body weights that are not representative of a 6–10-year range. To be more accurate for the target age range, we have used more granular WHO data for age 6-10 years as available online at:
(https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/weight-for-age-5to10-years
https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/weight-for-age-5to10-years).

Table 12: Intake in mg/kg/day of methylparaben in mouthwash

Age range (y)	Estimated Intake of product (mg/person/day)	P5 body weight (kg)	Mean body weight (kg)	Intake at P5 bw (mg/kg/day)	Intake at mean bw (mg/kg/day)
6-10*	9.5	16	20.2	0.594	0.470
10-14#	9.5	29.4	42	0.323	0.226
14-18#	9.5	45	60	0.211	0.158

* WHO body weight data. #EFSA body weight data.

SCCS comment
The Applicant has provided exposure estimates for toothpaste and mouthwash use by children. However, the values have not been aggregated. In addition, dermal exposure estimates for other cosmetic products were not provided. Therefore, a safety assessment for children and adolescents for the simultaneous use of Methylparaben in oral and dermal applications was not performed.

3.2.3 Inhalation exposure

According to the Applicant
In the Cosmetics Europe survey from 2016 (Cosmetics Europe report in 2017), Methylparaben is used in low levels in spray products. The worst-case systemic exposure dose as inhaled (SED_{inh}) is expected to be from propellant hairsprays. In the 11th revision of the SCCS Notes of Guidance (2021), a simple model for inhalation exposure was presented and this model has been used below to estimate the very low level SED_{inh} (0.003 mg/kg/day) to Methylparaben from a hairspray product. All other spray products would be even lower than this. According to the Applicant, inhalation exposure is not a concern for Methylparaben at the maximum level used.

Table 13: Inhalation exposure model (as per SCCS 11th NoG 2021) for Methylparaben in a propellant hairspray.

Description	Parameter	Propellant spray	Unit
Amount by application	A	6800[a]	mg/application
Fraction of MP in spray	C	0.35	%
Proportion of non-propellant in formulation	P	0.6	-
Airborne fraction	AF	1	-
Potential amount to be inhaled	EA(A*C*P*AF)/100	14.28	mg

First step: near-field, $1m^3$	V_1	1000	L
Breathing rate	BR	13	L/min
2 min in near field	t_1	2	min
Potential amount inhaled during t_1	IA_1 (EA/V_1*BR*t_1)	0.37	mg
Second step: far-field $10m^3$	V_2	10000	L
Breathing rate	BR	13	L/min
10 min in far-field	t_2	10	min
Potential amount inhaled during t_2	IA_2 (EA/V_2*BR*t_2)	0.19	mg
Substance availability fraction	G	0.75	
Respirable fraction	RF	0.2	
Frequency of application	F	2	d^{-1}
Default body weight	BW	60	kg
SED_{inh}	$(IA_1+IA_2)*G*RF*F/BW$	**0.003**	**mg/kg/day**

a. As derived in the ConsExpo factsheet (Bremmer, 2006).

Ref: Brenner, 2006

SCCS comment

The SCCS noted that for the airborne fraction a worst-case assumption has been used. Assumptions regarding the size of boxes and time, as well as the breathing rate, are all in accordance with the SCCS Notes of Guidance.

The Applicant has provided an assessment of inhalation exposure to Methylparaben, resulting in a SED_{inh} of 0.003 mg/kg bw/day. This value was not aggregated with the oral and dermal exposure.

Since inhalation exposure from hairspray (assuming 100% uptake) results in a lower systemic exposure than dermal exposure from hairstyling products (0.0253 mg/kg bw/day), which are included in the deterministic calculations presented in Table 9, inhalation exposure to hair spray is assumed to be covered by the aggregate exposure value of 0.671 mg /kg bw/day.

3.4. TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

<u>According to the Applicant</u>

<u>Animal data</u>

Table 14: Skin irritation studies in animals.

Test material % & pH	Test conditions	Results	Reference
Rabbit			
10% MP in a hydrophilic ointment paste	Test substance applied to the shaved back for 48h	Not irritant	Sokol (1952); as cited in CIR 2012
0.1 ml neat MP	Draize test using n=9 rabbits. Shaved skin was treated and occluded for 24h. Skin sites were graded at 24 and 72h	Primary irritation index was 0.67 with a maximum score of 4.0, indicative of very mild irritation	CTFA (1976a); as cited in CIR 2012

Human data

Table 15: Skin irritation studies with Methylparaben in humans.

Test material % & pH	Test conditions	Results (classified as)	Reference
0.8% methyl paraben	24h single insult patch test n=20 subjects	Not irritant	CTFA 1978a
0.2% methyl paraben in a hairdressing formulation	5 day cumulative irritancy daily occlusive patch test. n=50 subjects	No cumulative irritation	CTFA 1981a
0.2% methyl paraben in a white cream	21 day cumulative irritancy – 23h occluded patch per day. n=12 subjects	Not irritant	Hill top research 1979a, 1981

Conclusion of the Applicant: overall, there is no evidence of a skin irritation potential of Methylparaben at concentrations up to 10%. Minor signs of irritation may only be observed when neat Methylparaben is applied to skin. Methylparaben is regarded as not irritating to skin.

RIVM report, 2017
Methylparaben did not irritate the skin in the OECD TG404 studies on acute dermal irritation/corrosion. Data regarding human exposure are available and parabens are not irritating in people with normal, undamaged skin.

Ref: Brand *et al.*, 2017

Cherian *et al.*, 2020
In vitro
Parabens were tested individually for irritancy and sensitisation potential in cocultured keratinocytes and peripheral blood mononuclear cells (PBMCs). Categorization as potential irritants was based on EC50 calculated from concentration-response data for cell death. Methylparaben showed no potential for irritation in the *in vitro* test.

Human data
Parabens have been considered relatively non irritating at levels used in current formulations, as verified in extensive experience with the mix at current applied patch test concentrations. In one retrospective analysis, 1,363 cumulative irritation test studies in more than 45,000 subjects, who use-tested 151 different paraben-containing formulations (along with other ingredients), did not demonstrate parabens to be irritating in typical in-use conditions and irritation scores did not correlate with preservative concentrations.

A recent *in vitro* study showed no skin irritation (Svobodova *et al.*, 2023).

SCCS comment
Based on all available data, Methylparaben is not considered to be irritating to the skin.

3.4.2.2 Mucous membrane irritation / eye irritation

According to the Applicant

No *in vitro* data were available.

Two *in vivo* eye tests have been performed using pure Methylparaben. Methylparaben at 0.1 and 0.2% did not induce ocular irritation when it was instilled into the eyes of rabbits and guinea-pigs. 100% Methylparaben instilled into the eyes of six albino rabbits induced slight transient irritation with an eye irritation score of 1/110 on day 1. The majority of products containing 0.1-0.8% Methylparaben when tested in the 1970's and 80's in rabbit eye irritation studies produced no signs of eye irritation.

Conclusion of the Applicant: Methylparaben, under the conditions of cosmetics use, is not irritating to the eye.

RIVM report, 2017
Methylparaben did not irritate the eyes.
Ref: Brand *et al.*, 2017

A recent *in vitro* study reported no eye irritation (Svobodova *et al.*, 2023).

SCCS comment
On the basis of available information, the SCCS considers that Methylparaben is not irritating to the eyes.

3.4.2 Skin sensitisation

According to the Applicant

Animal data

Table 16: Skin sensitisation studies for Methylparaben in animals

Test type	Method	Observations	Reference
Guinea-pig (n=4)	Intradermal injections of 0.1% MP into shaved dorsal skin for 5 days per week for 8 weeks. Sites were scored 24h after each injection.	Skin reactions were seen but decreased in severity over time	Aldrete & Klug (1970)
Guinea-pig (n=20)	Intradermal injections of 0.1% MP into shaved dorsal skin every other day for 3 weeks (10 injections). Sites were scored 24h after each injection. 2 weeks after the last induction injection, a challenge injection was given. 10 days after this a 5% MP patch was placed on the skin.	3/20 reacted to the patch but it was not considered significant above control.	Maurer *et al* (1980)
Guinea-pig (n=5 male; n=5 female)	0.2% MP in a product formulation. 0.5 ml was applied topically, and the site occluded for 6h. a total of 9 applications were made, 3 per week for 3 weeks. A challenge path was applied 14 days later.	Slight irritation was observed but no reactions at challenge.	CTFA (1981c), as reported in CIR 2012
Guinea-pig maximisation test (GPMT)	1% MP applied in 50% Freunds completet adjuvant, followed by a booster of 10% sodium lauryl sulphate and 50% MP in pretrolatum 24h later. 5% and 10% MP in petrolatum was used for challenge.	No reaction	CTFA (1981d), as reported in CIR 2012

*Klimisch ratings from ECHA/REACH evaluation

Human data

Typically, human repeat insult patch tests (HRIPT) have been performed on parabens mixtures and not Methylparaben alone. Only one evaluation could be found analysing Methylparaben (Table 17).

Table 17: Human data for skin sensitisation.

Vehicle and test conditions	Test material Concentration/pH	Test type	Positive Responses	Reference
Test material applied to the backs of human volunteers (n=25 males; n=25 females)	5% in propylene glycol; applied 4-8h every other day for 3 weeks (10 applications). Challenge 3 weeks later for 24-48h.	HRIPT	None	Sokol 1952

RIVM report, 2017
Methylparaben was not considered to be a skin sensitiser when tested in skin sensitisation OECD TG406 studies.

Ref: Brand *et al.*, 2017

Cherian *et al.*, 2020
In vitro
Parabens were tested individually for sensitisation potential in cocultured keratinocytes and peripheral blood mononuclear cells (PBMCs). Categorization as potential skin sensitiser was based on EC50 calculated from concentration-response data for CD86 expression. Methylparaben showed no potential for irritation in the *in vitro* test. Methylparaben was classified as a weak skin sensitiser in this *in vitro* test.
In a recent publication, Methylparaben and other parabens were tested in three NAMs for skin sensitisation: DPRA, LuSens and h-CLAT. The DPRA was negative, whereas both LuSens and h-CLAT were positive (Svobodova *et al.*, 2023).

Human data
Paraben sensitisation has occurred, especially when paraben-containing medicaments have been applied to damaged or broken skin. Even when applied to patients with chronic dermatitis, parabens generally induce sensitization in less than 3% of such individuals. Of 27,230 patients with chronic skin problems, 2.2% were sensitized by preparations of parabens at concentrations of 1% to 30%. Many patients sensitized to paraben-containing medications can wear cosmetics containing these ingredients with no adverse effects.
Parabens were designated "nonallergen" of the year in 2019 by the American Contact Dermatitis Society. Monitoring for paraben allergy followed with studies reporting paraben testing in standard screening fashion since 1940. The frequency of allergic contact sensitization to parabens has remained low and remarkably stable for many decades despite wide use.
Allergic contact dermatitis caused by paraben mixture was analysed on the basis of data collected by the European Surveillance System on Contact Allergies (ESSCA) network between 2009 and 2012 from 12 European countries.124 Of the 52,586 tests during the study period, parabens yielded less than 1% positive reactions. Of the results obtained from 2,362 TRUE-Test, the paraben mixture yielded only 0.4% positive reactions. The allergic contact dermatitis data are summarized in Table 16.

SCCS comment
Methylparaben was positive in *in vitro* tests for skin sensitisation, but not in the DPRA. Methylparaben was negative when tested in animal studies. All human data are based on results from patch tests conducted with paraben mixtures and show that paraben sensitisation is rare and is related to medical applications and not to cosmetics. Human skin sensitisation data specifically for Methylparaben are not available. Taking all the data into consideration,

together with the data from animal tests, the SCCS considers that Methylparaben is not a skin sensitiser.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

According to the Applicant
Animal data: there are a number of studies from the 1970's in animals covering the acute oral toxicity of Methylparaben.

Table 18: Acute oral toxicity studies for Methylparaben.

Reference	Species	Dosing	Oral LD_{50} (mg/kg bw)	Observed effects
Litton Bionetics (1974)	Rat; groups of 5 to 10	100 to 5000 mg/kg in 0.85% saline solution	2100	All 10 animals dosed 5000 mg/kg died within 24h. Reddened gastric mucosa and congested lungs. No animals died at 100 or 500 mg/kg
CTFA (1976c, 1979a, 1979b)	Female rat n=5	Products containing 0.2% or 0.8% MP and dosed up to 15,000 mg/kg by gastric intubation	>15000	No gross lesions at day 7; no deaths.

NR = not reported

3.4.3.2 Acute dermal toxicity

According to the Applicant

Table 19: Acute dermal toxicity study for Methylparaben.

Reference	Species	Dosing	Dermal LD_{50} (mg/kg bw)	Observed effects
CTFA (1981b)	Rabbit n=3 male, n=3 female	0.2% in a hairdressing product. Doses of 2ml/kg applied to intact and abraded skin. Occluded for 24h.	NC	No toxic effects were seen 14 days post treatment

3.4.3.3 Acute inhalation toxicity

According to the Applicant

Table 20: Acute inhalation data for Methylparaben.

Reference	Species	Dosing	Inhalation LD_{50} (mg/kg bw)	Observed effects
Jian & Po (1993)	Male Wistar rat	Inhaled concentration of 1.18 mM for 4h exposure	NC	Mildly toxic to the respiratory mucus membranes (ciliotoxic)

Overall conclusions of the Applicant
Methylparaben is not acutely toxic.
The LD50 via the oral route in rats was 2100 mg/kg in a saline solution.
The LD50 via the dermal route in rabbits was not calculable. No toxicity observed at the dose studied.
The LC50 via the inhalation route was not calculated, but it was not acutely toxic in the Lung.
SCCS comment
The SCCS agrees with the Applicant's conclusion that Methylparaben is not acutely toxic.

3.4.4 Repeated dose toxicity

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

According to the Applicant

Table 21: Subchronic studies

Study	Species	Duration	Dose (mg/kg/day)	Observations
Subchronic 28-day studies				
Bijlsma (1928)	Dog	28 days	18	No toxicity and no gross lesions upon necropsy.
CTFA (1980a)	Rat n=10 male, n=10 female	28 days	0.2% MP & 0.2% PP: 0, 40, 200 mg paraben /kg/day as 2ml/kg in corn oil	All rats survived except one due to misdosing by gavage. No signs of toxicity were seen. Body weight gain and food consumption were unaffected. Slight changes in blood chemistry parameters were not statistically significant.

Beerens-Heijnen (2009) (as cited in EU REACH 2021).	Wistar rat (n=5/sex/dose)	28 days; n=5 animals also had 14 days recovery.	0, 50, 250, 1000 mg/kg/day of MP in propylene glycol by oral gavage	Two animals appeared to have suffered misdosing and were sacrificed due to ill health. Some effects in spleen to body weight ratio observed in males in high dose group only. Some females displayed rales and gasping, with piloerection. All observations resolved. No histopathological findings; No other adverse observations in any toxicological parameters. No effects on oestrous cycle or spermatological parameters. NOAEL 1000 mg/kg/day

SCCS comment
From oral subchronic (28 days) repeated dose toxicity studies provided by the Applicant, a NOAEL of 1000 mg/kg/day was derived for Methylparaben.

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

According to the Applicant

Table 22: Subchronic dermal toxicity studies.

Study	Species	Duration	Dose (mg/kg/day)	Observations
3 month/13 week studies				
CTFA (1980b)	Albino rabbits (n=5 male; n=5 female). n=7 male and n=7 female in control group.	Daily topical exposure for 3 months	0.2% MP in product formulation; 5.5 mg/cm^2 over 8.4% total body surface area	Body weight gain, food consumption, organ pathology and blood chemistry were not affected by treatment. Mild inflammation at the skin treatment site
CTFA (1980c)	Albino rabbits (n=5 male; n=5 female). n=7 male and n=7 female in control group.	Daily topical exposure for 3 months	0.2% MP in product formulation; 6.6 and 11 mg/cm^2 over 8.4% total body surface area	Body weight gain, food consumption, organ pathology and blood chemistry were not affected by treatment. Mild inflammation at the skin treatment site
CTFA (1981f)	Rat (n=10)	Daily topical exposure for 13 weeks	0.7% MP in a medicated cream; 4.12 g/kg to dorsal shaved skin 10-15% body area	Decreased body weight in males. No systemic toxicity observed. Inflammation only at the skin site.

Other routes:

According to the Applicant
A repeat dose subcutaneous toxicity study was performed in Fischer rats by Mason *et al.* (1971) where Methylparaben was administered via subcutaneous injection at doses of 3.5, 2, 1.1 and 0.6 mg/kg/ to groups of 80, 60, 40, and 20 rats, respectively. Doses were twice weekly for 52 weeks. Some rats were sacrificed at 52 weeks, and some were observed for an additional 6 months and scheduled for necropsy. Methylparaben treated rats showed no significant differences in mortality, weight gain or lesions from control animals.

Oral 90-day repeated dose toxicity
Based on Article 41 of Regulation (EC) No 1907/2006 (REACH-Regulation), the European Chemicals Agency (ECHA) has requested an oral subchronic toxicity study in rats according to EU BH.26/OECD TG 408. The original study report has been made available as a result of the EC call for data and is described briefly below.

Guideline: OECD Test Guideline 408
Species/strain: Rat, Wistar, Crl: WI(Han), 7-8 weeks old

Group size:	10/sex/dose + vehicle recovery group (5/sex) + high dose recovery group (5/sex)
Test substance:	Methyl 4-hydroxybenzoate (Methylparaben, MP)
Batch:	BM18020221 (Material No. 16690126894)
Purity:	100 %
Vehicle:	1% hydroxyethylcellulose
Dose levels:	0 (C), 100 (LD), 300 (MD) and 1000 (HD) mg/kg bw/day
Administration:	Oral gavage (5 ml/kg bw)
GLP:	In compliance
Study period:	Jan 2019 – Nov 2019

Information from the study report (shortened):
The test item was administered daily for a treatment period of 90 days. Control animals received the vehicle 1 % aqueous hydroxyethyl-cellulose, a recovery group was kept for a period of 28 days following the last administration. Once before the first exposure and once in the last week of exposure as well as in the last week of the recovery period functional observational battery tests were performed. At the conclusion of the treatment period, all animals were sacrificed and subjected to necropsy. A full histopathological evaluation of the tissues was performed on high dose and control animals. Gross lesions macroscopically identified were examined microscopically in all animals.

Results:
Test-item related alterations with respect to ophthalmoscopy, functional observation battery (FOB), urinalysis and histopathology were not observed. Male animals of all treatment groups showed lower body weight gain in week 8. In the low and high dose males, lower body weight gain was also observed during week 9. In female animals, slight to moderate decrease in mean body weight gain was observed on weeks 8, 10, 11, 12 and 13. Haematological changes consisted in a slight increase in mean white blood cells (WBC) in MD males and in LD and MD females; a tendency towards lower or higher percent differential leucocytes counts in males and females. After recovery, WBC were increased in male animals of the high dose and decreased in high dose females. Apart from statistically significant increase in percent reticulocytes in HD males (112 % above control) at the end of recovery, haematological parameters were within the range of historical controls.
With respect to clinical biochemistry, dose-related increases in potassium were observed for both sexes, in females also LDL was dose-dependently increased. In female animals, increases were also observed for TBIL, TBA, Cholesterol and aPTT. The observed changes remained in the range of historical control data (HCD), however, HCD data were not available for thyroid hormones, LDL and HDL. In male animals, TSH was dose-dependently decreased.
Compared to concurrent controls, the mean total number of abnormal and normal sperms/findings (sperm morphology) in HD males showed statistically significant differences at the end of the treatment period. However, the values were within the range of historical values provided along with the study report. Amongst the observed organ weight changes, a moderate but statistically significantly higher relative mean thymus weight was observed in males of the HD recovery group when compared to concurrent controls; a moderate increase in absolute and relative mean uterus with cervix weight was observed in females of the HD recovery group, when compared to the controls.
Slight but statistically significantly lower mean calculated weight of testicular parenchyma in HD groups was observed, when compared to concurrent controls at the end of treatment of main groups. No treatment related effects on the mean testis weight and mean testicular sperm counts in the recovery periods were observed.
The study authors derived a NOAEL of 1000 mg/kg bw/day from that study.

SCCS comment

In male animals, TSH was dose-dependently decreased (statistically significant in HD, p<0.01, 0.57 ng/ml versus 1.06 ng/ml in controls) and this decrease remained present after recovery. Testis weight was reduced at HD (statistically not significant) at the end of treatment and there was a (statistically not significant) trend for increased weight of Tunica Albuginea in all dose groups. In HD males, the calculated weight of testes parenchyma was statistically significantly (p<0.05) decreased when compared to controls.
Sperm motile count parameters in high-dose males showed statistically non-significant changes (sperm motile count: dose-dependent decrease of [%]: 76.8 high-dose group vs 84 in controls; static count: increase of [%]: 23.2 HD vs 16 in C; rapid count: decrease of [%]: 54.7 in HD vs 65.6 in C. The mean testicular sperm count was increased (statistically not significant) in HD males at the end of treatment, but not after recovery. The following sperm morphology parameters (head and neck and tail) were changed in HD males at the end of treatment: increased number of sperm with head only (1.6 ± 0.84 in HD vs 0.8 ± 0.79 in C (n=10 animals)) and increased number of sperm with broken tail 0.7 ± 1.06 in HD vs 0.10 ± 0.32 in C (n=10 animals); increased total number of abnormal sperm (p<0.05), 7.3 ± 1.34 in HD vs 5.7 ± 1.7 in C (n=200 sperm)); decreased total number of normal sperm (p<0.05); increased percentage of abnormal sperm (p<0.05), 3.65 ± 0.67 in HD vs 2.85 ± 0.85 in C (n=200 sperm)). Importantly, all the values related to sperm parameters were found to be within the normal range of the historical control data provided along with the study report.

Table 23: Sperm Morphology.

Mean Sperm Morphology – End of Treatment

Group	Animal No.	Head and Neck					
		Amorphous Head (AH)	Banana Shaped head (BH)	Hookless sperm (HkS)	Pin Head (PH)	Head only(HO)	Bent neck
C	Mean	0.00	0.00	0.00	0.00	0.80	0.00
	SD	0.00	0.00	0.00	0.00	0.79	0.00
	N	10	10	10	10	10	10
HD	Mean	0.00	0.00	0.00	0.00	1.60	0.00
	SD	0.00	0.00	0.00	0.00	0.84	0.00
	N	10	10	10	10	10	10

Group	Animal No.	Tail			
		Bent Tail (BT)	Broken Tail (BrT)	Coiled tail (CT)	Tail Only (TO)
C	Mean	2.90	0.10	0.00	1.90
	SD	1.29	0.32	0.00	0.99
	N	10	10	10	10
HD	Mean	3.00	0.70	0.00	2.00
	SD	1.70	1.06	0.00	1.49
	N	10	10	10	10

In conclusion, the effects on 49 functional observation parameters were slightly or inconsistently changed throughout the dose groups before and at the end of treatment/recovery. These can therefore not be related to adverse effects and are not useful for the derivation of the NOAEL or LOAEL.
In the absence of histopathological changes and due to reversibility of some of the effects observed, the SCCS considers the highest dose as NOEL. The sperm findings may be indicative for an anti-androgenic MoA (see also section3.4.10).

Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test

In addition to the oral 90-day repeated dose toxicity study described above, based on Article 41 of Regulation (EC) No 1907/2006 (REACH-Regulation), the European Chemicals Agency (ECHA) has requested an oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test according to OECD TG 422. The original study report has been made available as a result of the EC call for data and is described briefly below.

Guideline:	OECD Test Guideline 422 (version 29 July 2016)
Species/strain:	Rat, Wistar, Crl: WI(Han), approx. 14-15 weeks old
Group size:	10/sex/dose
Test substance:	Methyl 4-hydroxybenzoate (Methylparaben, MP)
Batch:	BM18020221 (Material No. 16690126894)
Purity:	100 %
Vehicle:	1% hydroxyethylcellulose
Dose levels:	0, 100 (LD), 300 (MD) and 1000 (HD) mg/kg bw/day
Administration:	Oral gavage (5 ml/kg bw)
GLP:	In compliance
Study period:	Dec 2018 – Dec 2019

Information from study report (shortened):
The test item was administered daily during 14 days of premating and maximum 14 days of mating in both males and females, during the gestation period and up to post-natal day 12 in females. Males were dosed after the mating period until the minimum total dosing period of 28 days was completed. Before dosing, all females were screened for two weeks for regular oestrous cyclicity and animals (10 females/group) with regular oestrous cycle (4–5-day cycle) were used in the study. The study was performed according to the 2016 version of OECD TG 422.

Results:
All animals survived the scheduled study period. Compared to concurrent controls, treatment with the test item had no significant effect on the oestrous cycle analysed during the 2-week premating period. There were no test item-related effects on litter data including total number of male and female pups, sex ratio and number of stillbirths and runts. Treatment with the test item had no statistically significant effects on litter weight data on PND 0, 4 and 13 when comparing test item-treated groups and the controls. Pre-coital interval and duration of gestation were not affected. Slight differences in number of corpora lutea, implantations sites, live pups on PND 0, 4 and 13 as well as preimplantation loss and post implantation loss were within the range of historical control data (HCD) provided along with the study report as were slight differences in the reproductive indices. Mean mortalities in treated pups were comparable to concurrent and historical controls. Compared to concurrent controls, mean male pup nipple retention was statistically significantly lower, only in the HD group compared to the control group, but was within the range of HCD. Female pups at MD had a statistically significantly higher mean pup weight (control: 5.76, MD: 6.04) and mean cube root of pup weight (control: 1.79, MD: 1.82). LD females had statistically significantly lower absolute

(control: 1.31, LD: 1.07) and relative (control: 0.73, LD: 0.60) anogenital distance. In HD male parental animals, T4 levels were statistically significantly lower compared to concurrent controls. Treatment did not cause gross external pup findings in any of the test-item treated groups or the control group.
Apart from a statistically significant increase in PT levels in HD male animals, there were no statistically significant changes in haematological parameters. In male animals, mean total bile acids (TBA), ALAT and ASAT showed a dose-dependent decrease, however none of the decreases was statistically significant. Females at the highest dose had a considerably higher mean in TBA (statistically not significant, exceeding HCD), due to extremely high values observed in 2 of 5 animals.
Only single or occasional macroscopic findings without corresponding histopathological findings were noted in the groups during necropsy of the animals. In HD males, mean relative pituitary gland weight was higher when compared to the concurrent controls. Absolute and relative mean adrenal gland weight was statistically significantly lower in females of the MD group.
There was no histological evidence of toxicity in the reproductive organs and tissues including testes, epididymides, prostate gland, seminal vesicles, coagulating glands, ovaries, uterus, cervix, and vagina. No treatment-related effects on the testicular histomorphology and interstitial cell structure were noticed. Substance treatment did not induce histomorphological effects in the reproductive organs of the non-pregnant females and their pairing partners.
The study authors derived a NOAEL of 1000 mg/kg bw/d from that study.

SCCS comment
Most of the changes observed in this study were not statistically significant and for several observations, dose-dependency was not observed. In addition, the effects reported were not accompanied by histopathological changes.
Regarding AGD, no difference was observed in male and female pups on PND 0 and in nipple retention of male pups on PND 12.
In female pups, the relative AGD in the LD group was statistically significantly lower when compared to controls, which was due to decreases that were only observed in two animals out of ten. Statistically significantly lower mean thyroxine hormone (T4) levels in male rats (64.51 ± 11.87 in HD vs 78.16 ± 12.08 nmol/L in C (n=10 animals) were measured without corresponding histopathological findings in the thyroid/parathyroid. No statistically significant effects were observed on pup thyroid weight and T4 level in PND 13 pups (male and female) of the Methylparaben-treated groups when compared to the controls.
Based on the results of this study, the SCCS concurs with the study authors and considers 1000 mg/kg bw/d as NOAEL.

3.4.4.3 Chronic (> 12 months) toxicity

According to the Applicant

Table 24: Chronic toxicity studies

Study	Species	Duration	Dose (mg/kg/day)	Observations
Chronic 1-2 years				
Matthews (1956)	Rat n=24	96 weeks	2 or 8% Methylparaben in the diet	Rats dosed at 8% MP had decreased body weight in the early part of the study, which resolved and there were no other toxic effects.

Matthews (1956)	Dog	378-422 days	1g/kg/day dosed to n = 6 dogs; 0.5g/kg/day dosed to n = 3 dogs	No toxicity was observed. All animals were in excellent condition throughout the study,

Conclusion of the Applicant: Although the study by Matthews was performed in 1956, prior to the development of OECD Test Guidelines, the study has been used for many decades to define an oral NOAEL for general toxicity for Methylparaben as 1000 mg/kg/day. Subsequent studies investigating reproductive and developmental effects (i.e. Oishi (2004) and the Charles River 2005 study published as Hoberman *et al.* 2008, and a 28-day study by Beerens-Heijnen (2009) have further corroborated this oral NOAEL. There are no effects seen in dermal toxicity studies.

RIVM report, 2017
Based on the available repeated-dose toxicity studies, repeated oral exposure to methyl-, ethyl- or propylparaben is not considered to cause serious effects to health. No data were available on toxicity relating to repeated dermal exposure and inhalation.

Ref: Brand *et al.*, 2017

SCCS comment
All repeated dose toxicity studies provided and discussed in the Applicants dossier and the information provided by RIVM in their 2017 report during the call for information point to a NOAEL of 1000 mg/kg bw/d for repeated dose toxicity. Two further oral *in vivo* repeated dose toxicity studies that had been requested in the context of another legislation have been made available to the SCCS. While the combined repeat-dose toxicity/reproductive toxicity study confirmed a NOAEL of 1000 mg/kg bw/d, the 90-day RDT study was indicative of changes pointing to an endocrine mediated MoA and effects on male reproductive parameters, however without histopathological findings. Therefore, the highest dose of 1000 mg/kg bw/d can be regarded as the NOAEL.

3.4.5 Reproductive toxicity

From the Applicant

Table 25: Reproductive toxicity and developmental toxicity studies

Species	Method	Route of exposure	Dosage	Results	Reference
Male Reproduction					

Male Crj:Wistar 25-27-day old rats (n=5 per group)	8 weeks study	Oral	0.1% and 1% in rat diet	No effects on organ weights. MP did not exhibit antispermatogenic effects. There was no effect on male reproduction. There were no changes in testosterone, LH and FSH hormones. NOAEL 1000 mg/kg/day	Oishi 2004
Crj:Wistar rat (n=16 per group)	Repetition of the Oishi study (2004) under GLP with MP using the same strain of rats at a higher number of animals per group. In addition to the parameters of the Oishi study, blood samples were taken weekly for the analysis of LH (luteinizing hormone), FSH (follicle stimulating hormone) and testosterone	Oral	0, 100, 1000 and 10,000 ppm in food	There were no relevant treatment related effects on testes, ventral prostates and preputial glands in any of the groups. There were no relevant effects on male reproductive parameters. The small but statistically significant increase in abnormal sperm in the 10,000 ppm group was not considered relevant due to the low magnitude and the fact that no other reproductive parameters were altered. The highest dose level in food corresponds approximately to a NOAEL of 1000 mg/kg bw/day	Hoberman *et al.* 2008; Charles River 2005.

Applicant conclusions from reproduction and developmental toxicology studies:
Methylparaben showed no relevant adverse effects in reproductive and developmental studies. The NOAEL of 1000 mg/kg/day can be used as the point of departure in safety evaluation.

RIVM report, 2017
For Methylparaben no OECD TG studies on reproductive toxicity were performed, but relevant peer-reviewed studies were performed and summarized in this paragraph. All these studies investigated the effects of methyl-, ethyl- and propylparaben exposure on the reproduction of male and female animals. As previously stated, for Methylparaben, a NOAEL of 1000 mg/kg bw/day was derived from the study by Oishi (2004). The NOAEL of 1000 mg/kg bw/day from this study also supported the establishment of the ADI for Methylparaben by EFSA. Oishi (2004) did not find any reproductive effects in rats after Methylparaben exposure up to a level

of 1000 mg/kg bw/day. This NOAEL does not take possible spermatogenic effects identified by Hoberman *et al.* (2008) into account, nor the delay in the date of vaginal opening in pre-pubertal rats and decrease in length of the oestrous cycle with a NOAEL of 250 mg/kg bw/day identified by Vo *et al.* (2010). The Vo *et al.* (2010) study was also taken into account by the SCCS. Vo *et al.* (2010) identified a NOAEL of 250 mg/kg bw/day and a LOAEL of 1000 mg/kg bw/day (effects on the date of vaginal opening, the length of the oestrous cycle and affected organ weight (thyroid, liver, adrenal gland and ovary)). The SCCS concluded that this study could not be used to determine the NOAEL since it was not an OECD TG study and the effects were not dose-response related. The RIVM does not completely agree with the SCCS opinion, since effects on the oestrous cycle and organ weights occurred only at the highest dose level tested (1000 mg/kg bw/day). The study by Vo *et al.* (2010) was well designed and the measured effects on vaginal opening, oestrous cycle and organ weights are relevant. Nevertheless, the RIVM recommends that further study for these or comparable effects is needed at the same dose levels.

Ref: Brand *et al.*, 2017

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH-Regulation), the European Chemicals Agency (ECHA) has requested an extended one-generation reproductive toxicity study in rats according to EU B.56/OECD TG 443. The original study report has been made available as a result of the EC call for data and is described briefly below. As a range-finder for the OECD TG 443 study, a combined repeated dose oral toxicity study with the reproduction/developmental toxicity screening test (OECD 422/OECD 421) has been performed. The original study report was also made available to the SCCS and is described in the section on repeated dose toxicity.

Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422)

See section 3.4.4.

Extended one Generation Reproductive Toxicity Study (EOGRTS, OECD TG 443)

Guideline:	OECD Test Guideline 443
Species/strain:	Rat, Wistar, Crl: WI(Han), approx. 12-13 weeks old
Group size:	30/sex/dose at high dose (P Generation) 25/sex/dose at mid and low dose (P Generation)
Test substance:	Methyl 4-hydroxybenzoate (Methylparaben, MP)
Batch:	BM18100811 (Material No. 16690126894)
Purity:	99.9 %
Vehicle:	1% hydroxyethylcellulose
Positive Control:	Cyclophosphamide (cohort 3)
Dose levels:	0 (C), 100 (LD), 300 (MD) and 1000 (HD) mg/kg bw/day
Administration:	Oral gavage (5 ml/kg bw)
GLP:	In compliance
Study period:	Aug 2019 – March 2020 (experimental completion) Report dated 5 May 2021

The test item was administered daily in doses of 0 (control - C), 100 (low dose – LD), 300 (mid dose – MD) and 1000 (high dose – HD) mg/kg bw/d groups of test animals. The parental (P)-generation animals were exposed with the test item by oral gavage 2 weeks during pre-mating (males and females), 2 weeks during mating (males and females), 6 weeks post-mating up to termination after weaning - 10 weeks total treatment (males), during pregnancy and lactation up to termination after weaning- 8-10 weeks' total treatment (females).

At weaning, selected F1 offspring were assigned to specific cohorts for the investigations comprising sexual maturation, reproductive organ integrity and function, neurological and behavioural endpoints, and immune functions. In F1 males and females, the direct exposure to test item was started at weaning until the scheduled termination, i.e., until an age of 13 weeks (Cohort 1A, twenty animals per sex and group) or until study termination (weeks 20-25: Cohort 1B, twenty animals per sex and group). Furthermore, Cohort 2A animals were sacrificed at an age of 12 weeks (Cohort 2A, ten animals per sex and group).
Cohort 2B animals served for developmental neurotoxicity and were sacrificed at weaning (ten animals per sex and group). Cohort 3 animals underwent evaluation of developmental immunotoxicity and were sacrificed at an age of 8-10 weeks (ten animals per sex and group). Cohort 4 contained ten animals per groups and sex for learning and memory testing that was sacrificed after completion of the test on post-natal day 38-39. During the period of administration, the animals were observed each day for signs of toxicity. Animals that died were examined macroscopically and at the conclusion of the test, surviving animals were sacrificed and observed macroscopically.

Results:
Clinical Observations:
During the weekly detailed clinical observation, no toxicologically relevant differences between the groups were observed in parental and F1 cohorts (Cohort 1A, 1B, 2A and 3) during the entire study period. There were statistical significances observed in few parameters in parental and F1 cohorts on few occasions.

Body weight:
Overall, in all parental and F1 cohorts, the body weight and body weight gain remained unaffected by the treatment with test item and values were in the normal range of variation throughout the treatment period when compared to the control group and also the mean body weights were found to be within the historical control range of this strain.

Litter weight data:
There was no test item related effect on pup mean weight, total litter weight, male and female litter weight on postnatal day (PND) 0, PND 4, 7, 14 and PND 21 observed in parental and Cohort 1B treatment groups when compared to the controls. There was no statistically significant change in dose groups compared to control except slight but statistically significantly lower pup mean weight from parental females on PND 0 in the Cohort 1B and male mean litter weight on day 7 in HD group in parental females when compared to control.

Anogenital Distance and nipple retention:
In male pups from parental females on PND 0, marginal but statistically significantly lower pup weight, cube root of pup weight and relative anogenital distance (AGD) in HD groups were observed when compared to the control. No effect was seen on absolute AGD. In MD, marginal but statistically significantly higher absolute AGD was observed. In male pups from Cohort 1B females on PND 0, slight, but statistically significantly lower pup weight, cube root of pup weight and absolute AGD were observed in the LD and HD group when compared to the control. Statistically significantly lower absolute AGD in MD group and lower relative AGD in HD groups were observed. In female pups from Cohort 1B females on PND 0, slight but statistically significant effect was observed on pup weight and cube root of pup weight parameter.

Oestrus Cyclicity:
There was no biologically significant effect on oestrus cyclicity in parental and cohort 1A females and no biologically significant effect in cohort 1A females on the time between vaginal opening and first oestrus cycle when compared with controls.

Haematology:

In parental generation, some changes in haematological parameters were observed in treated animals when compared to the controls.

Clinical Biochemistry:
There were some statistically significant differences in clinical biochemistry parameters of male and female parental and Cohort 1A animals.
Changes in haematology and clinical biochemistry were not associated with histopathological findings, did not show dose response relationships and/or within the range of historical controls.

Thyroid hormones:
In parental males and females (10/sex/group), group mean T4 and TSH levels were comparable with the control, except statistically significantly higher group mean TSH values in LD group parental females and statistically significantly higher group mean T4 values in MD group parental males.

Pathology:
Few spontaneous gross pathological changes were recorded for male and female animals from parental generation and various cohorts and were not considered to be treatment related by the study authors.

Organ weight:
Slight changes in organ weights observed in Cohort 1A, *i.e.* statistically significantly lower absolute and relative kidneys weight in LD group, absolute heart weight in LD and HD group, absolute and relative epididymides weights in LD and HD groups and absolute and relative liver weight in the LD group males and those findings were not associated with histopathological findings.
Weights of lymph nodes, spleen and thymus of Cohort 1A animals revealed no considerable changes that could indicate a test item related immunotoxic effect.
There was no test item-related effect on brain, spleen and thymus weights in F1 pups not selected for cohorts and F2 pups from Cohort 1B females at weaning.

Sperm findings:
In parental animals, difference between controls and Methylparaben dosed animals were not observed with respect to sperm number or number of normal/abnormal sperms. In cohort 1A males, statistically significantly ($p<0.01$) lower percentage of static sperm count was observed in all Methylparaben dosed groups. However, the variability within the control group was quite high and the finding might be compensated by increasing percentages of motile and rapid sperm counts observed at the same time.

Cohort 2
There were 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 as demonstrated by Haematoxylin & Eosin staining and Fluoro-Jade staining. No morphometric changes were observed in dose groups compared to control.

Cohort 3
On PND 56 ± 3 days, Cohort 3 animals (10 males and 10 females from each treatment group; 1 male or 1 female per litter; all litters represented by at least 1 pup; randomly selected) were used in a T-cell dependent antibody response assay.

The positive control group (C2) was administered with Cyclophosphamide 7 days before immunization until the day before the last blood sampling. Approximately one week after the start of the treatment with Cyclophosphamide or vehicle or test item, each animal of group (C, C2, LD, MD and HD) was injected intravenously into the tail vein with 0.300 µg/kg of KLH as single dose (at 0.75 mL/kg of dose volume). On PND 56 ± 3 days, a T-cell dependent antibody response assay was performed. The response was evaluated by determining the titre of KLH-specific IgM antibody in the serum by ELISA, at the peak of the response before and after immunization (day 6). Additionally, KLH-specific IgG antibody response was performed, before and after immunization on day 14. In addition, Total IgM and Total IgG were evaluated before and after immunization (day 6-IgM and day 14-IgG) with KLH.

There was no sign of immunotoxicity in this study. The results of the TDAR indicate a functional immune system. KLH-specific IgM levels indicate some variability similar to the negative control and did not show any sign of effect on the specific immune response. An integrated evaluation of all immunologically relevant data of the study comes to the conclusion that this is not considered clinically relevant.
These data comprise clinical observations including phenotyping of splenocytes subpopulations, clinical pathology parameters, weight of immune organs, macroscopic and

histopathological evaluation of lymph nodes, Peyer's patches, spleen and thymus of parental and Cohort 1A animals, where no test item related effects were observed.

In the absence of indication of toxicity, the NOAEL for developmental and reproductive toxicity, developmental neurotoxicity and developmental immunotoxicity the study authors derived a NOAEL of 1000 mg/kg body weight/day from this study.

SCCS comment

Cohort 1
The most remarkable findings in Cohort 1 was the statistically significantly ($p<0.001$) reduced relative AGD in male F2 pups at the highest dose tested, which can be considered indicative for an anti-androgenic mode of action. Based on this observation, a NOAEL of 300 mg/kg bw/day is derived from this study.
The statistically significantly reduced percentage of static sperm count in all Methylparaben treated groups of cohort 1A might be attributed to the high variability observed in the control group. In addition, the finding might be compensated by increasing percentages of motile and rapid counts observed at the same time. For this reason, the SCCS did not use this observation for the PoD derivation.

Cohort 2
The SCCS has carefully evaluated the effects on developmental neurotoxicity parameters assessed in cohort 2. For developmental neurotoxicity, no conclusion can be drawn because this part of the study was not performed according to guidelines and the findings were poorly documented. In addition, the study report did not include historical control data for the parameters assessed. According to the study authors, Methylparaben did not induce statistically significant changes in motor activity in cohort 2. The SCCS noted that changes in motor activity could be observed, but only when data for all measurements of one session were combined. However, due to insufficient description in the study report and lack of historical control data for the parameters assessed, the SCCS considers the findings not sufficiently robust to derive a PoD. In addition, there were neither macroscopical nor histhopathological findings in brains and nerve tissues and brain weights were not affected. Only the combination of all the available evidence points out to an indication of DNT effects, but this is not conclusive enough to enable derivation of a PoD.

Cohort 3 (developmental immunotoxicity)
In cohort 3, variability in anti-KLH IgM both within the treated and control groups immunised with KLH is high. OECD TG 443 gives no indication on the preferred KLH dose to be used for the assessment of developmental immunotoxicity but refers to Gore *et al.* (2004) where an immunosuppressive test substance caused a statistically significant suppression of anti-KLH IgM and IgG antibody production in response to immunisation with either 100 or 300 µg/kg bw KLH, but not with 30 µg/kg bw KLH. The intravenous KLH dose used by the Applicant was only 0.3 µg/kg, which is orders of magnitude lower than the optimal dose identified by Gore *et al.* (2004). This may explain the high variability in anti-IgM KLH IgM titers within the groups. Hence, the SCCS has concerns that the KLH dose was too low to mount an appropriate antigen-response, which was evident from the larger variability within the groups. This limits the identification of potential immunosuppressive effects of Methylparaben and precludes drawing any conclusions on immunosuppressive effects.
Despite this, the SCCS performed a statistical analysis and found that anti-KLH IgM levels were statistically significantly lower in the low-dose and high-dose groups for males compared to the control group indicating a suppressed immune response. There were, however, no statistically significant trends or differences between the control group compared to all the dose groups for anti-KLH IgM and IgG in females and anti-KLH IgG in males. These results should be interpreted with caution, since the study was not performed with the correct KLH dose, which resulted in high variation between the groups.

SCCS overall conclusion on Reproductive toxicity
Apart from studies provided by the Applicant and described in the RIVM report, two further studies were made available to the SCCS (one OECD TG422 study and dan OECD TG 443 study). Findings from the Vo *et al.* (2010) study (effects on the date of vaginal opening, the length of the oestrous cycle and affected organ weight (thyroid, liver, adrenal gland and ovary)) were not confirmed by the new guideline studies (OECD TG 422/421; OECD 443). However, reduction of AGD was observed in F2 pups from cohort 1B at the highest dose tested. The decrease of AGD was considered as the parameter to determine the PoD. It can be considered as an indication for an anti-androgenic MoA. The latter is also supported by effects on sperm which were observed in an oral 90-day repeated dose toxicity study.
From this study, a NOAEL of 300 mg/kg bw/day could be derived.
The SCCS in parallel did BMD modelling, according to the new BMD guidance from EFSA (2022). This resulted in a $BMDL_{5\%}$ of 374 mg/kg bw/day. As the $BMDL_{5\%}$ value is the preferred PoD value according to the SCCS Notes of Guidance, this will be used in the MoS calculation.

3.4.6 Mutagenicity / genotoxicity

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

According to the Applicant
The *in vitro* mutagenicity and genotoxicity studies that have been performed are summarised in Tables 23 and 24.

Table 26: *In vitro* bacterial assays for Methylparaben

Method	Test Article	Method details	Results	Reference	SCCS comment
Ames Test	50 µg MPB per plate	Salmonella typhimurium strains TA1538, TA1537, TA1535, TA100 and TA98 with and without rat liver S9	Not mutagenic	Unnamed Study 1981 as per ECHA REACH dossier[1]	Ames test was negative Testing was performed only with one concentration which s is not according to OECD TG 471. One bacterial test strain recommended by OECD TG 471 (E. coli WP2 strains or S. typhimurium TA102) has not been used.
Ames Test	4 to 6 doses, the highest concentration being 10 mg/plate. The test was repeated with 4 to 6 doses, the highest concentration being 3 mg/plate	Salmonella typhimurium strains TA1537, TA1535, TA100 and TA98 with and without rat liver S9	Not mutagenic	Unnamed Study 1982 as per ECHA REACH dossier[1]	Ames test was negative One bacterial test strain (E. coli WP2 strains or S. typhimurium TA102) recommended by OECD TG 471 has not been used.
Ames Test	0, 0.033, 0.10, 0.33, 1.00, 3.30,	S. typhimurium strains TA98,	Not mutagenic	Unnamed Study 1991 as per ECHA REACH dossier1	Ames test was negative

	10 mg/plate in DMSO	TA100, TA1535, TA1537, TA1538 and the E. coli strain WP2, with and without S9.			

[1]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/14310/7/7/2/?documentUUID=c6b99e26-baed-4b2e-8f35-6dc55c1bafed

All of the Ames tests were reported to be conducted according to OECD Test Guideline 471 (Bacterial Reverse Mutation Assay) but none were performed according to GLP.

Table 27: *In vitro* mammalian gene mutation.

Method	Test Article	Method details	Results	Reference	SCCS comment
OECD Test Guideline 476 (In Vitro Mammalian Cell Gene Mutation Test) in Hprt and xprt genes. GLP study	99.8% pure MP; 0.25, 0.50, 1 and 2 mg/ml	Chinese Hamster Ovary (CHO) with and without S9. The derivative of the CHO-K1, CHO AA8 cells were used as the test system as recommended in OECD Test Guideline 476	Not mutagenic	Unnamed study report (2019) as per ECHA REACH dossier[1]	Test is negative

[1]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/14310/7/7/2/?documentUUID=c676251a-638f-45c9-bc9e-231b22aaead7

From ECHA and public literature:
Besides the *in vitro* studies provided by the applicant, additional *in vitro* mammalian gene mutation test and chromosomal aberration studies with Methylparaben were presented in the ECHA REACH dossier. In addition, the SCCS conducted an additional literature search and found more *in vitro* studies. All are summarised in Table 28.

Table 28: *In vitro* chromosomal aberration.

Method	Method details	Results	Reference	SCCS comment
Chromosomal aberration study	WI-38 human fibroblasts exposed at concentrations: 1, 10, 100 µg/mL for 24 and 48 h. Positive control: triethylene melamine (0.1 µg/mL).	Negative	Litton Bionetics, 1974	Negative Metabolic activation system was not used.

	Percent of cells in mitosis: 200 cells observed/dose level.			
Chromosomal aberration study	Chinese hamster lung fibroblasts (V79) were incubated with the test chemical at 1 mg/mL both in the presence and absence of S9 fraction. Other concentrations not specified. D20 value was determined (D20 (mg/mL): The dose at which chromosome aberrations were detected in 20% of metaphase cells observed. D20 (mg/mL) for Methylparaben: 1 mg/mL D20 (mg/mL) for 4-Aminoquinoline-1-oxide: 10(exp-1) mg/mL D20 (mg/mL) for N-Ethyl-N-nitrosourea: 10(exp-3) mg/mL D20 (mg/mL) for Benzo[a]pyrene: 10(exp-1) mg/mL	Positive	Unnamed Year: **1980** as per ECHA REACH dossier[1]	Result positive but they are not reliable. Only one concentration was used. Cytotoxicity data not available.
In Vitro Mammalian Chromosome Aberration Test	Chinese hamster lung fibroblasts (V79) were used. Concentration of MP: 125 µg/mL; with and without metabolic activation system.	Methylparaben did not induce chromosome aberrations in the absence of S9 mix but was positive in the presence of S9 mix (5 to 9.9% aberrations).	Unnamed Year: **1978** as per ECHA REACH dossier1	Positive with S9 fraction but the results are not reliable. Only one concentration was used. Only benzo(a)pyrene was used as a positive control.
Human peripheral lymphocytes from 1	Cells were exposed toMP with or without S9 fraction. 48 hours after the start of the culture, the cells were treated for 4 hours ±S9-mix with MPB	Negative At >25 µg/mL inconclusive	Chrz J *et al.*, 2020	Inconclusive Only 1 lowest concentration was analysable; 3 highest

female volunteer Chromosomal aberrations	(10, 25, 50, 100 µg/mL) or for 26 h –S9-mix. Positive controls: Thio-TEPA without S9 and cyclophosphamide with S9. Cells stained with 5% Giemsa. At least 200 well-spaced metaphases were analysed.	due to cytotoxicity		concentrations were considered too toxic. Lack of data on historical controls significantly hampers drawing conclusions. Range of cells with CA in the study negative controls is 2-5.5% vs. 9% in the cells exposed for 26 h –S9. 200 metaphases were analysed which is not in line with OECD TG 473 (recommending scoring of 300 metaphases). THIOTEPA is not among the positive controls recommended by OECD TG 473.
Human lymphocytes from blood of healthy female donors Chromosomal aberrations	Cells treated with MPB at 0.1, 0.25 or 0.5 mg/L for 24 h. Staining with 10% Giemsa solution. For each treatment, four replicates were made. The analysis included the frequency of cytotoxic and genotoxic markers as well as assessment of the Mitotic Index. The frequencies of apoptotic and necrotic cells (cytotoxicity endpoints) and MI were analysed in a total of 4000 cells per each tested concentration and controls. CAs were evaluated in a total of 400 well-spread metaphases per each treatment and controls.	Significant increase in the number of acentric fragments was observed at 0.25 mg/L as compared to the both controls. Increased number of polyploidies (0.10 mg/L) was observed.	Todorovac *et al.*, 2020	Equivocal Although the authors suggest an increased number of polyploidies for MPB, the result is not clear considering the 0.5% polyploidy observed in DMSO (0.1%) control. Any firm conclusions cannot be drawn without reliable data on historical negative control data. No standard positive control was used to validate the system.

[1] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/14310/7/7/2/?documentUUID=b2839dd7-dee8-4304-bc70-60333e11aac3

SCCS comment *on vitro* mutagenicity testing of Methylparaben
Methylparaben was tested for gene mutations in 3 Ames tests, out of which one was considered as valid by the SCCS. All studies were negative. Methylparaben was also tested in one valid mammalian cell gene mutation study on CHO cells with a negative result.
Methylparaben was tested for chromosomal aberration in 5 studies: on WI-38 human fibroblasts with a negative result, on CHO and V79 cells with positive results, in 2 studies on human lymphocytes with inconclusive or equivocal results. All the results on chromosomal aberrations testing were considered of limited or low reliability and relevance. Therefore, based on the results on *in vitro* chromosomal aberration, a genotoxic effect of Methylparaben cannot be excluded.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

From the Applicant dossier
The *in vivo* mutagenicity and genotoxicity studies that have been performed are summarised in Table 29.

Methylparaben was tested in the dominant lethal assay in rats. The test item was suspended in 0.85% saline and dosed at 5, 50, 500 and 5000 mg/kg bodyweight to male rats (acute: single dose; subacute: 5 doses at 5 consecutive days), upon the results of a previously conducted dose range finding study. According to the test procedure the animals were sequentially mated to two females per week for 8 weeks (7 weeks in the subacute study). Females were killed at 14 days after mating and at necropsy the uterus was examined for the number of Corpora lutea, early deaths, late foetal deaths and total implantations.

Table 29: *In vivo* genotoxicity studies for Methylparaben.

Method	Method details	Results	Reference	SCCS comment
Mammalian Bone Marrow Chromosome Aberration Test	10- to 12-week-old, male, albino Sprague Dawley rats. Dosed by oral gavage **Test I:** acute single administration: 5, 50, 500, 5000 mg/kg Time of kill after administration: 6, 24, 48 hours. **Test II** subacute study, 5 consecutive applications, each 24 hours apart: 5, 50, 500, 5000 mg/kg. 50 metaphase spreads were scored per animal. Mitotic indices were obtained by counting at least 500 cells in duplicate and the ratio of the number of cells in mitosis/the number of cells observed was expressed as the mitotic index.	Not genotoxic	Litton Bionetics, 1974 Unnamed study report 1974 as per ECHA REACH dossier1.	Negative results in both tests conducted.

	Vehicle 0.85% saline, positive control: triethylene melamine (0.3 mg/kg) after 48 h.			
Rodent Dominant Lethal Test	10 to 12 week old, male, albino Sprague Dawley rats. Dosed by oral gavage **Test I:** acute single administration: 5, 50, 500, 5000 mg/kg Time of kill after administration: 6, 24, 48 hours. **Test II** subacute study, 5 consecutive applications, each 24 hours apart: 5, 50, 500, 5000 mg/kg Following treatment, the males were sequentially mated to 2 females per week for 8 weeks (7 weeks in the subacute study). Post exposure period: 8 weeks (sequential matings) Tissues and cell types examined: - determination of fertility index - necropsy of the uteri of mated females: - early deaths (deciduomata) - absorptions - dead implantations - total implantations - number of corpora lutea. Vehicle 0.85% saline, positive controls: triethylene melamine (0.3 mg/kg) intraperitoneally.	Not mutagenic	Litton Bionetics, 1974 Unnamed study report 1974 as per ECHA REACH dossier[1].	Not mutagenic

[1]https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/14310/7/7/3/?documentUUID=93134f51-cec3-4d8d-afc2-b0c9f0dfc111

Conclusion of the Applicant: Methylparaben is not mutagenic/genotoxic under any conditions.

SCCS comment
Methylparaben was tested in one valid study on chromosomal aberrations in rats with a negative result, and in one Rodent Dominant Lethal Test in rats with a negative result.

SCF, 1994
In vitro and *in vivo* mutagenicity studies provided no evidence of genotoxicity for Methylparaben.

Ref: SCF. Opinion on p-hydroxybenzoic acid alkyl esters and their sodium salts expressed on 25 February 1994. European Commission, Reports of the Scientific Committee for Food (Thirty-fifth series), http://europa.eu.int/comm/food/fs/sc/scf/reports/scf_reports_35.pdf

NICNAS evaluation
The majority of *in vitro* studies conducted with Methylparaben were negative. A chromosomal aberration test was positive in the presence of S9 metabolic activation. Therefore, it was concluded that Methylparaben was slightly mutagenic with metabolic activation. All *in vivo* genotoxicity tests were negative. Based on the weight of evidence from the available *in vitro* and *in vivo* studies, Methylparaben is not considered to be genotoxic.

SCCS overall comment on genotoxicity of Methylparaben
Methylparaben was tested for gene mutations in bacteria and mammalian cells with negative results.
Methylparaben was tested for chromosomal aberrations in 5 *in vitro* studies: on WI-38 human fibroblasts with a negative result, on CHO and V79 cells with positive results, in 2 studies on human lymphocytes with inconclusive or equivocal results. All the results on chromosomal aberrations testing were considered of limited or low reliability and relevance. Therefore, based on the results on *in vitro* chromosomal aberration testing a genotoxic effect of Methylparaben cannot be excluded.
However, as Methylparaben was tested in a valid study on chromosomal aberrations in rats with a negative result, the SCCS is of opinion that the *in vitro* results on chromosomal aberrations of limited or low reliability can be overruled by the in vivo data. Additionally, Methylparaben was tested in a rodent dominant lethal test, with negative results.
Taken all the data together, including the registration dossier that was submitted to ECHA, Methylparaben can be considered safe in regard to genotoxicity hazard.

3.4.7 Carcinogenicity

From the Applicant dossier
Three studies are of note relating to the investigation of Methylparaben and carcinogenicity. Two studies were performed using dosing of Methylparaben by injection (Homberger, 1968; Mason *et al.*, 1971) and one in diet (Rodrigues *et al.*, 1986).
The applicant concludes that Methylparaben is not a carcinogen in animals.

Table 30: Carcinogenicity studies in animals for Methylparaben

Study	Species	Duration	Dose (mg/kg/day)	Observations
Homberger, 1968	50 CF-1 strain A and 50 A/Jax female mice	7 months	2.5mg methyl paraben was injected into the tail vein	Lungs were examined for the presence of tumours and no significant difference was seen with controls
Mason *et al.*, 1971	Weanling Fischer rats	52 weeks	3.5 (n=80), 2 (n=60), 1.1 (n=40) and 0.6 (n=20) mg/kg; twice weekly	Incidence of injection site tumours, pituitary adenomas, uterine polyps and leukemias were no different from controls. Mammary fibroadenoma incidence was 8%; negative control 1%.
Rodrigues *et al.*, 1986	Weanling Fischer 344 rats n=8	9 days	4% MP in diet orally	No effect was seen in the prefundic region of the animal

Homburger (1968) studied the carcinogenicity of several compounds, including Methylparaben. Methylparaben was found to be non-carcinogenic in mice and rats by different routes. The study with Methylparaben involved various techniques for ascertaining carcinogenicity such as sc injection/secondary host transfer, iv injection/observation of lung adenomas and co-carcinogenesis. A group of 100 male C5BL/6 mice were injected sc with 2.5 mg Methylparaben (in tricaprylin) into the groin. Five weeks later, the injection site skin was excised, minced and pooled. The resulting mix was injected subcutaneously into each of 25 male mice. Eighteen weeks later, animals were killed and examined microscopically for tumours. Positive and negative controls were used. Six of the 25 test animals died by the eighth week. By the 10th week, 12 animals had died. The cause of death was not further investigated. At the injection sites, multiple granulomas with numerous giant cells scattered throughout the tissue were observed. Scar tissue and numerous cysts were present. There were no instances where fibroblasts in granulation or scar tissue suggested malignant transformation. The authors concluded that Methylparaben was not carcinogenic under these test conditions.

Homburger (1968) in a second, more sensitive study, injected 2.5 mg Methylparaben as a single dose into the tail vein of each of 50 CF1 strain A and 50 A/Jax female mice. Another group of 20 CF1 mice received ip injections of 2.5 mg Methylparaben daily for 7 months. Positive and negative controls were used. All mice were killed at 7 months, and the lungs were examined for the presence of tumours. Methylparaben did not significantly induce pulmonary adenoma formation as compared to controls.

In a third study, Homburger (1968) treated mice sc with 12.5 µg dibenzo[a,i]pyrene (DBP) in tricaprylin. Twenty-four hours after the injection of DBP, 2.5 mg Methylparaben was injected in the same site. Additional injections of Methylparaben were made on day 7 and 14. Positive and negative controls were included. All animals were killed between 29 and 30 weeks. Sites were examined microscopically for tumours. Methylparaben was not carcinogenic. However, since positive control animals treated with croton oil showed no effect, the studies were inconclusive.

The carcinogenic potential of Methylparaben was studied by Mason *et al.* (1971). Methylparaben was injected sc at doses of 0.6, 1.1, 2.0, 3.5 mg/kg to groups of 20, 40, 60 and 80 F344 rats, respectively, twice weekly for 52 weeks. Positive, negative and vehicle controls were used. All animals were necropsied after they died or were killed 26 weeks post-treatment. Of all tumours observed in Methylparaben-treated rats, only mammary fibroadenoma incidence was significantly higher than negative control groups (8% incidence for Methylparaben; 1% for negative control). The incidence of injection site tumours, pituitary adenomas, uterine polyps and leukaemia did not differ significantly from that of controls.

Rodrigues *et al.* (1986) studied the short-term effects of various phenols and acids, including Methylparaben, on the F344 rat forestomach epithelium. Methylparaben (4%) was fed to eight rats for 9 days to determine effects on the [^{3}H]thymidine labelling index and the histological appearance of the forestomach. Methylparaben feeding did not affect the labelling index in the prefundic and mid-region of the rat forestomach. Similarly, histopathological observations did not show mucosal changes after Methylparaben feeding. Also, Clayson *et al.* (1986) reported that feeding 4% Methylparaben in the diet to eight F344 male rats for nine days did not affect the [3 H]thymidine labelling index of the rat forestomach.

Ref: Sonia *et al.*, 2002

From SCCP 0874/05

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

addressed parabens and breast cancer in the "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.

From SCCP 0874/05 and SCCS/1348/10
A more recent review article (Darbre and Harvey 2008) repeats the arguments that have all been refuted in SCCP/0874/05. It does not add new data nor adds any conclusive evidence. Therefore, this issue will not be reconsidered in the present Opinion.

SCCS comment of carcinogenicity
The SCCS analysed the studies cited by the applicant and concluded as follows:

Homburger *et al.* (1968):
The SCCS has some doubts on designs of the studies, e.g.: one species (particular selected mice strains were used), dosing, exposure route, time of observations (maximum 7-8 months), reliability of the design for detecting potentially weak carcinogens (in the studies Methylparaben was compared with quite strong carcinogen dibenzo(a,i)pyrene). These are not standard procedures for assessing carcinogenicity potential according to OECD TGs.

Mason, 1971
The results may indicate a slight increase in cancer incidence in females but considering range of cancer incidence in controls and animals from groups exposed to other chemicals it does not seem to be of biological meaningfulness. The SCCS is not able to conclude as historical range of cancer incidence in this breeding colony is unknown. However, based on compiled data for Fischer F344 rats (Haseman, 1998) all these values seem to be in the normal range (certainly there is a difference in observation period, 1.5 in the study by Mason *et al.* (1971) vs. 2 years in NTP).

Rodriguez, 1986
This study was designed to investigate potential pro-proliferation activity of MPB (also other parabens were studied, including butylparaben, which showed the strongest activity comparable to reference chemical, butylated hydroxyanisol). Also, the effects can be questionable because they were investigated in forestomach, which is not present in humans, and has quite different histology from glandular part.

To conclude, the SCCS is of the opinion that the studies analysed have limited value in the WoE approach to carcinogenicity of Methylparaben. However, as the available evidence shows that Methylparaben is not mutagenic/genotoxic and that there are no indications of carcinogenicity in the available literature, the SCCS considers that further testing for carcinogenicity is not necessary.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

In a 3 month dermal toxicity study (CTFA 1981e), a product formulation was used containing both 0.2% Methylparaben and 0.2% propylparaben. The formulation was administered at doses of 2 and 6 mg/cm2 on 10% surface area of rabbits. The 6 mg/cm2 group was exposed daily to one-half the minimal dose of ultraviolet light (4 min at 6 inches from Westinghouse FS-20 lamps, producing a continuous spectrum of 2800 to 4000 A). Ultraviolet light exposure had no effect on the degree of irritation observed.

Product formulations 0.2 ml containing 0.2% Methylparaben (alone or in combination with 0.2% propylparaben were applied to the volar forearm of 10 to 12 human volunteers (Food & Drug research Labs 1978a, 1978b, 1979 & 1984; as cited in CIR 2012). The test material was occluded for 24h. An ultraviolet light source was applied to the test site for 15 minutes at a distance of 10-12 cm from the forearm. A UVA dose of 4400 µW/cm2 was applied. There were no observations of phototoxicity.

Over decades of use, there has been no human evidence of phototoxicity.

Conclusion: Methylparaben is not phototoxic.

3.4.8.2 Photomutagenicity / photoclastogenicity

SCCS comment
Methylparaben does not cause any acute dermal phototoxic effects, such as photo-irritation. No data were provided on photosensitisation nor on photomutagenicity/photoclastogenicity.

3.4.9 Human data

In recent years human studies have been published investigating possible relationships between e.g. urinary concentrations of Methylparaben/Methylparaben metabolites and cosmetic use, hormones such as oestradiol or thyroid hormones, certain health parameters or health outcomes, indicators of fertility or on birth outcomes.
Most studies concluded in a way that further studies would be warranted to confirm the observed outcomes or did not report on significant associations. Furthermore, for most studies, co-exposure to other substances hampers the interpretation of the outcomes, in particular in studies reporting on (statistically significant) associations between Methylparaben in spot urine and effects on certain sperm parameters. References are given in Annex IV.

Recently, the Human Biomonitoring (HBM) Commission in Germany has defined 'reference values' for parabens. Reference values are checked continuously and are updated if new information becomes available.' Therefore, a reference value is not regarded as a safe value in urine, but as a measure to enable human biomonitoring of a substance over time to see how it may change with exposure pattern changes. For Methylparaben, the provisional reference value set by the German HBM Commission is 400 µg/L for women and 240 µg/L for men (Apel *et al.* 2017).

SCCS comment
Human data support observations from animal studies, that some male reproductive parameters might be modulated. However, these studies do not provide sufficient evidence for adverse effects in humans. Biomonitoring data are gaining interest as they provide total values of exposure from different sources. These are, however, not always known. In the SCCS Opinions, usually conservative deterministic data are considered for aggregate MoS calculations.

3.4.10 Special investigations

From the Applicant:
A few reviews exist in the literature relating generally to parabens that discuss the potential of the parent paraben substance to be endocrine active (Golden *et al.* 2005; Boberg *et al.*

2010; Nowak *et al.* 2018). A number of *in vitro* and *in vivo* studies have been performed to investigate endocrine activity specifically for Methylparaben.

3.4.10.1.1. Level 1 Existing data and non-test information

From the Applicant:
A major criterion for substrate binding and endocrine activity appeared to be the presence of an unhindered phenolic OH group in the para position on an alkylphenol substance and a molecular weight of 140-250 Da (Miller *et al.* 2001).
Byford *et al.* (2002) performed molecular modelling, which indicated a mode by which paraben molecules can bind into the ligand binding pocket of the crystal structure of the ligand binding domain (LBD) of the estrogen receptor alpha (ERα) in place of 17β-oestradiol. The work showed that in theory, two paraben molecules could bind simultaneously into the receptor binding site. However, alkyl chain length and the branched nature of the R group on a paraben ester also has an influence on binding potency and activity. Generally speaking, the majority of evidence suggests that the longer/bulkier the alkyl chain the greater the binding and mimicking of bulky steroid-like hormones.
In silico profilers that the OECD QSAR Toolbox (OECD 2018) highlights as pertinent for reproductive toxicity – i.e. the DART scheme, Estrogen Receptor Binding, Retinoic Acid Receptor Binding – and the rtER Expert System from US EPA, were queried for methyl paraben alerts. The *in silico* profiling results (Ouedraogo *et al.* 2021) indicated methyl paraben and the metabolite pHBA exhibited weak binding propensities for the estrogen receptor as they both have a phenolic group; however, they were outside the applicability domain of the RAR-profiler. These ER profilers only provide theoretical binding alert predictions, but do not translate into in vivo effects due to the absence of relevant exposure of the respective target organs.
Molecular docking for methyl paraben and its principal metabolite pHBA was performed with twelve nuclear receptors (see Table 26 below) (Ouedraogo *et al.* 2021; OECD IATA case study ENV/JM/MONO (2020)). This showed, in comparison to other known receptors, that methyl paraben is not expected to bind to these hormone receptors.

Conclusion of the Applicant: Methylparaben, from its structure and chemical properties alone, is not expected to be a strong binder to hormone receptors.

Table 31: Docking scores towards sixteen structures belonging to twelve nuclear receptors for pHBA and short chain parabens. Docking simulations performed using the online docking tool 'Endocrine Disruptome'*

	pHBA	MP	EP	PP	BP	ZL(S, E)	ZL(R, E)	CE	GE	DD	AG	BPA
AR	-6.0	-6.1	-6.3	-6.6	-6.8	-3.8	-5.9	-9.9	-9.1	-9.3	-8.9	-8.5
AR an.	-5.9	-5.9	-6.0	-6.3	-6.3	-7.4	-8.1	-9.6	-9.1	-9.0	-9.1	-8.6
ER α	-5.6	-5.7	-6.0	-6.5	-6.7	-9.8	-9.0	-9.4	-9.2	-9.4	-8.8	-8.2
ER α an.	-5.6	-5.7	-6.0	-6.4	-6.5	-7.9	-7.5	-9.1	-9.2	-9.9	-9.2	-8.5
ER β	-5.7	-5.8	-6.1	-6.4	-6.5	-10.5	-9.7	-9.8	-8.6	-8.7	-8.2	-8.2
ER β an.	-5.6	-5.7	-6.1	-6.4	-6.5	-9.5	-8.0	-9.6	-8.6	-8.8	-8.9	-8.2
GR	-5.7	-5.9	-6.1	-6.3	-6.4	-8.4	-8.2	-9.4	-9.0	-9.0	-8.8	-7.8
GR an.	-5.2	-5.4	-5.6	-5.8	-5.8	-8.2	-8.5	-8.0	-7.6	-7.3	-7.8	-7.4
LXR α	-5.5	-5.6	-5.9	-6.3	-6.4	-7.5	-8.3	-9.4	-8.7	-9.0	-8.8	-8.6
LXR β	-6.0	-6.1	-6.3	-6.7	-6.9	-6.9	-7.8	-9.7	-9.6	-9.9	-9.5	-8.0
PPAR α	-5.5	-5.5	-5.8	-6.4	-6.5	-6.6	-7.9	-8.3	-8.1	-7.7	-9.3	-7.9
PPAR β	-5.8	-5.7	-6.0	-6.0	-6.0	-7.0	-6.3	-9.3	-8.6	-7.9	-8.3	-7.8
PPAR γ	-5.3	-5.4	-5.9	-6.5	-6.7	-8.5	-8.0	-9.0	-8.1	-8.6	-9.3	-7.1
RXR α	-6.3	-6.0	-6.5	-6.9	-7.0	-7.6	-7.6	-8.7	-8.9	-9.5	-9.7	-7.9
TR α	-5.9	-5.9	-6.3	-6.7	-6.8	0.4	-2.2	-8.0	-9.6	-9.4	-9.6	-8.9
TR β	-5.7	-5.8	-6.1	-6.6	-6.7	-3.2	-3.5	-8.4	-9.6	-9.3	-9.4	-8.6

***** (http://endocrinedisruptome.ki.si/). Green and yellow indicate low and intermediate binding probabilities respectively. The code "an." indicates receptors in antagonistic conformations. AR = androgen receptor; ER = estrogen receptor; GR = glucocorticoid receptor; LXR = Liver X receptor; PPAR = peroxisome proliferator-activated receptor; RXR = retinoid X receptor; TR = thyroid hormone receptor. Zeralenone (ZL, two stereoisomers), Coumestrol (CE), Genistein GE), Daidzein (DD), Apigenin (AG), bisphenol-A (BPA).

3.4.10.1.2. Level 2 *In vitro* assays

From SCCP/0873/05
Estrogenic effects of parabens
In a number of *in vitro* studies, such as the recombinant yeast estrogen screen, parabens have proven to be able to bind to the estrogen receptor, to activate genes controlled by these receptors, and to stimulate cell growth and increase the level of immune-reactive estrogen receptor protein.
The estrogenic potency increases with increasing length and branching of the alkyl side chains (methyl < ethyl < propyl < butyl < isobutyl), but remains at all times 1000 to 1,000,000 times below the potency of 17β-oestradiol. p-Hydroxybenzoic acid, the common metabolite of all parabens, appeared to be inactive in the *in vitro* assays.

From SCCP/1348/10
Update on the hormonal (estrogenic / anti-androgenic) properties of parabens
In vitro studies show the potential of endocrine modifying effects of parabens, including methylparaben, with estrogenic activity as a function of chain length. PHBA, the common metabolite does not seem to exhibit endocrine modifying effects.

From the Applicant:
An overview of studies where Methylparaben has been investigated for endocrine activity *in vitro* can be found in Annex III.

Conclusion of the Applicant: There is no evidence of a relevant Estrogen, Androgen, Thyroid, Steroidogenesis-related (EATS) activity *in vitro* for Methylparaben.

RIVM report, 2017
New *in vitro* studies have been performed since the last SCCS Opinion. Overall, a MOA has been identified in these studies showing estrogenic and anti-androgenic properties of Methylparaben *in vitro*. Estrogenic and anti-androgenic activity and effects on adipogenesis

seem to increase as a function of chain length. The relevance of this *in vitro* data to the measurement of possible adverse effects in vivo is still under debate.

Ref: Brand *et al.*, 2017

From registration dossier ECHA[12]

Methylparaben was tested for its estrogenic activity using several *in vitro* assays. Methylparaben was assessed for its estrogenic activity by using the yeast two-hybrid assay incorporating either the human or medaka estrogen receptor α and by using hERα competitive enzyme-linked immunosorbent assay (ER-ELISA). Methylparaben did not show any estrogenic properties in the yeast two-hybrid assay (up to 10,000 nM) and ER-ELISA (up to 38,000 nM). The estrogenic activity of Methylparaben towards estrogen receptors α and ß was measured by using three reporter cell lines HELN, HELN ERα and HELN ERß. Methylparaben did not show any estrogenic activity when applied to HELN, HELN ERα and HELN ERß cells up to 10 µM.

A validated estrogen receptor competitive-binding assay to determine the estrogen receptor (ER) affinity for Methylparaben was utilized. Uteri from ovariectomized Sprague-Dawley rats were the ER source for the competitive binding assay. Methylparaben was assayed using a wide range of concentrations (10 nM to 0.1 mM) to determine the relative binding affinity value (RBA). Methylparaben exhibited a weak binding to the ER (Relative Binding Affinity: 0.0004% of 17ß-Estradiol Binding Affinity). The calculated IC50 (50% inhibition of the 17ß-Estradiol binding) was 0.25 mM compared to an IC50 of 0.9 nM for 17ß-Estradiol.

The effect (competitive inhibition of [^{3}H]-Estradiol binding, expression of estrogen-regulated genes) of Methylparaben on MCF7 human breast cancer cells was investigated. The binding of Methylparaben to the ER was rather weak, requiring a minimum concentration of 500,000-fold molar excess over 17ß-Estradiol. Where 17ß-Estradiol acts maximally between 10^{-10} and 10^{-8}M in MCF7 cells Methylparaben acts in 10^{-4}M range. Methylparaben gave a very weak effect on cell proliferation at 10^{-4}M. No significant antagonist properties of Methylparaben were found on cell proliferation stimulated by 10^{-10}M 17ß-Estradiol for concentrations of Methylparaben in up to 105 molar excesses.

MCF7 human breast cancer cells were grown for 7 days under conditions of estrogen deprivation, sufficient time to deplete the estrogen memory without development of loss of response. Gene expression was studied after a further 7 days with 0.5 mM Methylparaben or 17ß-Estradiol (10 nM, positive control). Methylparaben increased the cell growth. However, the extent of overlap in identity of the genes up- or downregulated by Methylparaben did in majority not follow the same pattern of regulation as under 17ß-Estradiol.

Taking into account all above mentioned results the estrogenic properties of Methylparaben are negligible. The binding to the estrogenic receptor is very weak and was shown at 106molar excess compared to ß-Estradiol. This is an artificial concentration and very unlikely to occur within the organism since Methylparaben is demonstrated to be metabolized and excreted rapidly. Methylparaben was not found to be a 17ß-Estradiol antagonist.

Therefore, no concern is arising from Methylparaben with reference to the estrogenic activity.

[12] https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/14310/7/10/4

3.4.10.1.2. Level 3 *In vivo* assays providing data about selected endocrine mechanisms/ pathways

From SCCP/0873/05
Estrogenic effects of parabens
The *in vivo* estrogenic activities of parabens have been tested in uterotrophic assays employing either immature female rodents or adult ovariectomized female rodents after oral, subcutaneous or dermal administration. Again, butyl paraben appeared being more potent than propyl, ethyl and Methylparaben, and again the values remained several magnitudes of order below the potency of 17β-estradiol. Conflicting results have been reported for p-hydroxybenzoic acid tested *in vivo*. One study claims that it has no estrogenic effect; another study gives potency values 1000-fold below the 17β-estradiol level.

From SCCP/1348/10
In vivo studies on parabens published between 2008-2010 showed effects with relatively high dosage levels (mainly about 1000 mg/kg bw/day) of paraben esters, including Methylparaben.

From the Applicant:
An overview of studies where Methylparaben has been investigated for endocrine activity in vivo can be found in Annex IV.

Conclusion of the Applicant: There is no evidence of a relevant *in vivo* endocrine activity for Methylparaben

RIVM report, 2017
The new *in vivo* studies on ED properties are summarised and discussed below.

Sun *et al.* (2016)
The uterotrophic activity of Methylparaben was investigated in immature Sprague Dawley rats. The expression of the following genes was affected in the Methylparaben-exposed group (0.8, 4 and 20 mg/kg bw/day): Icabp, Itmap1, CaBP-9k, Pgr. Relative uterine weight was increased in the Methylparaben-exposed group (20 mg/kg bw/day).
RIVM concluded that the study was performed properly; however, it focused on a limited set of effects. The measured effects (gene expression, uterine weight) suggesting an ED MOA should be confirmed by other studies. By themselves the results are not sufficient to derive a NOAEL.

Manservisi *et al.* (2015)
This study determined whether low doses of Methylparaben affect the development and proliferative activity of the mammary glands. Female animals treated with Methylparaben (0.1050 mg/kg bw/day) showed evident histological differences from controls: the alveoli of the mammary gland were not always milk-filled and an increase in adipose tissue was noted. The collapsed alveolar and duct structures showed residual secretory content. Gene expression was affected.
The RIVM noted that part of this study was performed in a low number of animals (n=3 dams per group). Additionally, it was not described how the statistical significance of mortality and pup numbers was identified. Furthermore, the quantification of the histopathological findings was not explained; therefore, the quality of the study was poor.

Lee *et al.* (2017)
The influence of Methylparaben on ovarian folliculogenesis and steroidogenesis in Sprague Dawley rats was investigated. Methylparaben-treated rats showed a regular estrous cycle.

There was no effect on the number of primary follicles, and secondary follicles showed a decrease in total number in all treated groups. The RIVM noted that only one dosage was used and questions whether the control group was representative, since this group seemed to deviate from the other groups.

Gopalakrishnan *et al.* (2017)
In this study the effects of Methylparaben on the histology and transcriptome profiles of normal (noncancerous) mammary glands of Sprague Dawley rats were studied. Dosages were chosen that mimicked human exposure (0.105 mg/kg bw/day, orally). Animals were exposed across several key developmental stages: perinatal (GD1–GD20, n=10 or PND1–PND21, n=10), prepubertal (PND21–PND42, n=5) and pubertal (PND42–PND63, n=5). There were also long-term exposures from birth to lactation (PND1–PND146, n=3). Perinatal Methylparaben exposure decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad. Prepubertal Methylparaben treatment was associated with a significant reduction in adipose tissue and more abundant glandular tissue. Pubertal Methylparaben exposure elevated the amounts of glandular tissue compared with controls. This was visible as a higher degree of branching relative to the total gland area. Long-term Methylparaben treatment from birth to lactation did not result in significant histological changes. In the pubertal window gene expression, changes were observed.
The RIVM opinion is that all these effects were intermediate effects, suggesting an ED MOA.

Costa *et al.* (2017)
The ED effects of Methylparaben on the adult gerbil prostate were studied. Methylparaben caused morphological changes in gerbil prostates in all experimental groups. These animals displayed similar alterations, such as prostate epithelial hyperplasia, increased cell proliferation, and a higher frequency of AR-positive cells.
The RIVM noted that no adverse effects were measured, but it is unclear how the morphological effects were quantified. Only one dosage was measured.

RIVM conclusion: Findings in the *in vivo* studies performed after the SCCS Opinions did not contradict the current NOAEL of 1000 mg/kg bw/day for Methylparaben. The available *in vivo* studies all have weaknesses in study design (e.g. with regard to statistics, small number of animals, no dose–response relationship measured) and in some, no adverse ED effects were found. The (intermediate) endpoints measured in the studies described above suggest an endocrine MOA for all the parabens evaluated, but more data with regard to *in vivo* effects are needed.

Ref: Brand *et al.*, 2017

3.4.10.1.3. *In vivo* assays providing data on adverse effects on endocrine relevant endpoints (level 4)

The Applicant concluded that based on the *in vivo* studies that were described in his dossier, there is no evidence of relevant *in vivo* adverse effects for Methylparaben.

Overall Conclusion of the Applicant on Endocrine Activity:

Level 1: Methylparaben from its structure and chemical properties alone is not expected to be a strong binder to hormone receptors.

Level 2: Some investigative *in vitro* studies have shown weak activity for Methylparaben at 10,000 – 100,000-fold lower potency, and on one occasion 2,500,000-fold lower than

endogenous substrates such as 17β-estradiol. MP is not a potent substrate in hormone receptor assays and does not mimic estrogen.

Level 3: Some weakly positive responses have been observed in a few uteroptrophic assays. However, several other uterotrophic assays failed to show any in vivo estrogenic activity at doses up to 800 mg/kg bw MP per day after oral or subcutaneous administration. Overall, uterotrophic assays only indicate some biological activity, but no adverse effect.

Level 4: Studies by Beerens-Heijnen (2009), Matthews (1956), Oishi (2004) and Hoberman *et al.* (2008) showed no adverse effects with respect to carcinogenicity, reproductive or developmental toxicity.
Level 5: There are no level 5 assays for Methylparaben. However, there is sufficient evidence from level 4 assays that up to 1000 mg/kg/d Methylparaben shows no convincing evidence of endocrine activity *in vivo*.

Overall SCCS comment on ED activity
In addition to the studies that were used by the Applicant to assess ED modality, further *in vivo* toxicity studies were made available to the SCCS.

In level 2 *in vitro* assays, weak estrogenic activity was observed in some of the studies investigated, albeit at high concentrations only.

In the majority of level 3 *in vivo* (uterotrophic) assays, no effect was found on relative uterine weight. In two studies, however, an increase in the relative uterine weight could be observed at all doses investigated, which is indicative of an estrogenic MoA. These studies cannot be used to derived a PoD for the safety assessment.

In level 4 *in vivo* (OECD TG408, 421/422 and 443) studies, estrogenic effects were not observed. However, there were indications for an anti-androgenic mode of action, based on a 90d oral repeated dose toxicity study and an EOGRT study. In Cohort 1 of the latter study, statistically significantly ($p<0.001$) reduced relative AGD in male F2 pups was observed, which can be considered as indication for an anti-androgenic MoA. An anti-androgenic MoA is further supported by effects on sperm which were observed in an oral 90-day repeated dose toxicity study

Based on available data on thyroid and thyroid hormones, T modality was not clearly affected. Although some scattered effects were observed, including decreased TSH in males in the 90-days repeated dose toxicity study and decreased T4 in males in the reproduction/developmental toxicity screening test, the results were not considered strong enough to conclude on the T modality.

3.5. SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

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

Pivotal study for calculating oral PoD:
In the EOGRT study, reduction of AGD was observed in F2 pups from cohort 1B at the highest dose tested (1000 mg/kg bw/d), which can be considered as an indication for an anti-

androgenic MoA. The latter is also supported by effects on sperm which were observed in an oral 90-day repeated dose toxicity study at 1000 mg/kg bw/d.

For the determination of the POD, the SCCS performed benchmark dose (BMD) modelling according to the 2022 EFSA Guidance on the use of the BMD approach in risk assessment (EFSA Scientific Committee, 2022). More guidance is included on criteria for acceptability of the results of the modelling than in previous versions (EFSA Scientific Committee, 2019).

Benchmark dose (BMD) analysis was carried out according to the latest EFSA guidance (EFSA Scientific Committee, 2022). To perform the BMD modelling, the SCCS used the Bayesian BMD Modelling web-app (https://zenodo.org/record/7334435#.Y5osYXbMLD4) available at the EFSA R4EU platform (https://efsa.openanalytics.eu/).

The reports of the modelling are shown in Annex V.

A $BMDL_{5\%}$ of 374 mg/kg bw/day could be derived. The modeling of the reduction in relative in AGD in male F2 pups is presented below and fulfils the EFSA criteria of acceptance. The default BMR of 5% was chosen.

Model	Type	BMDL	BMD	BMDU
Model Averaged	LP	374.414	903.241	2,032.377

Due to high oral absorption, the PoD is not adjusted.

The POD as an oral $BMDL_{5\%}$ for use in MoS calculation is 374 mg/kg bw/day

In conclusion, the SCCS considers the BMDL of 374 mg/kg bw/day as the POD for MOS calculations.

Safety evaluation

Table 27: Margin of Safety (MoS) calculation for individual cosmetic product types and aggregate exposure to Methylparaben

Product	**Calculated SED (mg/kg bw/day)[1]**	**MoS[2]**
Shower gel	0.006152	60793
Hand wash	0.007343	50933
Shampoo	0.00333	112312
Hair conditioner	0.001477	253215
Hair styling	0.01266	29541

Body lotion	0.27166	1377
Face cream	0.05323	7026
Hand cream	0.07210	5187
Liquid foundation	0.01742	21469
Lipstick/salve	0.0039	95897
Make-up remover	0.01837	20359
Eye shadow	0.000728	513736
Mascara	0.000926	403888
Eyeliner	0.000176	2125000
Non-spray deodorant	0.04869	7681
Toothpaste	0.0095	35368
Mouth wash	0.1435	2606
Aggregate	**0.671**	**557**

[1]SED calculations can be found in Table 9.
[2]MoS = POD (374 mg/kg bw/day)/SED (see Table 9).

The aggregate SED for Methylparaben using a worst-case deterministic aggregate scenario is 0.671 mg Methylparaben/kg bw/day. Using a PoD of 374 mg/kg bw/day, the Margin of Safety (MoS) is 557.

3.6 DISCUSSION

Physicochemical properties
Details of the analytical methods used for the determination of purity of the test substance should be provided.

No data on impurities of the test substance were provided by the Applicant. Details of the analytical methods used for the determination of impurities along with the results of these studies should be provided.

Toxicokinetics
In vitro and in vivo skin absorption studies
As pHBA is considered as the common inactive metabolite of parabens, it is the systemic availability of intact (parent) compound that may be of concern for systemic adverse effects. Valid dermal penetration studies to estimate systemic availability of parent (intact) Methylparaben after dermal application in humans are not available. There are indications in the literature that there are differences in metabolism between animals and humans. *In vivo* pharmacokinetic data in humans are therefore required and have been requested from

Applicants in the past. Up to now, this data has not been provided. *In vitro* dermal penetration studies using human skin that comply with the SCCS requirements have not been performed. A human pharmacokinetic study published in 2023 by Shin *et al.* does not inform on a dermal absorption percentage (due to the lack of oral data for comparison), however it informs about important toxicokinetic parameters for Methylparaben.
The key study (*in vitro* using pig ear skin) presented by the applicants suffers from several shortcomings. It does, however, indicate that a value of 3.7%, which was used in previous SCCS Opinions for dermal absorption of non-metabolised (parent) paraben, might not be protective in the case of Methylparaben. Therefore, in the absence of a proper dermal penetration study using human skin, a default value of 50% for non-metabolised Methylparaben will be used by the SCCS in the MoS calculation.

Other toxicokinetics studies
Apart from Campbell 2015 study, the studies presented by the Applicant were already considered in the previous SCCS/SCCP evaluation and therefore do not lead to a change in the conclusion drawn earlier: "The toxicokinetic study confirms that, in rats, short-, mid- and long-chain parabens are rapidly absorbed and eliminated after single oral or subcutaneous administration. After dermal administration, they are partly (15 to 27%) absorbed and rapidly eliminated. Blood analysis only showed the presence of PHBA.". Based on the study by Moos *et al.*, 2016 (Table 2) using 3 male volunteers, 17.4% of dermally applied Methylparaben was excreted as parent (as the sum of free Methylparaben and glucuronide and sulfate conjugates) compound, 63.8% as PHHA, 3.0 % as PHBA and 0.1 % as ring hydroxylated Methylparaben. *In vivo* animal studies point to high oral absorption (clearly above 50%). Therefore, adjustment for oral absorption is not necessary when MoS calculation is based on an oral study. 100 % oral absorption will be used for MoS calculation (i.e. no adjustment of PoD from oral study).

Exposure
The SCCS assumes that the values presented on the Methylparaben occurrence from the Cosmetics Europe percentage use survey data (Table 6) relate to the ester and not to the acid form, and that therefore the level in mascara does not exceed the level permitted under the regulation.
The SCCS accepts Scenario A that uses maximum allowed concentrations according to regulation. The Applicant has used a dermal uptake of 15%, but the SCCS will use a default value of 50%. The SCCS has recalculated the adjusted aggregate SED by using this default value, except for lipstick, toothpaste and mouthwash, for which a dermal absorption of 100% is used. After recalculation, the adjusted aggregate SED for Methylparaben exposure of adults is 0.671 mg Methylparaben /kg BW/day.
The SCCS noted that for the airborne fraction, a worst-case assumption has been used. Assumptions regarding the size of boxes and time, as well as the breathing rate, have all been made in accordance with the SCCS Notes of Guidance.
The Applicant has provided an assessment of inhalation exposure to Methylparaben, resulting in a SED_{inh} of 0.003 mg/kg bw/day. This value was not aggregated with the oral and dermal exposure.
Since inhalation exposure from hairspray (assuming 100% uptake) results in a lower systemic exposure than dermal exposure from hairstyling products (0.0253 mg/kg bw/day), which are included in the deterministic calculations presented in Table 9, inhalation exposure to hair spray is assumed to be covered by the aggregate exposure value of 0.671 mg /kg bw/day.
The Applicant has provided exposure estimates for toothpaste and mouthwash use by children. However, the values have not been aggregated. In addition, dermal exposure estimates for other cosmetic products were not provided. Therefore, a safety assessment for children and adolescents for the simultaneous use of Methylparaben in oral and dermal applications was not performed.

Toxicological Evaluation

Irritation and corrosivity
Based on all available data, Methylparaben is not considered to be irritating to the skin nor the eyes.

Skin sensitisation
Methylparaben was positive in *in vitro* tests for skin sensitisation, but not in the DPRA. Methylparaben was negative when tested in animal studies. All human data are based on results from patch tests conducted with paraben mixtures and show that paraben sensitisation is rare, and is related to medical applications and not to cosmetics. Human skin sensitisation data specifically for Methylparaben are not available. Taking all the data into consideration, together with the data from animal tests, the SCCS considers that Methylparaben is not a skin sensitiser.

Acute toxicity
Methylparaben is not acutely toxic.

Repeated dose toxicity
From oral subchronic (28 days) repeated-dose toxicity studies provided by the Applicant, a NOAEL of 1000 mg/kg/day was derived.

All repeated-dose toxicity studies provided and discussed in the Applicants dossier and the information provided by RIVM in their 2017 report during the call for information point to an NOAEL of 1000 mg/kg bw/d for repeated dose toxicity. Two further oral *in vivo* 90-day repeated-dose toxicity studies that had been requested in the context of another legislation have been made available to the SCCS. While the combined repeat-dose toxicity/reproductive toxicity study confirmed a NOAEL of 1000 mg/kg bw/d, the 90-day repeated-dose toxicity study was indicative of changes pointing to an endocrine mediated (anti-androgenic) MoA and effects on male reproductive parameters, however without histopathological findings. Therefore, the highest dose of 1000 mg/kg bw/d can be regarded as the NOAEL.

Reproductive toxicity
Apart from studies provided by the Applicant and described in the RIVM report, two further studies were made available to the SCCS (one OECD TG422 study and dan OECD TG 443 study). Findings from the Vo *et al.* (2010) study (effects on the date of vaginal opening, the length of the estrous cycle and affected organ weight (thyroid, liver, adrenal gland and ovary)) were not confirmed by the new guideline studies (OECD TG 422/421; OECD 443). However, reduction of AGD was observed in F2 pups from cohort 1B at the highest dose tested, which can be considered as an indication for an anti-androgenic MoA. The latter is also supported by effects on sperm which were observed in an oral 90-day repeated dose toxicity study.
From this study, a NOAEL of 300 mg/kg bw/day could be derived. The SCCS in parallel did BMD modelling, which resulted in a $BMDL_{5\%}$ of 374 mg/kg bw/day. As the $BMDL_{5\%}$ value is the preferred PoD value according to the SCCS Notes of Guidance, this will be used in the MoS calculation.

Mutagenicity / genotoxicity
Methylparaben was tested for gene mutations in three Ames tests, out of which one was considered as valid by the SCCS. All studies were negative. Methylparaben was also tested in one valid mammalian cell gene mutation study on CHO cells with a negative result.
Methylparaben was tested for chromosomal aberration in 5 studies: on WI-38 human fibroblasts with a negative result, on CHO and V79 cells with positive results, in 2 studies on human lymphocytes with inconclusive or equivocal results. All the results on chromosomal aberrations testing were considered of limited or low reliability and relevance. Therefore,

based on the results on *in vitro* chromosomal aberration, a genotoxic effect of Methylparaben cannot be excluded.

Carcinogenicity
The SCCS analysed the three carcinogenicity studies cited by the Applicant. There were several deficiencies noted: the studies did not meet the standard procedures for assessing carcinogenicity potential according to OECD TGs, the values were in the historical range of cancer incidence or the human relevance of the effects was questioned.
To conclude, the SCCS is of the opinion that the studies analysed have limited value in the WoE approach to carcinogenicity of Methylparaben. However, as the available evidence shows that Methylparaben is not mutagenic/genotoxic and that there are no indications of carcinogenicity in the available literature, the SCCS considers that further testing for carcinogenicity is not necessary.

Photo-induced toxicity
Methylparaben does not cause any acute dermal phototoxic effects, such as photo-irritation. No data were provided on photosensitisation nor on photomutagenicity/photoclastogenicity.

Human data
Human data support observations from animal studies, that some male reproductive parameters might be modulated. However, these studies do not provide sufficient evidence for adverse effects in humans. Biomonitoring data are gaining interest as they provide total values of exposure from different sources. These are, however, not always known. In the SCCS Opinions, usually conservative deterministic data are considered for aggregate MoS calculations.

Special investigation
In addition to the studies that were used by the Applicant to assess ED modality, further *in vivo* toxicity studies were made available to the SCCS.

In level 2 *in vitro* assays, weak estrogenic activity was observed in some of the studies investigated, albeit at high concentrations only.

In the majority of level 3 *in vivo* (uterotrophic) assays, no effect was found on relative uterine weight. In two studies, however, an increase in the relative uterine weight could be observed at all doses investigated, which is indicative of an estrogenic MoA.

In level 4 *in vivo* (OECD TG408, 421/422 and 443) studies, estrogenic effects were not observed. However, there were indications for an anti-androgenic mode of action, based on a 90d oral repeated dose toxicity study and an EOGRT study. In Cohort 1 of the latter study statistically significantly ($p<0.001$) reduced relative AGD in male F2 pups was observed, which can be considered as indication for an anti-androgenic MoA. An anti-androgenic MoA is further supported by effects on sperm which were observed in an oral 90-day repeated dose toxicity study

In the 90-day repeat-dose toxicity study, TSH was dose-dependently decreased in males. These effects were only statistically significant in males treated with 1000 mg Methylparaben/kg bw/day. In the EOGRT study statistically significantly lower mean thyroxine hormone (T4) levels were observed in males treated with 1000 mg Methylparaben/kg bw/day. No effects were seen in the corresponding histopathological findings in the thyroid/parathyroid. No statistically significant effects were observed on pup thyroid weight and T4 level in PND 13 pups (male and female) of the Methylparaben-treated groups when compared to the controls.

Although scattered effects were observed in the 90-day repeat-dose toxicity study and in the EOGRT study the SCCS considers the results were not strong enough to conclude on the T modality.

The MoS calculated for deterministic aggregate exposure to Methylparaben with a dermal absorption of 50% in all cosmetic categories, results in values above 100. Therefore, the SCCS is of the opinion that the concentration of 0.4% Methylparaben present in different cosmetic product categories is safe.
A well-performed absorption study could further support this conclusion.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Methylparaben, does the SCCS consider Methylparaben safe when used as a preservative in cosmetic products up to a maximum concentration of 0.4% (as acid) when used on its own and up to 0.8% (as acid) for mixtures of esters as indicated in entry 12 of Annex V to the Cosmetics Regulation?*

   On the basis of the safety assessment considering all available data and the concerns related to endocrine activity, the SCCS is of the opinion that the use of Methylparaben as a preservative in cosmetic products at concentrations of up to 0.4% (expressed as acid) is safe. It is also safe when used up to 0.4% in a mixture of esters for which the total concentration of all esters does not exceed 0.8% (as acid), as indicated in entry 12 of Annex V to the Cosmetics Regulation.

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

   /

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

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

5. MINORITY OPINION

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

Further Abbreviations used in this Opinion:

MP	Methylparaben
pHBA	para-Hydroxybenzoic acid
pHHA	para-Hydroxyhippuric acis

Annex I: Detailed information provided by the Applicant on dermal penetration and skin metabolism of Methylparaben

1) Details on the dermal penetration study used as key study for dermal penetration as provided in Applicants dossier.

From Applicant dossier

Nine formulations (see Table 1 below), representing the most frequently types of MP-containing topical leave-on products, were prepared; for comparison a simple aqueous solution was also prepared.

Table 1: Composition of the vehicles/formulations tested with 0.1% w/w methyl paraben, with enhancers urea, transcutol or propylene glycol as used by Pažoureková *et al.* (2013).

Ingredient[a]	Aqueous solution (%, w/w)	Hydrogel (%, w/w)				Emulsion oil-in-water (%, w/w)			
		1 without E	2 with UR	3 with TC	4 with PG	1 without E	2 with UR	3 with TC	4 with PG
Methylparaben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Aqua	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100
Urea	–	–	5	–	–	–	5	–	–
Ethoxydiglycol[b]	–	–	–	5	–	–	–	5	–
Propylene glycol	–	–	–	–	5	–	–	–	5
Olea Europaea oil	–	–	–	–	–	18	18	18	18
Glyceryl stearate	–	–	–	–	–	5	5	5	5
C12-14 Pareth-3	–	–	–	–	–	3	3	3	3
Cetyl alcohol	–	–	–	–	–	2	2	2	2
Carbomer[c]	–	1	1	1	1	–	–	–	–
Sodium hydroxide		to pH 5.5	to pH 5.5	to pH 5.5	to pH 5.5	–	–	–	–
Lactic acid	to pH 5.5	–	–	–	–	to pH 5.5	to pH 5.5	to pH 5.5	to pH 5.5

E: enhancer; UR: urea; TC: Transcutol; PG: propylene glycol.
[a] Name by the International Nomenclature of Cosmetic Ingredients (EC, 2006).
[b] Commercial name Transcutol® CG.
[c] Commercial name Carbopol® 940.

Preparation of pig skin ear: Fresh ears from 6 months old domestic pigs (Slovak large white) were obtained from a local abattoir immediately post-mortem and prior to steam cleaning. Following brief cleaning with tap water, the sheet of the full-thickness skin (FTS, consisting of the SC, viable epidermis, and dermis) was separated from the underlying cartilage on the upper half part of ear using a scalpel.
Hairs were cropped to a length of 3 mm with an electric hair clipper. The FTS sheets with some visible imperfections were excluded. Four freshly excised FTS sheets were used in the same day and four were stored at 4 °C for 18 h) until the next day. For all other experiments FTS sheets were wrapped individually in an aluminium foil and stored at -20°C for up to 6 weeks before use. One hour prior to the experiment, frozen FTS sheet was allowed to thaw at room temperature.

Franz cell method: Pre-calibrated static unjacketed Franz-type diffusion cells were used with a receptor chamber volume of 5.5 ± 1 mL and an area of 2.00 cm2. N=3 FTS discs were obtained from each previously frozen intact FTS sheet, as well as stripped FTS sheets. Barrier integrity was checked with transdermal electrical conductivity experiments. The test formulation was left in contact with the skin for 24 h.

Receptor fluid (50 µL) samples were taken (and replaced with the same volume of fresh RF) at 1, 2, 3, 4, 5, 6, and 24 hours. The sample was immediately assayed for a concentration of MP and PHBA via HPLC.
24h application to previously frozen skin: The results for 24h experiments are shown in Table 2 below. The vast majority of penetration is in the form of the pHBA metabolite. The penetration of unmetabolised methyl paraben was typically low (and was below the limit of detection (LOD) in some cases in the 4h experiments (see Figure 2). The highest absolute

amount for skin penetration of unmetabolised Methylparaben into receptor fluid is seen in experiment 3, with Transcutol penetration enhancer, and in stripped skin (0.76 µg/cm2). Penetration was typically lower in intact skin. NB. it is expected that fresh skin would be less penetrable and more metabolically active than frozen skin. Of the total penetrant (MP + pHBA), Methylparaben ranged from 4.9-7.4%, the majority of penetrant (approximately 92-95%) was pHBA.

Table 2: The amount of unmetabolised Methylparaben and pHBA metabolite in receptor fluid after 24h penetration through previously frozen intact and stripped full-thickness pig-ear skin in each of nine formulations containing 0.1% MP w/w as per Pažoureková *et al.* (2013)

Membrane	Permeant	Aqueous solution (µg/cm²)	Hydrogel (µg/cm²)				Emulsion oil-in-water (µg/cm²)			
			1 without E	2 with UR	3 with TC	4 with PG	1 without E	2 with UR	3 with TC	4 with PG
Intact FTS	PHBA	3.85 ± 0.35	3.70 ± 0.47	4.05 ± 0.35	4.30 ± 0.42	4.15 ± 0.20	5.50 ± 0.45	6.20 ± 0.30	7.30 ± 0.41	6.85 ± 0.45
	MP	0.20 ± 0.03	0.22 ± 0.02	0.23 ± 0.03	0.26 ± 0.03	0.24 ± 0.02	0.34 ± 0.06	0.40 ± 0.05	0.58 ± 0.08	0.52 ± 0.04
	UnmMP	*4.9%*	*5.6%*	*5.4%*	*5.2%*	*5.5%*	*5.8%*	*6.1%*	*7.4%*	*7.1%*
Stripped FTS	PHBA	5.67* ± 0.56	5.60* ± 0.65	5.80* ± 0.42	6.60* ± 0.40	6.25* ± 0.45	8.00* ± 0.42	8.75* ± 0.55	9.50* ± 0.40	9.20* ± 0.35
	MP	0.29* ± 0.04	0.29* ± 0.02	0.33* ± 0.03	0.39* ± 0.02	0.38* ± 0.04	0.50 ± 0.07	0.64* ± 0.04	0.76* ± 0.07	0.70* ± 0.03
	UnmMP	*4.9%*	*4.9%*	*5.4%*	*5.6%*	*5.7%*	*6.3%*	*6.8%*	*7.4%*	*7.1%*

E: enhancer; UR: urea (5%, w/w); TC: Transcutol® CG (5%, w/w); PG: propylene glycol (5%, w/w).
UnmMP (%): a percentage of unmetabolised MP from the total amounts of permeants (MP + PHBA) in the RF (SD values are not included).
Values are the mean ± SD ($n = 3$); no significant differences were found ($p > 0.05$).
* Denotes the amount of MP or PHBA permeated through previously frozen stripped FTS significantly different ($p < 0.05$) from the amount of the same compound permeated through previously frozen intact FTS in the RF.

4h application to both fresh and previously frozen skin: The results for 4h experiments with Methylparaben are shown in Table 5 below. These present a 'mass balance' of both Methylparaben and pHBA recovery in the experiments.

Table 3: Distribution of unmetabolised methyl paraben and its metabolite pHBA in a compartment of the diffusion system after 4h exposure of intact fresh, previously frozen (-20°C for 6 weeks max) intact and stripped pig ear skin, to each of the four emulsions with penetration enhancers (Reproduced from Pažoureková *et al.* 2013).

Membrane	Compartment of the diffusion system	Emulsion 1 without E		Emulsion 2 with UR		Emulsion 3 with TC		Emulsion 4 with PG	
		MP	PHBA[c]	MP	PHBA[c]	MP	PHBA[c]	MP	PHBA[c]
Intact freshly excised FTS[a]	Surface (µg/cm²)	2.10 ± 0.25	<LOD	2.01 ± 0.31	<LOD	1.59 ± 0.19	<LOD	1.69 ± 0.28	<LOD
	Skin (µg/cm²)	1.19 ± 0.10	2.84 ± 0.10	1.13 ± 0.42	<LOQ	0.98 ± 0.31	<LOQ	1.00 ± 0.29	<LOQ
TEC 0.21–0.40 (mS/cm)	Receptor fluid (µg/cm²)	<LOQ	4.06 ± 0.56	<LOQ	4.55 ± 0.46	0.23 ± 0.02	5.70 ± 0.49	0.21 ± 0.02	5.45 ± 0.67
Thickness 1.025–1.200 (mm)	*MP from (MP + PHBA[c]) in receptor fluid (%)*	*Incalculable*		*Incalculable*		*3.9*		*3.7*	
	Total recovery (MP + PHBA[c]) (%)	*101.9*		*76.9*		*85.0*		*83.5*	
Intact frozen stored FTS[b]	Surface (µg/cm²)	1.28 ± 0.33*	<LOD	1.26 ± 0.30*	<LOD	1.07 ± 0.18*	<LOD	1.24 ± 0.22	<LOD
	Skin (µg/cm²)	2.41 ± 0.65	<LOQ	2.33 ± 0.72*	<LOQ	1.66 ± 0.39*	<LOQ	1.49 ± 0.51	<LOQ
TEC 0.24–0.42 (mS/cm)	Receptor fluid (µg/cm²)	0.23 ± 0.05*	4.58 ± 0.39*	0.23 ± 0.04*	4.66 ± 0.53	0.33 ± 0.09	6.09 ± 0.55	0.28 ± 0.07	5.59 ± 0.72*
Thickness 1.020–1.195 (mm)	*MP from (MP + PHBA[c]) in receptor fluid (%)*	*4.8*		*4.7*		*5.1*		*4.8*	
	Total recovery (MP + PHBA[c]) (%)	*85.0*		*84.8*		*91.5*		*86.0*	
Stripped frozen stored FTS[a]	Surface (µg/cm²)	1.08 ± 0.22	<LOD	0.98 ± 0.18	<LOD	1.02 ± 0.05	<LOD	0.98 ± 0,05	<LOD
	Skin (µg/cm²)	1.32 ± 0.48	<LOQ	1.02 ± 0.14	<LOQ	1.05 ± 0.16**	<LOQ	1.13 ± 0.23	<LOQ
TEC 1.85–2.81 (mS/cm)	Receptor fluid (µg/cm²)	0.32 ± 0.08**	6.10 ± 0.93**	0.37 ± 0.05**	6.52 ± 0.42**	0.55 ± 0.15**	7.26 ± 0.60**	0.42 ± 0.05	6.60 ± 0.90**
Thickness 1.000–1.160 (mm)	*MP from (MP + PHBA[c]) in receptor fluid (%)*	*5.0*		*5.4*		*7.0*		*6.0*	
	Total recovery	*88.2*		*88.9*		*98.8*		*91.3*	

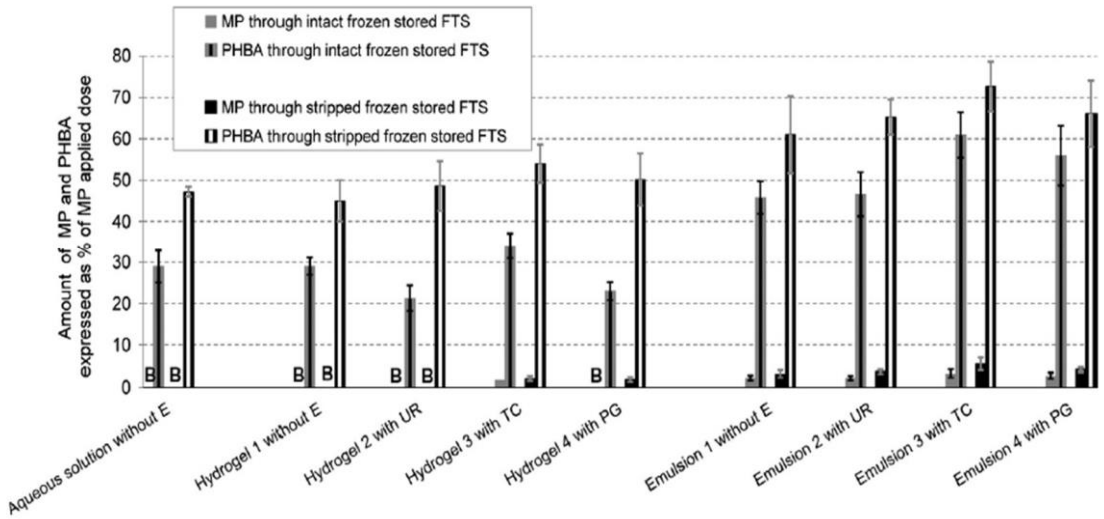
(MP + PHBA) (%)

E: enhancer; UR: urea (5%, w/w); TC: Transcutol® CG (5%, w/w); PG: propylene glycol (5%, w/w).
<LOD: below the limit of detection; LOD for MP: 0.020 µg/mL; LOD for PHBA: 0.330 µg/mL (see Section 2.9).
<LOQ: below the limit of quantification;
LOQ for MP: 0.066 µg/mL, i.e. 0.066 µg/cm² on surface; 0.165 µg/cm² in skin; 0.182 µg/cm² in receptor fluid (see Section 3).
LOQ for PHBA: 0.990 µg/mL, i.e. LOQ for PHBA[c]: 1.089 µg/cm² on surface; 2.475 µg/cm² in skin; 2.995 µg/cm² in receptor fluid (see Section 3).
* Denotes the amount of MP and PHBA[c] permeated through intact frozen stored FTS significantly different ($p < 0.05$) from the amount of the same compound through intact freshly excised FTS in a given compartment of the diffusion system.
** Denotes the amount of MP and PHBA[c] permeated through stripped frozen stored FTS significantly different ($p < 0.05$) from the amount of the same compound through intact frozen stored FTS in a given compartment of the diffusion system.
[a] Values are the mean ± SD ($n = 6$); no significant differences were found ($p > 0.05$) in a given compartment of the diffusion system (surface, epidermis plus dermis, and receptor fluid).
[b] Values are the mean ± SD ($n = 6 + 2$); no significant differences were found ($p > 0.05$) in a given compartment of the diffusion system (surface, epidermis plus dermis, and receptor fluid).
[c] PHBA is expressed as MP, i.e. PHBA × 1.1 (see Section 3).

As expected, the main compound in the receptor fluid was seen to be pHBA; 45.8–60.9% of the applied dose (AD) for previously frozen intact FTS and 61.0–72.6%AD for stripped FTS. Low amounts of unmetabolised Methylparaben (2.3–3.3%AD and 3.2–5.5%AD) penetrated into receptor fluid through previously frozen intact FTS and stripped FTS, respectively.

Amounts of parent methyl paraben remained on the surface of intact (10.7–12.8%AD) and stripped (9.8–10.8%AD) skin. Levels of pHBA on the surface of both intact and stripped skin was <LOD in all cases.

The amounts of unmetabolised Methylparaben in the skin at 4h was: in intact frozen skin (14.9–24.1%AD) and stripped frozen skin (10.2–13.2%AD). With all vehicles and in both intact and stripped frozen skin, amounts of pHBA within skin extracts were <LOQ (limit of quantification).
When using intact fresh pig ear skin, the amount of pHBA and unmetabolised Methylparaben in receptor fluid was 40.6–57.0%AD and <LOQ–2.3%AD, respectively. A considerable amount of the parent Methylparaben remained on the surface of freshly excised FTS (11.6–20.1%AD). No pHBA could be detected on the skin surface. The levels of unmetabolised methyl paraben within intact fresh skin was 9.8–11.9%AD.
Based on these observations, a value for unmetabolised Methylparaben absorbed (penetrated plus within skin would be **2.3 + 11.9% = 14.2%.** For test emulsions 2,3 and 4 with fresh skin, only negligible amounts of pHBA (above LOD and below LOQ) were detected; for emulsion 1 without enhancer the amount of 28.4%AD of pHBA was measured in fresh intact skin. The total recoveries of the test substance from experiments with intact freshly excised skin ranged from 76.9% to 101.9%.



Further studies on dermal penetration as provided in the Applicants dossier

1) In vitro human skin absorption studies

From Applicant dossier

Studies that have been used to investigate absorption of Methylparaben in vitro using human skin are summarised in Table 4.

Table 4: Summary of observations from in vitro skin penetration studies using human skin.

Exposure concentration/vehicle	Application site details	Observations	Reference
200 mg MP/200ml of acetone or water, water/propylene glycol, water/PEG 400, liquid paraffin, 3 cosmetic formulations (Type I, II and III)	Abdominal skin from human cadavers. The epidermis was removed and mounted in a diffusion cell.	Steady state Flux values Jmax were calculated (µg/cm^{2}h) Water 3.83 Water/propylene glycol 6.5 Water/PEG400 4.01 Liquid paraffin 0.42 Type I 32.5 Type II 22.54 Type III 5.13	Dal Pozzo & Pastori, 1996
5 mg MP/cm^2 in a commercial allergy test ointment, acetone and ethanol.	Human female abdominal skin. Epidermal membranes. Franz cell diffusion. Samples were occluded with highdensity polyurethane. Paraben concentration in receptor fluid was performed by HPLC.	Unoccluded penetration at 10h (µg) Ointment 27.0 ± 1.3 Acetone 86.4 ± 15.7 Ethanol 90.3 ± 28.3 Occluded penetration at 10h (µg) Ointment 11.9 ± 0.6 Acetone 531.6 ± 68.6 Ethanol 593.2 ± 43.0	Cross & Roberts 2000
0.8% MP in an oil in water emulsion	OECD 428 study. Fresh human skin dermatomed to 450 µm. n=13 samples from 3 donors. 24 h application. 8 - 10 mg/cm² (10 µl/cm²). Flow-through diffusion cells. HBSS receptor fluid. Exposure area 0.64 cm^2. Not occluded. Samples were analysed by radiochromatography and LC-MS.	Total penetration: 79.36% of applied dose. 35.1% was recovered as pHBA. Receptor fluid 79.36 ± 15.62% Receptor wash 0.46 ± 0.11% Skin 4.88 ± 2.01% Total % applied dose absorbable = 84.69 ± 15.46% (total radioactivity) Skin wash 14.65 ± 8.76% Donor chamber 0.42 ± 0.94% Tape strips 6.13 ± 12.01%	Fasano 2004

		Total unabsorbed dose = 21.21 ± 20.48% Total recovery = 105.91 ± 15.10	
25 µg MP/cm^2 in DMSO	Human skin (n=3), previously frozen at -70°C. Dermatomed to 350 µM. 25 µg in DMSO 10µl/cm^2. Skin 1cm^2 application area, held in a glass ring sat in a 12 well plate. Bespoke method of skin absorption to assess potential of fresh skin to metabolise MP.	Total absorption: At 6h: 5.56 ± 1.4% in receptor fluid At 24h: 27.80 ± 3.92% in receptor fluid 28.6 ± 11.52% in skin A complete mass balance was not performed.	Jewell *et al.*, 2007
0.1% MP as contained in commercial body lotion	Human abdominal skin (previously frozen at -20°C) from cosmetic surgery females n=8 samples. Franz diffusion cells. Physiological saline receptor fluid. Single application 100µl of 45 mg. Repeat dose x3: 0, 12 and 24h 100µl of 45 mg.	Parent methyl paraben estimated in receptor fluid Single Dose (mean ± SD) After 36h, a total of 0.057 ± 0.03%. Repeat Dose (mean ± SD) After 36h, a total of 0.6 ± 0.1% A mass balance was not performed in this study.	El Hussein *et al.*, 2007
0.1, 0.4 and 2% MP in oil-in-water cream emulsion	Human epidermis (~0.03mm thick)(previously frozen). 10mg cream applied to area 0.785 cm^2. Receptor fluid (1:1 ethanol:water). 24 hour analysis by HPLC of the parent compound.	Permeability coefficient Kp for MP was similar at all concentrations: 0.74±0.19 to 0.91±0.44 cm/h x 10^{-4}. A mass balance was not performed.	Seo *et al.* (2016)

Effects of occlusion:
Cross & Roberts (2000) provided some initial observations on the effects of occlusion on skin penetration of Methylparaben across epidermal membranes and using three different vehicles: an ointment, acetone and ethanol. With an oil-based ointment, penetration was apparently decreased by occlusion but with acetone or ethanol vehicles, occlusion significantly increased absorption.

3) In vivo human skin absorption studies

<u>From Applicants Dossier</u>
Ishiwatari *et al.* (2007) measured levels of methyl paraben in the stratum corneum after a single application. Cosmetic emulsions containing 0.15, 0.25 or 0.5% w/v methyl paraben were applied once to the forearm (42 cm^2) of one male and one female subject. At 1, 2, 5 and 12 hours after application, a small area was cleaned of emulsion using wet cotton and Methylparaben was extracted by applying a glass cylinder (3.1 cm^2) with 0.5ml ethanol for 5 minutes. Samples were analysed by HPLC/GC-MS. Methyl paraben reached a peak 2 hours after application and returned to baseline after 12 hours. Ishiwatari *et al.* (2007) also applied a methyl paraben-containing lotion (concentration not stated) twice a day for 1 month in n=6 subjects. GCMS was used to analyse for Methylparaben in stratum corneum at 1, 2, 3 and 4

weeks after the start of the study, and 2 days after the last application. Methyl paraben concentrations in the SC increased with repeated application, but 2 days after treatment stopped, levels returned back to pre-treatment levels.

Martins *et al.* (2019) performed a study to assess the exposure to methylparaben (MP) from antiperspirants in serum of 24 women aged 20-30 years old. An antiperspirant containing 0.2% w/w MP was given to the volunteers, to estimate the internal dose. An effective liquid chromatography-tandem mass spectrometry method for the determination of MP levels in serum was developed and validated in the range of 10-100 µg/L; the method was fast, simple, sensitive, linear, precise, and accurate. In addition, a simple and rapid liquid chromatography-ultraviolet detection method for the determination of MP levels in antiperspirants was developed and validated in the range of 2-26 mg/L, which presented satisfactory linearity, precision, and accuracy. Although MP permeated the skin, there was no correlation between antiperspirant use and paraben serum concentration in the volunteers.

4) Metabolism in Skin

From Applicant Dossier

The potential for carboxylesterases to be metabolically active and perform first pass effective clearance for parabens in the skin, has been investigated in multiple species in vitro and ex vivo, including human, rabbit, rat and pig (Williams *et al.* 2008). Lobemeier *et al.* (1996) showed that both the epidermal and dermal layers of human skin have the capacity to hydrolyse parabens, extensively though not 100% completely in the skin. Another study showed that parabens are metabolised by human and rat skin (Harville *et al.*, 2007). However, in that study, human and rat skin were found to have different rates of paraben hydrolysis to yield p-hydroxybenzoic acid (p-HBA), with human skin esterases appearing less metabolically active in producing pHBA than rat skin. Rates of hydrolysis were seen to be more similar between human and minipig (Jewell *et al.* 2007). As can be seen in the above sections, there is substantial metabolism of methyl paraben to pHBA in metabolically competent skin in vitro and low levels of parent methyl paraben is typically seen in the receptor fluid. Skin esterases act as effective first pass metabolism for parabens in the skin (Williams *et al.* 2008), and then if any small amount of parent parabens enters the blood, this would be rapidly metabolised by esterases which are ubiquitous in the rest of the body.

Annex II: Studies investigating endocrine activity of Methyl paraben in vitro

			androgen sulfation. No positive control was included.	
Test substances	**Test system**	**Test principle(s)**	**Result(s) and conclusion(s)**	**Reference**
Methyl paraben	Recombinant yeast assay screen	DNA sequence of the human estrogen receptor is integrated into the yeat genome. Substances are compared with the potency of estrogen at its receptor.	The potency of MP if approx. 2,500,000-fold lower as compared to 17β-oestradiol	Routledge *et al*. 1998 Miller *et al* 2001
Methyl paraben	Estrogen-receptor competitive binding assay. Ability of MP to displace ^{3}H-oestradiol.	Substance competes with estradiol in binding with the ER	IC_{50} for 225 µM, compared with an IC_{50} for 17β-estradiol of 0.0009 µM. MP had relative binding affinity of 0.0004%	Blair *et al* 2000
Methyl paraben	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Assaying estrogen-receptor (ER)-dependent proliferation of MCF-7 cells	MP 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.	Okubo *et al* 2001
Methyl paraben	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Competitive inhibition of [3H]estradiol binding to MCF7 cell estrogen receptors	Competitive inhibition of [^{3}H]oestradiol binding to MCF7 cell estrogen receptors could be detected at 1,000,000-fold molar excess of MP (21%)	Byford *et al* 2002
Methyl paraben	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Principle of gene expression profiling based on DNA microarray analysis with 120 genes selected as showing greater statistical reliability for estrogen-responses.	No significant effects were seen for methyl paraben	Terasaka *et al*. 2006
Methyl paraben	Skin and liver cytosol and **human** epidermal keratinocytes	Parabens elevate estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin	SULT activity was weakly inhibited in skin cytosol by MP, but not by PHBA. No inhibition of	Prusakiewicz *et al*. 2007

			androgen sulfation. No positive control was included.	
Methyl paraben	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	MP 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
Methyl paraben	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	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	A weak potential was noted (potency 5 to 6 orders of magnitude below that of 17β-oestradiol) and pHBA was not active	van Meeuwen *et al*. 2008
Methyl paraben	GH3 rat pituitary cancer cell line	Induction of an estrogenic biomarker gene - Calbindin-D(9k) (CaBP-9k)	Methyl paraben was weakly active in this assay, in terms of inducing CaBP-9k and PR via the ER pathway in GH3 cell line	Vo *et al* 2011

Test substances	Test system	Test principle(s)	Result(s) and conclusion(s)	Reference
In Vitro Assays ….continued				
Methyl paraben	Mouse and Human adipocytes	1) Murine 3T3-L1 fibroblasts 2) hADSC (human adipose-derived multipotent stromal cells) 3) GR-responsive luciferase reporter construct MMTV-Luc 4) PolarScreen GR competitor assay	1 & 2) MP effects were similar to controls re adipocyte morphology, lipid accumulation, and mRNA expression of adipocyte-specific markers 3) No activity (4) no activity	Hu *et al* 2013
Methyl paraben	Obesogen screening based on the 3T3-L1 cell line, a well-characterised adipogenesis model, and direct fluorescent measurement using Nile red lipid staining technique. Also PPARγ activation and antagonist experiments.	Positive controls: acknowledged obesogens rosiglitazone and tributyltin. 0.39-200 µM test concentrations of MP.	LOECs (3T3-L1 cell line): Rosiglitazone 16nM Tributyltin 6.25nM MP 100µm LOECs (PPARγ): Rosiglitazone 30nM Tributyltin 3nM MP 30µm	Pereira-Fernandes *et al* 2013
Methyl paraben	MCF-7 and MCF-10A cells	Analysed 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 exposure of MP 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 MP 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

Test substances	Test system	Test principle(s)	Result(s) and conclusion(s)	Reference
In Vitro Assays ….continued				
17 parabens; linear C1 to C12, plus 5 non-linear side chain parabens.	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. Among 12 parabens with linear alkyl chains (C_1 to C_{12}), heptylparaben (C_7) and pentylparaben (C_5) showed the most potent ERα and ERβ agonistic activity in the order of $10^{(-7)}$M and $10^{(-8)}$M. Activities decreased in a stepwise manner as the alkyl chain was shortened to C_1 or lengthened to C_{12}. Most parabens exhibited ERβ-agonistic activity at lower concentrations than ERα-agonistic activity. The estrogenic activity of butylparaben was markedly decreased by incubation with rat liver microsomes, and the decrease of activity was blocked by a carboxylesterase inhibitor. These results indicate that parabens are selective agonists for ERβ over ERα; their interactions with ERα/β are dependent on the size and bulkiness of the alkyl groups; and they are metabolized by carboxylesterases, leading to attenuation of their estrogenic activity.	Watanabe *et al* 2013
Methyl paraben	*In vitro* nuclear receptor coactivator recruiting assay	Antagonist competitive binding on the human estrogen-related receptor γ (ERRγ)	MP 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

Methyl paraben	MCF-7 and MCF-10A	MP (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 MP. In MCF-10A, MP increased the expression of G1 /S phase genes, and downregulated cell cycle inhibitors.	Wróbel & Gregoraszczuk 2014a
Methyl paraben	MCF-7 and MCF-10A	MP (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)	MP did not stimulate PGR mRNA expression in MCF-7 cells. MP increased ESR1 gene and protein expression in MCF-7. MP increased ESR2 mRNA and protein expression in MCF-7 cells, but in MCF-10A cells only ESR2 protein expression.	Wróbel & Gregoraszczuk 2014b
Methyl paraben	Human MDA-kb2 breast carcinoma cells	0 and 25 µM in DMSO. Cells stably transformed with MMTV-luciferase and express high levels of functional endogenous AR and GR which both act through MMTV promoter. Cells, cultured in Leibovitz's L-15 medium with 10% FBS, 100U/ml penicillin, 100 mg/ml streptomycin, then incubated 24h with and without test compound, and with or without the AR agonist flutamide (5µM).	MP did not enhance the hydrocortisone-induced GR signal.	Kolšek *et al* 2015

Test substances	Test system	Test principle(s)	Result(s) and conclusion(s)	Reference
In Vitro Assays ….continued				
Methyl paraben	*In vitro* testing of MP and pHBA for inhibition of 17β-HSD1 and 17 β-HSD2 activities. Molecular docking studies were performed using GOLD (Gold version 5.2, CCDC, Cambridge, UK)	Endogenous 17β-HSD1 activity assays were performed in intact COV434 cells. Lysates of HEK-293 cells expressing 17β-HSD1 or 17β-HSD2.	MP but not pHBA inhibited 17 β-HSD2 at 20μM. However, it is noted, regarding the very rapid metabolism of these compounds to the inactive p-hydroxybenzoic acid by esterases, it needs to be determined under which conditions low micromolar concentrations of these parabens or their mixtures can occur in target cells to effectively disturb estrogen effects *in vivo*.	Engeli *et al* 2017
Methyl paraben	Tox 21 Endocrine screening program assays	Estrogen receptor (ER) assays Androgen receptor (AR) assays Thyroid receptor (TR) assays Steroidogenesis assays	6/21 ER assays reported as positive. 4 assays close to cut off. All AR assays negative All assays negative for TR or steroidogenesis	US EPA Endocrine Screening program 2019*

* https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID4022529

Annex III: In vivo studies on endocrine system relevant endpoints

Test substances	Test system	Test principle(s)	Result(s) and conclusion(s)	Reference
Level 3 - *In Vivo* Experiments – female rodents				
Methyl paraben	Alpk:AP rats	**Uterotrophic assay** with immature rats. MP was administered on PND 21-22 once daily for 3 consecutive days at the following dosage levels: - MP orally at 40, 400 and 800 mg/kg bw/day - MP subcutaneously (sc) at 40 and 80 mg/kg bw/day **Uterotrophic assay** with ovariectomized (OVX)rats (8-10 weeks old): - MP subcutaneously (sc) at 800 mg/kg bw/day	Immature rat model: No effect MP administered sc or orally failed to increase uterus weights up to 80 and 800 mg MP/kg/day The positive control oestradiol exerted its effects at an oral dose of 0.4 mg/kg or 0.04 mg/kg bw/day (sc).	Routledge *et al.* 1998
Methyl paraben	B6D2F1 mice Appears compliant with OECD TG440 Non-GLP	**Uterotrophic assay** - **oral** (1, 10, 100 mg/kg) and **subcutaneous** doses (100 mg/kg (all)) for 3 days. Immature female mice	No effects Oral and s.c. NOEL (mice) 100 mg/kg (top dose oral).	Hossaini *et al* 2000
Methyl paraben	CD1 **mice** Wistar **rats** Appears compliant with OECD TG440 Non-GLP	**Uterotrophic assay** with both immature and ovariectomized adult mice and immature rats. Animals were **subcutaneously** (sc) treated for three consecutive days with 5.5, 16.5 or 165 mg/kg/day MP or E2 (0.036 micromol/kg). Estrogen receptor binding affinities of MP relative to E2 were determined.	Weak oestrogenic activity observed at 16.5 - 165 mg/kg/day, no activity at 5.5 mg/kg/day (Limitations of study described in the text)	Lemini *et al.*, 2003

Methyl paraben	Adult ovariectomized (Ovx) CD1 mice Appears compliant with OECD TG440 Non-GLP	**Morphometric analysis of uteri in uterotrophic assay. Subcutaneously** (sc) treated daily for three days with MP (55 and 165 mg/kg), E_2 (10 mg/kg; 0.036 mmol/kg), and vehicle (butyleneglycol; V, 10 mL/kg)	Weak oestrogenic activity observed at 55 and 165 mg/kg/day (Limitations of study described in the text)	Lemini *et al* 2004
Methyl paraben	Mated Sprague Dawley female rats**;** Prepubertal (8-week-old) females, N=200, n=10/group, 20 groups. 0, 62.5, 250 or 1000 mg/kg bw/day in corn oil (vehicle), by oral gavage. Non GLP Non guideline	*In vivo* assay to investigate whether oral-subacute exposure to MP may induce suppressive effects on reproductive organs in female rats during the critical juvenile-peri-pubertal stage. **Oral-subacute administration by gavage** of MP from postnatal day 21 to 40. Investigation of Calbindin-D9-k biomarker for estrogenic effects.	No significant changes to estradiol, prolactin and T4 levels. IC_{50} for binding to ERα and ERβ receptors was too low to be calculated for MP. No effect on vaginal opening. No significant change to estrous cycle. Slight decrease in ovary weight and increase in adrenal weight and thyroid gland weight.	Vo *et al*. 2010

Test substances	Test system	Test principle(s)	Result(s) and conclusion(s)	Reference
In Vivo Experiments (Level 3) continued – female rodents				
Methyl paraben 17ß-oestradiol (E2)	Neonatal Sprague Dawley female rats (n =5) Non GLP Non guideline	Effects of neonatal exposure to MP on development of early follicle stages and ovarian factors regulating follicular development and steroidogenesis after **subcutaneous** administration of MP at doses of 62.5, 250 or 1000 mg/kg bw/day or 17ß-oestradiol (40 µg/kg/day) once daily on PND 1-7. Ovaries were excised on PND 8 and prepared for histopathology. Follicles were counted and classified regarding their developmental stages. Relative mRNA expression of the following proteins was determined by quantitative real-time PCR: calbindin-9k (CaBP-9k, indicator of estrogenic activity in rat uterus), ovarian anti-Mullerian hormone (AMH), kit ligand/stem cell factor (KITL) and forkhead box protein I2 transcription factor (FoxI2), all three associated with follicle development in rat as well as the steroidogenic enzymes steroidogenic acute regulatory transport protein (StAR) and CYP11a1.	No effects on organ weight Methyl-paraben did not have any significant effect on CaBP-9k expression There were no significant effects in any parameters measured for MP.	Ahn *et al*. 2012
Methyl paraben	Wistar rat	Repetition of the Oishi study (2001) under GLP with MP using the same strain of rats but 16 instead of 8 animals per dose group, same oral route dosage levels of 0, 100, 1000 and 10,000 ppm in food. In addition of the Oishi study, blood samples were taken weekly for the analysis of LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone	There were no treatment-related effects on testes, ventral prostates and preputial glands in any of the groups. Unlike Oishi (2001), sperm parameters were found unaffected. The highest dose level in food corresponds approximately to a **NOAEL of 1000 mg/kg bw/day**	Hoberman *et al* 2008; Charles River 2005.

Annex IV: References used in Section 3.4.9

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Annex V Benchmark Dose Modeling: Report

European Food Safety Authority (EFSA)

Table of Contents

A. Data Description

Brief general description of the data. This section should include a table summarizing the data. In case that raw data is available, resulting in a too large table, summary statistics may be given instead. For quantal endpoints both the number of responding animals and the total number of animals should be given for each dose level; for continuous endpoints either the individual responses or the mean responses and the associated SDs (or SEMs) and sample sizes should be given for each dose level.

The endpoint to be analyzed is: mean.

Subset of the data is taken for dose, retaining value(s) 0, 100, 300, 1000.

Data used for analysis:

dose	mean	ec	n
0	1.67	0.18	107
100	1.62	0.18	102
300	1.61	0.14	91
1000	1.58	0.15	110

B. Software Used

Results are obtained using the EFSA web-tool for Bayesion BMD analysis, which uses the R-package [BMABMDR] version 0.0.0.9057 for the underlying calculations.

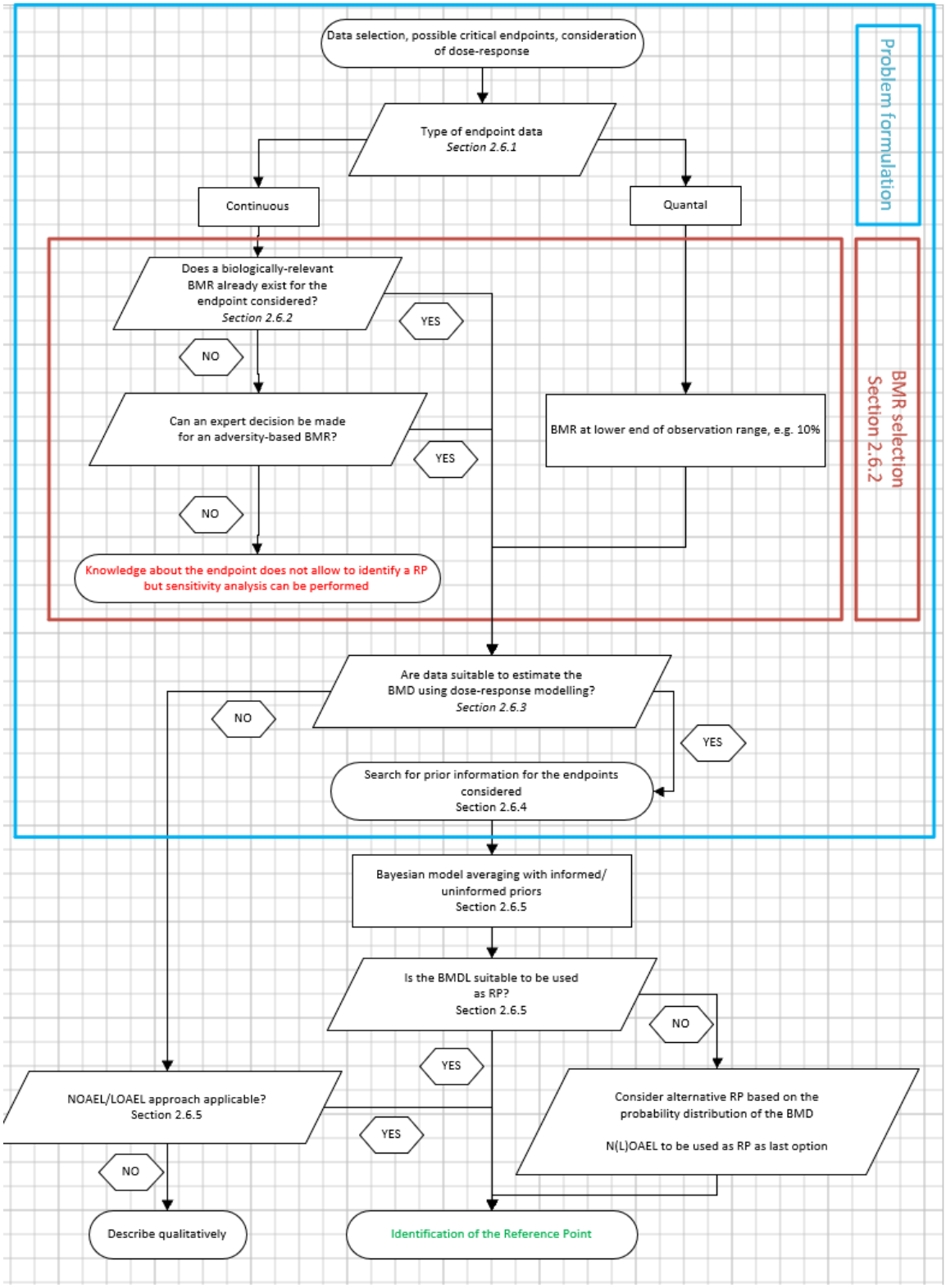
C. Justification of any deviation from the procedure and assumptions

- *In case another approach than Bayesian model averaging was used, the rationale and details for deviating from the recommended approach should be provided.*

- *Assumptions made when deviating from the recommended defaults in this guidance document (e.g. gamma distributional assumption instead of normal and log-normal, heteroscedasticity instead of homoscedasticity).*

- *Other models than the recommended ones listed in Tables 2 and 3 of this guidance document that were fitted should be listed, with the reasons to include them.*

- *Description of any deviation from the procedure described in the flow chart to obtain the final BMD credible interval.*



Flowchart to derive a Reference Point (RP) from a dose-response dataset of a specified endpoint, using BMD analysis

D. Results

Information pertaining to this endpoint.

Check for constant variance coefficient of variation

Distributional assumption of constant variance for the normal distribution is not met, Bartlett test p-value is 0.0235

Distributional assumption of constant variance (on log-scale) are met, Bartlett test p-value is 0.0641

Goodness of Fit

Best fitting model fits sufficiently well (Bayes factor is 1.75e+00).

Model Averaged BMD

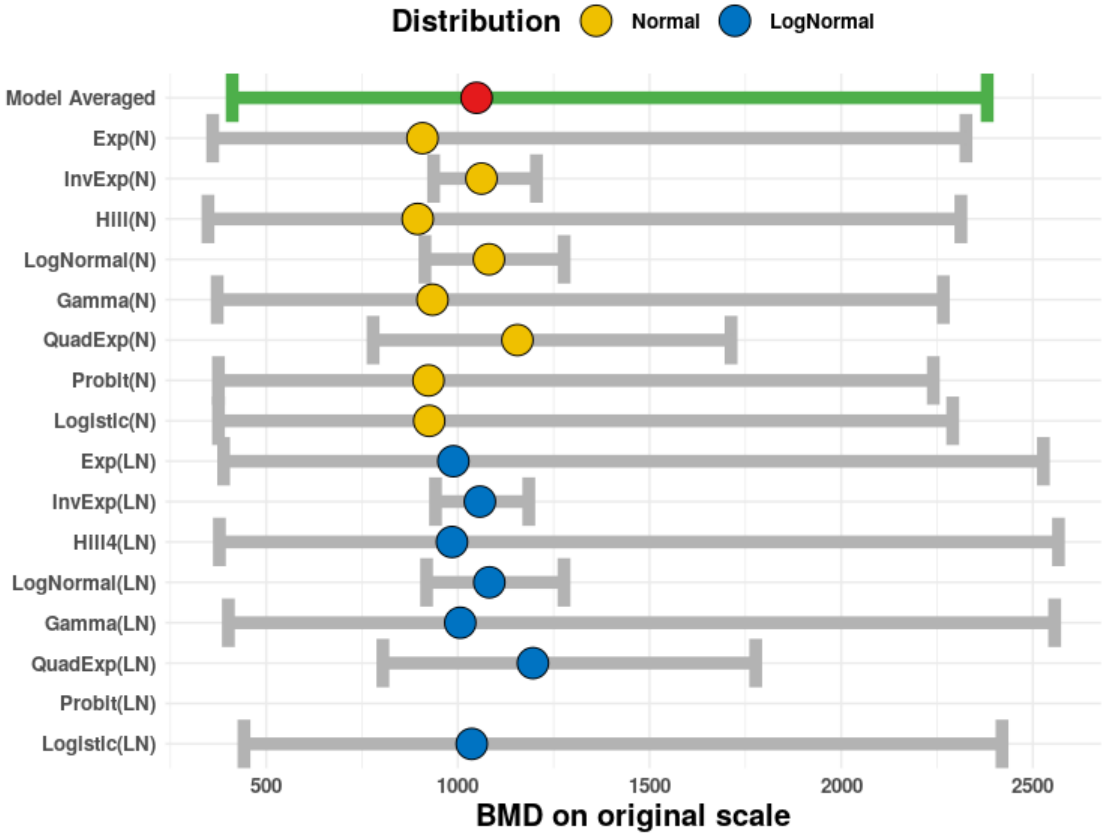
Model	Type	BMDL	BMD	BMDU
Model Averaged	LP	411.341	1,048.908	2,380.944
Model Averaged	LP	409.573	1,048.576	2,424.878
Model Averaged	LP	374.414	903.241	2,032.377
Model Averaged	LP	375.894	992.710	2,382.271

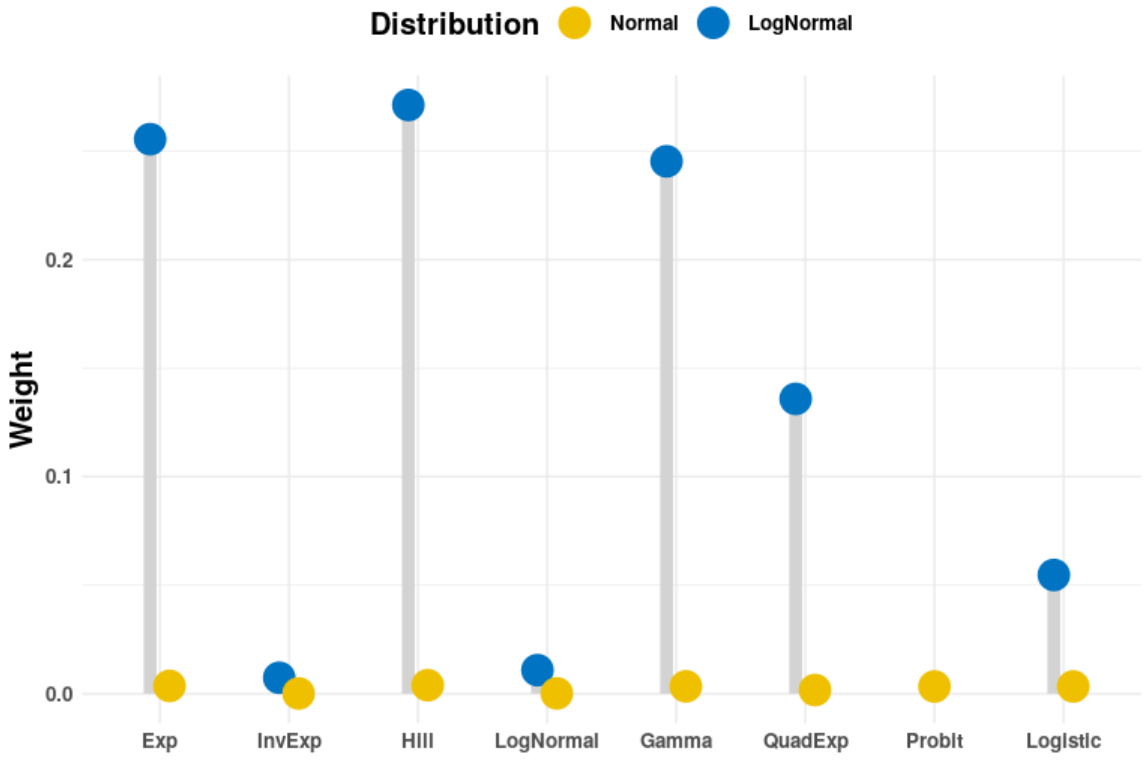
Estimated BMDs per model

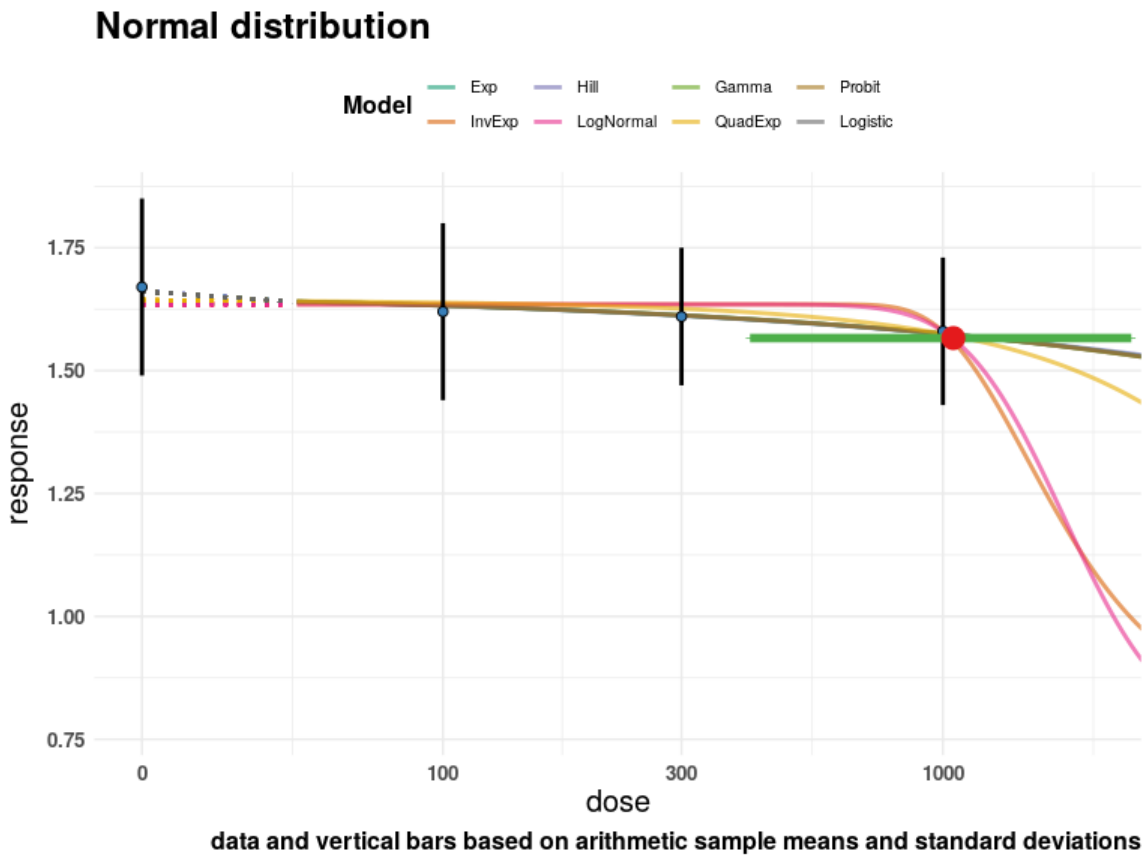
Model	BMDL	BMD	BMDU	Model Weights
E4_N	360.444	907.687	2325.615	0.004
IE4_N	936.764	1061.635	1205.054	0.000
H4_N	348.188	895.277	2312.261	0.004
LN4_N	914.164	1081.141	1277.165	0.000
G4_N	372.180	933.908	2266.566	0.003
QE4_N	778.909	1155.419	1712.635	0.002
P4_N	375.436	923.383	2240.312	0.003
L4_N	375.593	925.280	2290.954	0.003

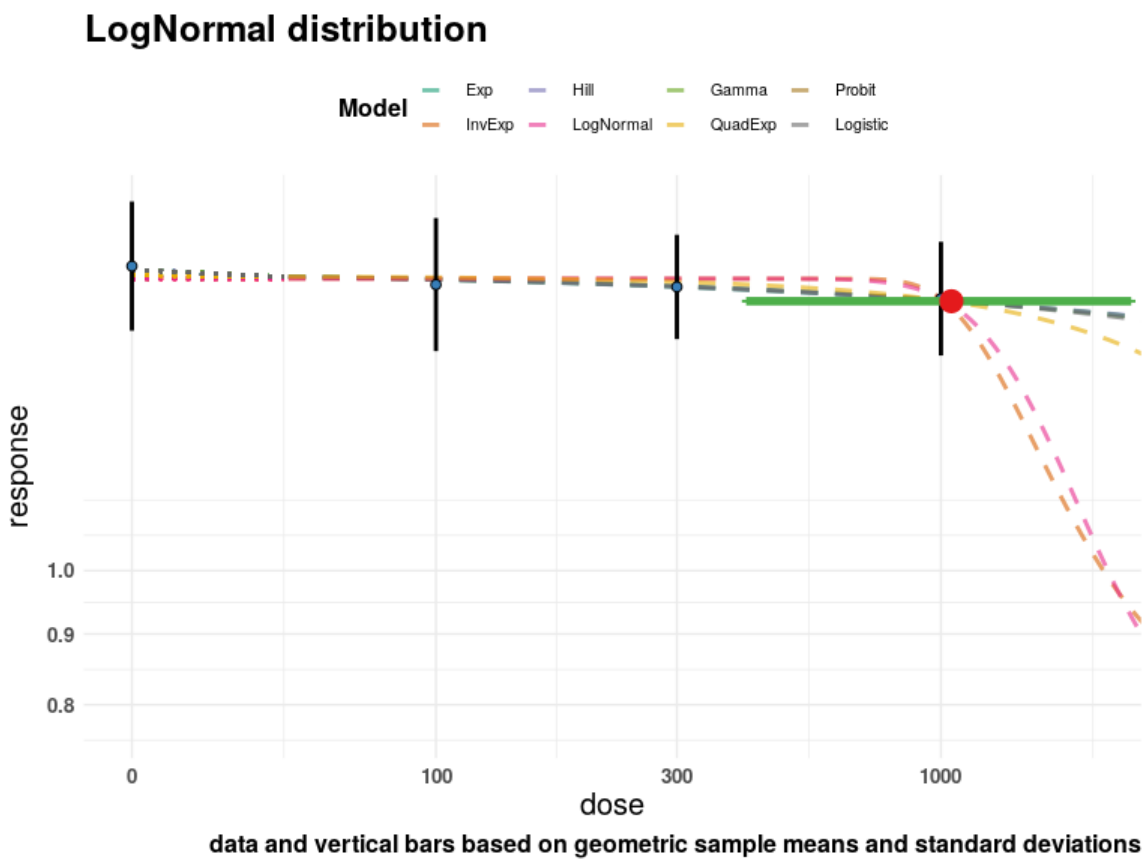
E4_LN	388.967	988.319	2527.481	0.255
IE4_LN	941.732	1057.501	1185.067	0.007
H4_LN	377.968	984.677	2566.855	0.271
LN4_LN	918.435	1082.100	1276.904	0.011
G4_LN	401.400	1006.207	2556.828	0.245
QE4_LN	804.424	1195.831	1776.946	0.136
L4_LN	442.056	1036.288	2419.550	0.055

Plots of Fitted Models






Normal distribution
Model
Exp
Hill
Gamma
Probit
InvExp
LogNormal
QuadExp
Logistic
response
1.75
1.50
1.25
1.00
0.75
0
100
300
1000
dose
data and vertical bars based on arithmetic sample means and standard deviations


LogNormal distribution
Model
Exp
Hill
Gamma
Probit
InvExp
LogNormal
QuadExp
Logistic
response
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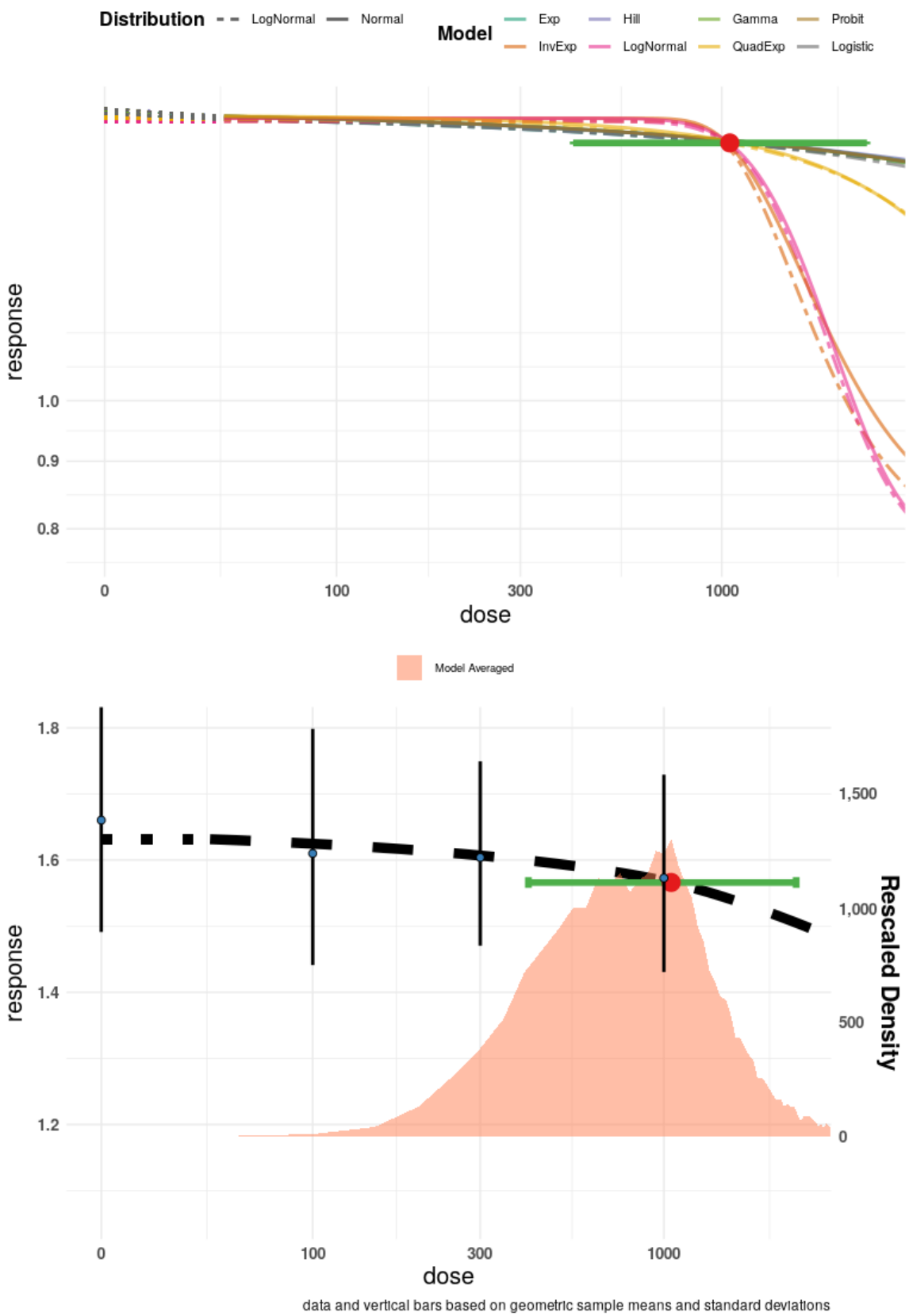
0.9
0.8
0
100
300
1000
dose
data and vertical bars based on geometric sample means and standard deviations


Distribution
LogNormal
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Model
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Hill
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LogNormal
QuadExp
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response
1.0
0.9
0.8
0
100
300
1000
dose
Model Averaged
1.8
1.6
1.4
1.2
response
1,500
1,000
500
0
Rescaled Density
0
100
300
1000
dose
data and vertical bars based on geometric sample means and standard deviations

E. Conclusions

This section should summarize the results for each endpoint (dataset) that was analysed and provide a discussion of the rationale behind selecting the critical endpoint. The BMD confidence interval of the critical endpoint (and the BMDL selected as RP) should be reported and discussed.