METHYLPARABEN

(CAS #99-76-3)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: June 21, 2023

Expiration Date: June 21, 2028

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GreenScreen® Executive Summary for Methylparaben (CAS #99-76-3)

Methylparaben is a commonly used preservative in food and cosmetics, and is naturally occurring in some foods, such as blueberries. It is a crystalline powder at room temperature, and is not explosive, oxidizing, or flammable. If released to the environment, methylparaben is expected to partition to soil and water. Methylparaben is soluble in water and has a very low vapor pressure, therefore it is unlikely to volatilize and is not a volatile organic compound (VOC).

Methylparaben is assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes"). This score is based on the following hazard score:

- Benchmark 2e
    - Moderate Group I Human Toxicity (endocrine activity-E)

The GreenScreen® Benchmark Score for methylparaben has not changed over time. The original GreenScreen® assessment was performed in 2018 under version 1.4 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. This BM-2 score was maintained in the current version 1.4 update. Several new studies were identified in the public literature and are incorporated herein. These studies add to the weight of evidence for numerous endpoints, and fulfill the previously identified data gap for reproductive toxicity.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* testing for mutagenicity, endocrine activity, and skin irritation, and *in silico* modeling for respiratory sensitization and bioaccumulation. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

Type I (input data) uncertainties in methylparaben's NAMs dataset include lack of experimental data and validated methods for assessing respiratory sensitization. Methylparaben's Type II (extrapolation output) uncertainties include reliance on *in vitro* data in which the exogenous metabolic activation does not entirely mimic *in vivo* conditions and extrapolation of skin sensitization data to respiratory sensitization which is incomplete in that it does not account for non-immunologic mechanisms of respiratory sensitization. Some of methylparaben's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for Methylparaben

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
L	**L**	**L**	**L**	*M*	**L**	*M*	**L**	*L*	**L**	**L**	*L*	**L**	**L**	**M**	*H*	**vL**	**vL**	**L**	**L**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Chemical Assessment for Methylparaben (CAS #99-76-3)

Method Version: GreenScreen® Version 1.4
Assessment Type[1]: Certified
Assessor Type: Licensed GreenScreen® Profiler

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Mouna Zachary, Ph.D.
Title: Toxicologist
Organization: ToxServices LLC
Date: July 12, 2018

Quality Control Performed By:
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Title: Senior Toxicologist
Organization: ToxServices LLC
Date: July 12, 2018

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Name: Nancy Linde, M.S.
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Organization: ToxServices LLC
Date: March 29, 2023; June 8, 2023

Quality Control Performed By:
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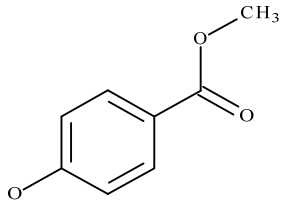
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: April 17, 2023; June 21, 2023

Expiration Date: June 21, 2028[2]

Chemical Name: Methylparaben

CAS Number: 99-76-3

Chemical Structure(s):



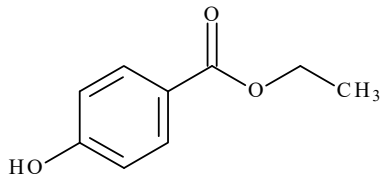
Also called: 4-Hydroxybenzoic acid methyl ester; methyl p-hydroxybenzoate; methyl-4-hydroxybenzoate; methyl parahydroxybenzoate; p-hydroxybenzoic acid methyl ester; p-methoxycarbonylphenol; benzoic acid, 4-hydroxy-, methyl ester; p-carbomethoxyphenol; FEMA No. 2710; CCRIS 3946; HSDB 1184; EINECS 202-785-7 (PubChem 2023).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Methylparaben has a relatively complete toxicological dataset. For the carcinogenicity endpoint, the available carcinogenicity studies were performed using routes of exposure (subcutaneous or intraperitoneal) that are generally outside the scope of the GreenScreen® criteria, but are useful in the weight of evidence assessment. These data are supplemented with carcinogenicity data for the

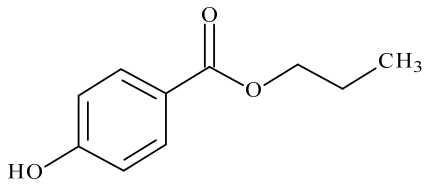
[1] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[2] Assessments expire five years from the date of completion starting from January 1, 2019. An assessment expires three years from the date of completion if completed before January 1, 2019 (CPA 2018a).

surrogates butylparaben (CAS #94-26-8) and isobutylparaben (CAS #4247-02-3). Available data indicate that smaller parabens are absorbed more rapidly, but as all 5 compounds (methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben) are rapidly absorbed and readily metabolized to the same primary metabolite 4-hydroxybutanoic acid and the corresponding alcohol within hours, following oral and dermal exposure, butylparaben and isobutylparaben are still reasonable surrogates for the shorter chain parabens (CIR 2008).

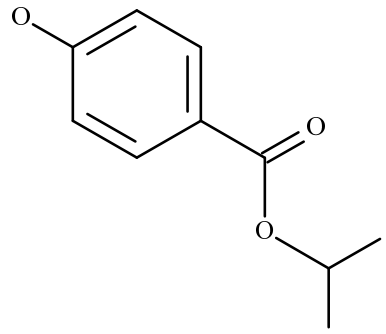
For some other endpoints, where data on the target chemical are limited, data for the other short chain parabens are used as supporting data.



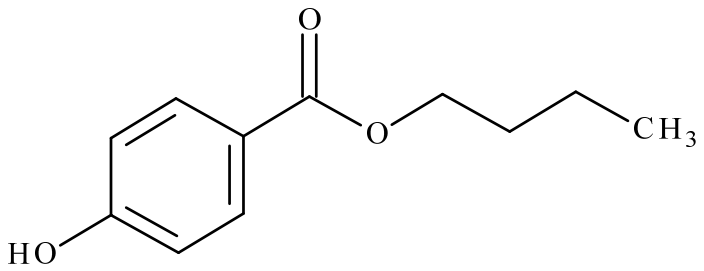
Ethylparaben (CAS #120-47-8)



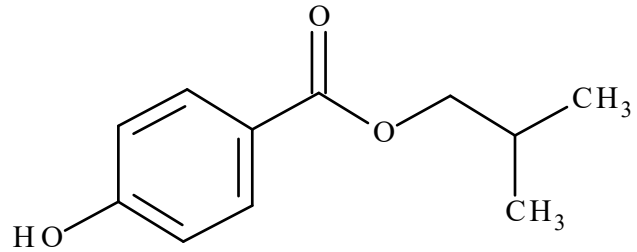
Propylparaben (CAS #94-13-3)



Isopropylparaben (CAS #4191-73-5)



Butylparaben (CAS #94-26-8)



Isobutylparaben (CAS #4247-02-3)

Identify Applications/Functional Uses:
A preservative in food, cosmetics, and numerous consumer and industrial products. Reported maximum use levels in cosmetics include 0.9% in rinse-off products (e.g. shampoo), 0.8% in leave-on products, 0.8% in products used near the eye (e.g., mascara), 0.5% bath oils, tablets, and salts, 0.4% in baby lotions, oils, and creams, and up to 0.41% in fragranced spray products (CIR 2020). In the United States, methylparaben is generally recognized as safe (GRAS) for use in food as an antimicrobial agent (21 CFR §184.1490); is prior sanctioned for use in food as an antimycotic (antifungal) (21 CFR §181.23) (U.S. FDA 2022); and is approved for use as an excipient (inactive ingredient) in pharmaceuticals (e.g., up to 1.8 mg/tablet or 2.6 mg/mL in an oral solution) (U.S. FDA 2023).

Known Impurities[3]:
p-Hydroxybutanoic acid is a commonly specified impurity at ≤ 0.1% based on multiple studies summarized in the REACH dossier (ECHA 2023a). This impurity is a starting compound in the manufacturing process of methylparaben, as well as a functional group, and primary metabolite. This GreenScreen®, however, is performed on the theoretical pure substance.

[3] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.

GreenScreen® Summary Rating for Methylparaben[4,5 6,7]: Methylparaben is assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes") (CPA 2018b). This score is based on the following hazard score:

- Benchmark 2e
    - Moderate Group I Human Toxicity (endocrine activity-E)

Figure 1: GreenScreen® Hazard Summary Table for Methylparaben

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
L	L	L	L	*M*	L	*M*	L	*L*	L	L	*L*	L	L	M	*H*	vL	vL	L	L

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

Environmental Transformation Products

Per GreenScreen® guidance (CPA 2018b), chemicals that degrade rapidly and completely (i.e., meet criteria for a Very Low for persistence) are not likely to form persistent biodegradation intermediates because the degradation intermediates will not persist long enough to be encountered after use or release of the parent chemical (i.e., relevant). As methylparaben is readily biodegradable, it is not expected to have relevant transformation products. It may be noted however, that methylparaben, ethylparaben, and butylparaben, have been identified in marine biota. McHugh (2022) reported detection of methylparaben in 46 out of 50 (approximately 96%) biota samples (primarily mollusks) collected over OSPAR Regions I to IV (primarily western European coastal regions of the Atlantic Ocean), with a maximum concentration of 719 µg/kg wet weight. Authors acknowledged that based on the widespread use of parabens, there is considerable potential for inadvertent cross-contamination of environmental samples (McHugh 2022); however, it is unclear what measures, if any, were employed to prevent cross-contamination of the samples, and whether investigators assessed for parabens in the laboratory equipment, reagents, etc. Additionally, methylparaben has been identified as naturally occurring in some plants, and it is unclear if the measured parabens were anthropogenic.

Introduction

Methylparaben is manufactured by esterifying 4-hydroxybenzoic acid in the presence of an acid catalyst, such as sulfuric acid, and an excess of methanol, followed by neutralization with caustic soda (CIR 2020). It is frequently used in cosmetics and personal care products, as a food additive for human food and animal feed, and as an excipient in pharmaceuticals (CIR 2020). Methylparaben is naturally

[4] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.
[5] See Appendix A for a glossary of hazard endpoint acronyms.
[6] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[7] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen® Guidance v1.4 Annex 2.

occurring in some fruits, such as blueberries, and prevents decomposition of foods by preventing the growth of fungi or bacteria (ChEBI 2023).

ToxServices assessed methylparaben against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2021).

U.S. EPA Safer Choice Program's Safer Chemical Ingredients List

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2023). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

Methylparaben is not currently present on the SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2023) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[8] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for methylparaben can be found in Appendix C.

- Methylparaben is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Methylparaben is not listed on the U.S. DOT list.
- Methylparaben is on the following GreenScreen®-specified lists for multiple endpoints:
  - GHS – Japan – H412: Harmful to aquatic life with long lasting effects Category 3
  - German FEA – Substances hazardous to waters Class 1 – low hazard to waters
- GreenScreen®-specified lists for single endpoints are presented under their respective endpoints below.

Hazard Statement and Occupational Control

No Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for methylparaben, however, self-classifications by the REACH registration dossier authors or the majority of notifiers in the EU are indicated in Table 1, below. General personal protective equipment (PPE) recommendations are presented in Table 2, below. No occupational exposure limits (OELs) were identified.

Table 1: GHS H Statements for Methylparaben (CAS #99-76-3) (ECHA 2023a,b)

H Statement	H Statement Details
H315	Causes skin irritation (majority of notifiers)
H319	Causes serious eye irritation (majority of notifiers)
H411	Toxic to aquatic life with long lasting effects (REACH dossier authors)
H412	Harmful to aquatic life with long lasting effects (majority of notifiers)

[8] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

Table 1: GHS H Statements for Methylparaben (CAS #99-76-3) (ECHA 2023a,b)	
H Statement	**H Statement Details**

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Methylparaben (CAS #99-76-3)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Respiratory protection – short term – filter apparatus, Filter P2; wear PVC gloves and safety goggles and protective clothing	ECHA 2023a	None	

Physicochemical Properties of Methylparaben

Methylparaben is a white or colorless crystalline powder. It has a low melting point of 125°C and decomposes prior to boiling. Its vapor pressure is very low, indicating that it is unlikely to volatilize. Methylparaben is soluble in water, and its partition coefficient of 1.98 suggests low bioaccumulation potential.

Table 3: Physical and Chemical Properties of Methylparaben (CAS #99-76-3)		
Property	**Value**	**Reference**
Molecular formula	$C_8H_8O_3$	PubChem 2023
SMILES Notation	COC(=O)C1=CC=C(C=C1)O	PubChem 2023
Molecular weight	152.15 g/mol	PubChem 2023
Physical state	Solid at 20°C	ECHA 2023a
Appearance	White crystalline odorless powder	ECHA 2023a
Melting point	125°C (OECD Test Guideline (TG) 102)	ECHA 2023a
Boiling point	Decomposes prior to boiling at around 275°C	HC 2020
Vapor pressure	0.000055 Pa at 25°C (EU Method A.4 and OECD TG 104, GLP)	ECHA 2023a
Water solubility	1,880 mg/L at 20°C (OECD TG 105, and EU Method A.6, GLP)	ECHA 2023a
Dissociation constant	pKa = 8.4 at 23°C (OECD TG 112, GLP)	ECHA 2023a
Density/specific gravity	1.3775 g/cm³ at 20°C (OECD TG 109)	ECHA 2023a
Partition coefficient	Log K_{ow} = 1.98 at 22°C (similar to OECD TG 107)	ECHA 2023a
Particle size	D10: 22.0 +/- 0.9 µm; D50: 141.7 +/- 18.4 µm; D90: 426.7 +/- 82.6; And 3.7 +/- 0.2% < 10 µm	CIR 2020

Toxicokinetics

Methylparaben is highly absorbed and rapidly metabolized in animals and humans following oral and dermal exposure. Absorption is faster for the shorter alkyl chain parabens compared to longer chain parabens for both the dermal and oral routes of exposure (HC 2020).

Parabens applied to the skin are rapidly hydrolyzed to 4-hydroxybenzoic acid and the corresponding alcohol by carboxylesterases present in the keratinocytes. The rate of hydrolysis in the skin is faster for rodents than humans, and is faster for intact skin compared to dermatomed skin. Chemicals that disrupt

the stratum corneum may increase the skin penetration of shorter parabens, such as methylparaben and ethylparaben, but do not affect the penetration of longer-chain parabens (CIR 2020). A single dermal radiolabeled dose of 100 mg/kg methylparaben administered to rats resulted in maximum plasma concentrations in less than 8 hours. A single peak in the plasma corresponding to that of para-hydroxybutanoic acid (PHBA), the primary metabolite, whereas methylparaben was not detected. Approximately 50% of the dermally applied dose was absorbed after 24 hours, 14-26% was excreted in the urine, <2% in the feces, and the remainder was purportedly in the external tissues (e.g., hair, nails) (Aubert et al. 2012 as cited in HC 2020). In humans exposed to methylparaben on the forearm, accumulation occurred in the stratum corneum but did not persist 48 hours after application ceased (Ishiwatari et al. 2007 as cited in HC 2020).

Ingested parabens are quickly absorbed from the gastrointestinal tract, and similar to the dermal route of exposure, are hydrolyzed to 4-hydroxybenzoic acid, conjugated, and excreted in the urine. A single oral radiolabeled dose of 100 mg/kg methylparaben administered to rats resulted in maximum plasma concentrations in less than 1 hour, with a single peak in the plasma, corresponding to that of PHBA, the primary metabolite, and methylparaben was not detected. For the oral dose, over 70% was excreted in 24 hours with <1% detected in the feces and <1% detected in the tissues (Aubert et al. 2012 as cited in HC 2020). *Ex vivo* studies indicate metabolism by carboxylesterases present in human liver, subcutaneous fat, and blood, and by UDP-glucuronosyltransferases in liver microsomes. Hydrolysis in human liver cells was approximately 2 orders of magnitude greater than in skin cells (Jewell et al. 2007; and Harville et al. 2007, as cited in HC 2020).

Chronic exposure studies indicate that parabens do not accumulate in the body (CIR 2020), however, methylparaben has been detected at low levels in tumorous breast tissue, human adipose tissue, and in the brain (free or conjugated not specified) (Barr et al. 2012; Wang et al. 2015, and van der Meer 2017, as cited in HC 2020).

Relative to propylparaben and butylparaben, methylparaben was hydrolyzed 2 to 10 times faster in human liver and skin subcellular fractions evaluated *ex vivo* (Jewell et al. 2007a, 2007b; Harville et al. 2007; Lobemeier et al. 1996; Abbas et al. 2010; and Prusakiewicz et al. 2006, as cited in HC 2020). In human plasma, methylparaben was stable after 24 hours, which is markedly different from the aforementioned *in vivo* studies in which methylparaben was nearly non-detect in plasma. Hydrolysis in liver microsomes provided a half-life of 22 minutes for methylparaben, compared to 87 minutes for butylparaben (Abbas et al. 2010, as cited in HC 2020). For rats, skin and liver cell fractions hydrolyze parabens at roughly comparable rates, which for skin cells, is about 2 to 3 orders of magnitude faster than human skin cells (Harville et al. 2007; Prusakiewicz et al. 2006, as cited in HC 2020). While hydrolysis of shorter chain parabens is comparable in rats and humans, hydrolysis by carboxylesterase increases with increasing chain length for rats, such that butylparaben is metabolized in rat liver about 10 times faster than in human liver cells (Harville et al. 2007, as cited in HC 2020).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): L

Methylparaben is assigned a score of Low for carcinogenicity based on lack of indications of carcinogenicity in non-standard carcinogenicity studies on the target chemical, and with surrogates. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate data are available and negative and they are not classifiable under GHS (CPA 2018b). Although there are

deficiencies in the dataset (e.g., lack of guideline studies, reduced numbers of parameters, fewer animals, and limited reporting), confidence in the score is high based on the overall weight of evidence including numerous studies on the target compound and close surrogates with multiple routes of exposure that collectively do not identify concerns for carcinogenicity.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9] (Note: for the carcinogenicity endpoint, several studies are summarized for methylparaben using exposure routes (subcutaneous or intraperitoneal) that are generally outside the scope of the GreenScreen® assessment; however, they are included in the weight of evidence to assess overall carcinogenic potential).
  - *Subcutaneous:* Methylparaben was evaluated in a pre-GLP, non-guideline, carcinogenicity study. Male and female Fischer 344 rats were administered subcutaneous injections of methylparaben (purity not specified) in saline, twice weekly, at 0.6, 1.1, 2.0 and 3.5 mg/kg/application for 1 year. The number of animals/sex for each dose group was 20, 40, 60, and 80, respectively. There was a concurrent negative control group (60/sex), vehicle control group (60/sex), and positive control group administered nickel sulfide (80/sex). Each animal was sacrificed and autopsied at 12 or 18 months. There were no increase in tumor incidence in animals administered methylparaben, and authors concluded the test substance was not carcinogenic (Klimisch 2, reliable with restrictions) (Unnamed 1971 and 1973 publications).
  - *Subcutaneous/intravenous:* Methylparaben was evaluated in another pre-GLP, non-guideline carcinogenicity study. Groups of C57BL/6 male mice were injected via subcutaneous or intravenous injection with methylparaben (purity not specified) at 2.5 mg/mouse.
    - Group A had 25 males, 7 weeks old, that were administered a single dose of methylparaben at 2.5 mg/mouse, into the groin. Positive control animals were exposed to 25 mg dibenzopyrene. Five weeks after injection, the sites were excised, the tissue suspensions were pooled and injected into 250 secondary hosts, and the host injection sites were examined weekly. At 23 weeks after injections into the primary hosts, and 18 weeks after transfer to the secondary hosts, all animals were sacrificed. The injection sites were excised and preserved for histological analyses, and gross autopsies were performed on all animals.
    - Group B had 50 female CF1 mice and 50 female A/jax mice administered a single intravenous injection at 2.5 mg methylparaben, and another group of 20 female CF1 and 20 female A/Jax mice was administered 7 intravenous injections at monthly intervals. Positive control animals were exposed at 0.05, 0.1, or 0.5 mg benzopyrene/mouse. At the end of 28 weeks, mice were sacrificed, the lungs were inflated with formaldehyde and inspected for microscopic lesions.
    - Group C had 50 C57BL/6 male mice administered 12.5 γ benzopentaphene in tracaprylic administered subcutaneously, followed immediately and at 7 and 14 days by methylparaben at 2.5 mg/mouse in the same test site. The injection sites were examined weekly for tumors ≥ 1 cm in diameter. Positive control animals were administered dibenzopyrene at 0.025 mg/mouse plus croton oil at 0.1 mg/mouse. Animals were sacrificed at 29 to 31 weeks and histopathology was performed.

    The positive control induced tumors as expected in Groups A and B, but not Group C. No carcinogenic effects were observed in any animals treated with methylparaben in Groups A,

[9] Throughout this GreenScreen®, only studies with sufficient details and reliability ratings (Klimisch 1, reliable without restriction, or Klimisch 2, reliable with restrictions) are included in this assessment, unless noted otherwise.

B, or C. Authors of the REACH dossier reported that the test substance was not carcinogenic under the conditions of the test (Klimisch 2, reliable with restrictions) (Unnamed 1968 publication).

- ECHA 2023c[9]
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a non-guideline study (GLP not specified) examining the induction of lesions of the forestomach, glandular stomach, and urinary bladder in hamsters. Fifteen male Syrian hamsters were administered the test substance (> 99.8% purity) in the feed (no vehicle) at 3% for 20 weeks (equivalent to 1,009.6 – 2,163.5 mg/kg/day, based on average body weight of 208 g and average daily food intake of 7-15 g). Animals were sacrificed at the end of the exposure period, and the liver and kidney weights were determined, and five sections from each animal were cut from the anterior and posterior walls of the forestomach, two from the glandular stomach, and four from the urinary bladder. Sections were stained for analysis of the labelling index. Counts were made on 4,000 cells of urinary bladder epithelium, 3,000 cells of pyloric gland epithelium (1000 cells each of the fundic side, middle portion and pyloric side), and 2,000 basal cells of the forestomach epithelium (1,000 cells each from regions proximal to the fundic gland of the greater curvature and of the lesser curvature of the anterior wall). The labelling index was expressed as the number of labelled cells per 100 cells. There were no mortalities during the treatment period, and no significant effect on body or liver weights in treated animals compared to controls. There were no findings of papillomatous lesions. No significant inflammation, hyperplasia, or tumorous lesions were identified in the urinary bladder. Labelling indices of the forestomach and pyloric region in treated animals was comparable to controls. The labelling index was significantly ($p < 0.05$) increased in the urinary bladder to 0.52 ± 0.18 for the treated group, compared to 0.08 ± 0.14 in the control animals, however, there were no corresponding histopathological findings (Klimisch 2, reliable with restrictions) (Hirose et al. 1986).
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a pre-GLP, pre-guideline, chronic oral repeated dose toxicity study. Male and female Mongrel dogs (negative control = 2 animals; 0.5 g/kg/day = 1 animal; 1.0 g/kg/day = 3 animals (sex not reported)) received 0, 0.5, or 1.0 g/kg/day (0, 500, and 1,000 mg/kg/day) propylparaben (purity not reported) in gelatin capsules 6 days per week. Negative control animals were treated for 195 and 422 days; the low dose animal was treated for 394 days; and the high dose animals were treated for 313 – 394 days. Animals were examined for clinical signs, body weight, and changes in blood and urine parameters. Pathology and histopathology was performed at termination of the study. Histopathological analysis focused on the kidney, liver, heart, lung, spleen, and pancreas. One control animal died after 195 days of pneumonia. Treatment had no effect on clinical signs, body weight and weight gain, hematology, urine parameters, gross pathology, or histopathology. The study authors identified a NOAEL of 1 g/kg/day (1,000 mg/kg/day; equivalent to 857 mg/kg/day after adjustment for a 7-day treatment period[10]), the highest dose tested (Klimisch 2, reliable with restrictions) (Matthews et al. 1956).
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a non-guideline study (GLP not specified) examining the induction of lesions of the forestomach and glandular stomach in rats. Five male Fischer 344 rats were administered the test substance (> 99.8% purity) in the feed (no vehicle) at 3% for 8 weeks (equivalent to 1,883.96 – 4,150.38 mg/kg/day, based on average body weight of 133 and 293 g, and average daily food intake of 18.4 g/rat). At week 8, the rats were injected i.p. with 100 mg/kg of bromodeoxyuridine (BrdU), 1 hour prior to sacrifice. Histopathological examination was performed on five

[10] 1,000 mg/kg/day * 6 days/7 days = 857 mg/kg/day

strips of forestomach tissue, and four strips of glandular stomach tissue. The numbers of cells incorporating BrdU into DNA per 2,000 basal cells of the forestomach (1,000 cells each from regions proximal to the fundic gland of the greater curvature and of the lesser curvature wall) and 1,000 cells of pyloric gland epithelium (pyloric side) were counted. The heights of pyloric glands were determined and the average numbers of pyloric gland epithelial cells comprising one crypt were calculated for each group. There were no mortalities during the exposure period. There were no significant effects on body weights, food and water consumption, histopathology and labeling indices, and no proliferative lesions in treated animals compared to controls (Klimisch 2, reliable with restrictions) (Shibata et al. 1990).

- *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a pre-GLP, pre-guideline, chronic oral repeated dose toxicity study. Male and female Wistar rats (6/sex/dose) were exposed to 0, 2, or 8% propylparaben (equivalent to 0, 0.9-1.2, and 5.5-5.9 g/kg/day[11]) in their diet for 96 weeks. Animals were examined for clinical signs, body weight, and changes in blood and urine parameters. Pathology and histopathology was performed at termination of the study. Histopathological analysis focused on the kidney, liver, heart, lung, spleen, and pancreas. Animals treated with 8% propylparaben had a slower rate of weight gain compared to control animals, which was more apparent in the early part of the study. By the end of the study, these effects were no longer apparent. Decreased weight gain was more apparent in male rats compared to females. No other treatment-related effects were reported. Histopathological examination found no abnormalities. The study authors identified a NOAEL of 8% propylparaben (equivalent to 5.5-5.9 g/kg/day or 5,500 – 5,900 mg/kg/day) (highest dose tested) (Klimisch 2, reliable with restrictions) (Matthews et al. 1956).
- *Transplacental: Surrogate Propylparaben:* Propylparaben was evaluated for carcinogenicity in a non-guideline transplacental assay, and a newborn assay (Odashima 1976).
  - In the transplacental assay, pregnant rodents (strain not reported) were administered the maximum dose which did not cause abortion or early death of neonates (dose not reported). Animals (number not reported) were treated every other day for 5 days during gestation days 15 through 19. Offspring were observed for 1 year after birth for tumor development. Authors concluded that propylparaben was not carcinogenic. No further details were provided.
  - In the newborn assay, rodent pups (strain not reported) were administered four subcutaneous injections of propylparaben (total dose = LD_{20}; dose not reported) on post-natal days (PND) 1, 8, 15, and 22. Animals (number not reported) were observed for 1 year after birth for tumor development. Authors concluded that propylparaben was not carcinogenic. No further details were provided.
- *Oral: Surrogates Butylparaben and isobutylparaben*: Male and female 8-week old ICR/Jcl mice (50/sex/group) were administered 0.15%, 0.3% or 0.6% butylparaben or isobutylparaben in their feed for 102 weeks. Animals surviving until the end of the study were sacrificed and necropsied. Data were compiled for animals surviving ≥ 78 weeks. Treatment did not significantly alter the incidence of tumors or the time to tumor development between treated mice and controls, or between different dose groups. Authors concluded that butylparaben and isobutylparaben were not carcinogenic under the conditions of this assay (Inai et al. 1985).

- CIR 2008

[11] Values reported in the ECHA REACH Dossier

  - "Ethylparaben, propylparaben, and butylparaben in the diet produced cell proliferation in the forestomach of rats, with the activity directly related to chain length of the alkyl chain, but isobutylparaben and butylparaben were noncarcinogenic in a mouse chronic feeding study. Methylparaben was non carcinogenic when injected subcutaneously in mice or rats, or when administered intravaginally in rats, and was not cocarcinogenic when injected subcutaneously in mice. Propylparaben was noncarcinogenic in a study of transplacental carcinogenesis."
- CIR 2020 – no new data were identified.
- SCCP 2005a
  - Parabens are not carcinogenic or co-carcinogenic.
- Darbre and Harvey 2008
  - Discussion of the possible role of parabens in breast cancer was sparked in 2004 when methylparaben, ethylparaben, propylparaben, and isobutylparaben were measured in human breast cancer tissue (Darbre et al. 2004). The Scientific Committee for Consumer Products (SCCP) (2005b) reviewed the available data and concluded that there was no evidence that demonstrated a risk of developing breast cancer with the use of 'underarm' cosmetics.
- HSDB 2017
  - A population-based, case-control, epidemiological study was performed to assess the carcinogenicity of paraben-containing (specific paraben not specified) underarm deodorant. Patients aged 20-74 (n=813) who developed breast cancer, and control subjects also aged 20-74 (n=793), were randomly assigned to frequency-matched 5-year age groups. Product use information was obtained by in-person interviews. The risk for breast cancer was not increased with application of antiperspirant or deodorant, or among those who shaved with a blade razor, or among those who applied the products within 1 hour of shaving. Authors concluded the results do not suggest that antiperspirant use increases the risk of breast cancer.

Mutagenicity/Genotoxicity (M) Score (H, M, or L): L

Methylparaben is assigned a score of Low for mutagenicity/genotoxicity based on the weight of evidence including numerous negative *in vitro* and *in vivo* mutagenicity and clastogenicity studies on the target compound. Although one *in vitro* study reported weak evidence of clastogenicity, the study is unreliable, and a higher reliability GLP-compliant, OECD guideline, *in vivo* micronucleus test demonstrated lack of clastogenicity. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high based on the overall weight of evidence from multiple reliable studies on the target compound.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - *In vitro:* Methylparaben was not mutagenic in numerous bacterial reverse mutation assays (Ames assays) at concentration ranging from 50 µg/plate to 10 mg/plate using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and TA1538. No increase in the number of revertants was seen in any of the bacterial strains in the presence or absence of metabolic activation.
  - *In vitro:* Methylparaben was clastogenic in an *in vitro* chromosome aberration test conducted according to OECD TG 473 (pre-GLP). Chinese hamster lung fibroblasts (V79) were exposed to methylparaben in ethanol or dimethyl sulfoxide at a concentration of 125

μg/mL with and without metabolic activation. No increase in the frequency of chromosome aberrations was observed with treatment in the absence of metabolic activation. A slight increase (in the range of 5-9.9%) in chromosome aberrations was observed in the presence of the S9 mix. The positive control substance was benzo(a)pyrene, which performed as expected. Authors concluded that methylparaben is non-clastogenic without metabolic activation but slightly clastogenic with metabolic activation (Klimisch 2, reliable with restrictions) (Unnamed 1978 study). *ToxServices notes numerous reporting and possibly methodological deficiencies for this study. Specifically, it is not clear why only one concentration was tested, which does not allow for observations of a dose-response, the reason for the chosen test substance concentration is not reported, cytotoxicity is not reported, and statistical significance for the positive findings are not reported relative to concurrent and/or historical controls. Due to these deficiencies, ToxServices considers this study summary unreliable and discounted it in the weight of evidence.*

- *In vitro:* Methylparaben was not mutagenic in a GLP-compliant *in vitro* mammalian cell gene mutation test performed according to OECD TG 476. Chinese hamster ovary (CHO) cells were administered methylparaben (99.8% purity) in DMSO, with and without metabolic activation (sodium phenobarbitone and β-naphthoflavone induced rat liver homogenate) at concentrations of 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL. The positive control substances were 4-nitroquinoline-N-oxide and benzo(a)pyrene. The highest concentration tested corresponded with the recommended dose limit, did not result in precipitation or change in pH, and was slightly cytotoxic based on 71.91 to 86.21% relative survival. There were no statistically significant increases in the number of mutant colonies at any concentration tested, with or without activation, compared to controls (Klimisch 1, reliable without restriction) (Unnamed 2019 study). *The summary in the REACH dossier does not specify which gene (Hprt or gpt) was tested, however, this deficiency does not affect the reliability of the study.*
- *In vivo:* Methylparaben was not mutagenic in a non-GLP, *in vivo* dominant lethal assay conducted according to OECD TG 478. Male Sprague-Dawley rats (10/sex/group) were administered 0, 50, 500, or 5,000 mg/kg methylparaben (purity not reported) in 0.85% saline via gavage. In the acute study, animals received a single dose, and in the subacute study animals were treated once per day on five consecutive days. Following treatment males were sequentially mated with 2 females per week for 8 weeks (acute study), or 7 weeks (subacute study). Females were sacrificed 14 days after separating from the treated male. At necropsy the uterus was examined for corpora lutea, early fetal deaths, late fetal deaths, and total implantations. Saline and triethylene melamine (TEM) were used as the negative and positive controls, respectively, and provided the expected results. No treatment-related effects were found. Authors concluded methylparaben was not mutagenic under the conditions of this assay (Klimisch 1, reliable without restriction) (Unnamed 1974 study).
- *In vivo:* Methylparaben was not clastogenic in a pre-GLP *in vivo* mammalian bone marrow chromosome aberration test conducted similar to OECD TG 475. Male Sprague-Dawley rats were administered 0, 5, 50, or 500 mg/kg methylparaben (purity not reported) in 0.85% saline via oral gavage. Animals (10/dose) received a single oral dose (acute study) or were treated once per day on five consecutive days. Animals were sacrificed 6, 24, or 48 hours after the single administration. Methylparaben treatment did not significantly alter the incidence of bone marrow cells with chromosomal aberrations. Saline and TEM were the negative and positive controls, respectively, and provided the expected results. There were no indications of toxicity reported, and no effect on mitotic index. The top dose was based on toxicity from a range-finding study in which deaths occurred at doses ≥ 1,000 mg/kg.

Authors concluded that methylparaben was not clastogenic under the conditions of this assay (Klimisch 2, reliable with restrictions) (Unnamed 1974 study).

- ECHA 2018
    - In its assessment on the dossier for methylparaben, ECHA identified a data gap for mutagenicity and specifically suggested performance of an *in vitro* gene mutation study in mammalian cells, such as OECD TG 476 or OECD TG 490. *Although ECHA has not re-evaluated the data, ToxServices suggests this request has been fulfilled by the OECD TG 476 study (Unnamed 2019) summarized above.*
- Prival et al. 1991, as cited in CCRIS 1992
    - *In vitro:* Methylparaben was not mutagenic in the bacterial reverse mutation assay in *S. typhimurium* TA98, TA100, TA1535, and TA1537, and in *Escherichia coli* WP_2 at concentrations up to 10 mg/plate in DMSO, with and without metabolic activation (rat liver S-9, Aroclor 1254), using the standard plate method (no further details provided).
- CIR 2008
    - Numerous genotoxicity studies, including Ames testing, dominant lethal assay, host-mediated assay, and cytogenic assays, suggest the parabens are generally non-mutagenic, although ethylparaben and methylparaben did increase chromosomal aberrations in an *in vitro* CHO cell assay.
- CIR 2020
    - *In vitro:* Methylparaben was evaluated in a non-guideline *in vitro* study in human spermatozoa exposed at 2.5 and 13 mM for 2 or 5 hours (Samarasinghe et al. 2018).
        - There was no significant effect on DNA fragmentation as measured by the TUNEL and sperm chromatin dispersion assays in human spermatozoa exposed to methylparaben at 13 mM.
        - A statistically significant decrease in spermatozoa motility was observed after 2 and 5 hours of exposure.
        - After 5 hours of exposure, significant increases were observed in Annexin V and fluorescently labeled inhibitor of caspase assay signals, mitochondrial and total superoxide generation, and 8-hydroxy-2'-deoxyguanosine (8OHdG) production.
        - At 2.5 mM for 5 hours, there were no significant changes in motility, vitality, mitochondrial reactive oxygen species (ROS) production, and 8OhdG formation.

      ToxServices notes that as this study was non-guideline, there is no discussion of concurrent or historical control values, and there is no indication of method validation, the study is included for completeness but the significance of the findings is unknown and this study is not included in the weight of evidence.
    - *In vitro: Surrogate Propylparaben:* Propylparaben was evaluated in a non-guideline *in vitro* study in Vero cells from the African green monkey kidney. The study summary suggests an effect on cell cycle arrest at the G0/G1 phase and a resulting statistically significant, dose-dependent decrease in percentage of mitotic cells (Perez et al. 2010). *ToxServices notes that as this study was non-guideline, there is no discussion of concurrent or historical control values, and there is no indication of method validation, the study is included for completeness but the significance of the findings is unknown and this study is not included in the weight of evidence.*
    - A mixture of methylparaben, ethylparaben, propylparaben, and butylparaben was evaluated in a non-guideline *in vitro* study in human spermatozoa (Samarasinghe et al. 2018).
        - A statistically significant decrease in spermatozoa motility was observed immediately after the treatment and was further exacerbated after 24 hours at concentrations of 1, 2, and 4 mM.

- After 24 hours the spermatozoa treated with 0.2 and 1 mM of the paraben mixture exhibited increased mitochondrial ROS which then declined with decreased cell viability.
- Acute total superoxide response was observed with dihydroethidium shortly after exposure to the parabens and was statistically significant at 2 and 4 mM.
- Caspase activation was observed at ≥ 1 mM of the paraben mixture and increased further at 24 hours.

ToxServices notes that as this study was non-guideline, there is no discussion of concurrent or historical control values, and there is no indication of method validation, the study is included for completeness but the significance of the findings is unknown and this study is not included in the weight of evidence.

Reproductive Toxicity (R) Score (H, M, or L): L

Methylparaben is assigned a score of Low for reproductive toxicity based on the lack of reproductive toxicity in a GLP-compliant, extended one-generation reproductive toxicity study performed according to OECD TG 443, in rats exposed at up to 1,000 mg/kg/day orally. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high based on high quality data for the target compound.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - *Oral:* Methylparaben was evaluated in a GLP-compliant extended one-generation reproductive toxicity study with F2 generation and developmental neurotoxicity (Cohorts 1A, 1B with extension, 2A and 2B), performed according to OECD TG 443. Wistar rats were administered methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg. Parental (P1) males were dosed from 14 days pre-mating, through mating, and until terminal sacrifice, for a total of 10 weeks. P1 females were dosed from 14 days pre-mating, through mating, and gestation, and until weaning on PND 21, for a total of 8-10 weeks. Pups were dosed from weaning on PND 22. In addition to standard parameters, pups were assessed for developmental neurotoxicity (Cohort 2 for auditory startle, functional observational battery, motor activity, and neuropathology assessments; Cohort 4 for learning and memory on PND 38-39), and developmental immunotoxicity (Cohort 3, using a T-cell dependent antibody response assay). There were no significant findings based on clinical observations, mortality, body weight and weight changes, hematology, clinical chemistry, urinalysis, organ weights, or histopathology for any generation. There were no significant findings based on reproductive function, including estrus cycles and sperm measures. There were no significant findings based on developmental toxicity, including developmental neurotoxicity, and developmental immunotoxicity parameters. The systemic toxicity, reproductive toxicity, and developmental toxicity NOAELs are reported at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2019 study).
  - *Oral:* Methylparaben was evaluated in a GLP-compliant combined repeated dose toxicity study with a reproductive and developmental screening test performed according to OECD TG 422. Wistar rats (Crl: WI(Han) (Full Barrier)) were administered methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg/day, 7 days/week (10/sex/dose). Females were exposed from 14 days pre-mating, during mating,

gestation, and up to PND 12, for up to 63 days total. Males were exposed for 2 weeks pre-mating, during mating, and after mating for a total of 28 days. Clinical signs of toxicity included moving the bedding (9/10 males and 10/10 females at 1,000 mg/kg/day). Increased salivation was noted in 1/10 females at 300 mg/kg, and 5/10 females at 1,000 mg/kg. As both clinical signs were in close approximation to dosing, or in anticipation thereof, investigators considered these to be indications of discomfort or local reactions as opposed to systemic effects. Piloerection was observed in control, low, mid, and high dose females at 4/10, 4/10, 8/10, and 7/10, and authors considered the findings to be not test item-related based on high incidence in control animals and lack of dose response. There were no mortalities in treated animals or their pups, and there were 2 mortalities in control pups that were considered incidental. There were no significant findings based on body weight and weight changes, food consumption, hematology, clinical chemistry, behavior, organ weights, gross pathology, or histopathology in any exposed group compared to controls. There were no significant findings based on reproductive or developmental endpoints, including estrous cycle, copulation, fertility and delivery indices, number of corpora lutea, implantation sites and live pups, pre- and post-implantation loss, number of male and female pups, sex ratio, still births, runts, litter weight data, anogenital distance, nipple retention, and external abnormalities. Thyroid hormone thyroxine (T4) was slightly (magnitude not specified) and statistically significantly lower in treated males compared to controls but there was no corresponding pathological finding in the thyroid or parathyroid and authors did not consider it adverse. The NOAEL for systemic, reproductive, and developmental toxicity was assigned at 1,000 mg/kg, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2018 study).

- *Oral:* Methylparaben was evaluated in a GLP-compliant repeated dose toxicity study conducted according to OECD TG 407, EU Method B.7, and OPPTS 870.3050. Wistar rats (5/sex/dose, plus an extra 5/sex for the control and high dose groups for a 14-day recovery period) were administered methylparaben (purity > 99%) by gavage in propylene glycol at 0, 50, 250, and 1,000 mg/kg/day for 28 days (25/sex/group at the low- and mid-dose, and 30/sex/group for controls and the high-dose). Testis and ovary weights were measured and these organs were subject to gross and histopathological examinations, including ovarian follicle counts and staging of spermatogenesis. No adverse effects were seen on reproductive organs and estrus cycle (Klimisch 1, reliable without restriction) (Unnamed 2009 study).
- *Oral:* Methylparaben was evaluated in a non-GLP, non-guideline subchronic feeding study in rats. Male Crj:Wistar rats were administered methylparaben (purity 99.9%) in the diet at 0.1 or 1.0%, equivalent to 102 or 1,030 mg/kg/day, respectively, for 56 days (8/dose). At the end of 8 weeks, the rats were sacrificed and the weights of the testes, epididymides, prostates, seminal vesicles and preputial glands were determined. There were no treatment-related effects on body weights and the absolute and relative organ weights. Additionally, the test substance did not exhibit anti-spermatogenic effects or elicit changes in testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels. The NOEL is reported at 1.0% (equivalent to 1,030 mg/kg/day as calculated by the study authors) (Klimisch 2, reliable with restrictions) (Unnamed 2004 study).
- *Oral:* Methylparaben was evaluated in a GLP-compliant, non-guideline study in male Crj.(WI)BR rats. Animals were exposed to methylparaben (99.9% purity) in the diet at 0, 100, 1,000, or 10,000 ppm, equivalent to 0, 11.2, 110.0, or 1,141.1 mg/kg/day (16/dose), beginning at 21 days postpartum for at least 56 days. Animals were evaluated for clinical signs of toxicity, body weight, and food consumption. In addition, reproductive organs from all rats, as well as the liver, thyroid and pituitary glands were weighed and histological

examination performed. Sperm evaluations were conducted to determine sperm concentration, motility and morphology and a detailed quantitative examination of the testes was performed, taking into account the tubular stages of the spermatogenic cycle. There were no treatment-related effects on any of the reproductive parameters measured (histopathology of reproductive organs and sperm analysis). Although there was a statistically significant reduction in the number of normal sperms and increase in the number of abnormal sperms (mostly no heads) at 1,000 ppm and 10,000 ppm, study authors did not consider this effect treatment related due to lack of dose-dependency. Authors established a NOAEL of 1,141 mg/kg/day for general toxicity, including histopathology of reproductive organs and sperm analysis; which was the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2005 study).

- CIR 2020
  - *Oral*: Several parabens were assessed for reproductive and developmental effects in a non-guideline study in prepubertal rats (Vo et al. 2010). Methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, and isobutylparaben were administered to groups of prepubertal Sprague Dawley rats (8 weeks old) at 0, 62.5, 250, or 1,000 mg/kg by gavage in corn oil once per day (10/group) on PND 21 to 40. EE was used as a positive control administered at 1 mg/kg/day. All rats were sacrificed at 24 hours following the final exposure.
    - A statistically significant delay in vaginal opening was observed in rats exposed to methylparaben at 1,000 mg/kg, and to isopropylparaben at ≥ 250 mg/kg, whereas there was a statistically significant accelerated date of vaginal opening for the positive control animals. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens based on lack of reproducibility with ethylparaben, propylparaben, and butylparaben.*
    - At 1,000 mg/kg, there was a statistically significant decrease in ovary weights for rats exposed to methylparaben and isopropylparaben; decreased kidney weights in rats exposed to ethylparaben and isopropylparaben; increases in adrenal gland weights in rats exposed to methylparaben, ethylparaben, and propylparaben, and increases in thyroid gland weights in rats exposed to methylparaben. Liver weights were increased for all doses of rats exposed to butylparaben. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there was no mention of corresponding pathological effects.*
    - Decreased number of corpora lutea with increased number of cystic follicles and thinning of the follicular epithelium was observed in the ovaries of rats (test substance(s) and dose(s) not specified). Myometrial hypertrophy in the uterus was identified in rats exposed to propylparaben and isopropylparaben at 1,000 mg/kg, and in rats exposed to butylparaben and isobutylparaben at ≥62.5 mg/kg. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens.*
    - Serum estradiol concentrations were significantly reduced in rats exposed to ethylparaben and isopropylparaben at 1,000 mg/kg, and prolactin concentrations were increased in rats exposed to methylparaben at 1,000 mg/kg. *ToxServices notes the severity and biological significance of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens.*

- Serum concentrations of T4 were statistically significantly reduced in rats exposed to methylparaben at 1,000 mg/kg, propylparaben and isopropylparaben at ≥ 250 mg/kg, and isobutylparaben, propylparaben, and isopropylparaben at ≥ 62.5 mg/kg. The IC50 (the concentration causing 50% inhibition activity) values for affinity to ERα and ERβ range from 2.07E-6 to 5.55E-5 in the following order: isobutylparaben > butylparaben > isopropylparaben = propylparaben > ethylparaben (the value for methylparaben was not reported); comparatively, the IC50 for 17 β-estradiol was approximately 3E-9. *ToxServices suggests these effects indicate the parabens have very weak affinity for ERα and Erβ.*

ToxServices notes the observations of myometrial hypertrophy in the uterus, and reduced concentrations of serum T4, are of questionable toxicological significance particularly as the study was not guideline, has limited reporting (e.g., severities are not reported), and there do not appear to be any corresponding pathological effects. Furthermore, these effects have not been reproduced in the other more comprehensive guideline reproductive toxicity studies.

- *Oral:* Methylparaben was evaluated in a non-guideline reproductive toxicity study. Groups of nulli-parous and parous Sprague-Dawley rats were exposed to methylparaben to examine effects on the mammary glands. The start of dosing was not specified for F0 animals, but they were dosed through lactation, therefore, F1 animals were exposed through lactation. After weaning on lactation day (LD) 28, F1 offspring were divided into 2 groups – nulliparous and parous, and were exposed orally through PND 181 (10 rats/group) at 0 or 0.105 mg/kg in olive oil by gavage. Parous F1 females were mated on PND97 and exposed through pregnancy and lactation of the F2 pups. Nulliparous females were exposed through PND 181. There was a statistically significant increase in the number of pups born to treated F1 females compared to controls. F2 pups had increased mortality at PND 7 and thereafter compared to controls. All non-parous F1 females exhibited normal mammary tissue morphology. **In treated parous F1 females, during lactation the mammary alveoli were not always milk-filled, increase in adipose tissue was noted, and collapsed alveolar and duct structures showed residual secretory content. Microscopic examination showed decreased lobular structures in treated F1 females compared to controls.** There were no significant findings based on histopathology of treated animals compared to controls (Manservisi et al. 2015). *ToxServices notes the severity, biological, and statistical significance of these findings are not discussed in the CIR report, and this was a non-guideline study with only one dose. Therefore, while the study suggests an effect on F2 pup mortality, and effects on lactation, the study is of low reliability. Furthermore, the effects were not reproduced in the previously summarized GLP-compliant extended one-generation reproductive toxicity study with F2 generation and developmental neurotoxicity performed according to OECD TG 443 (Unnamed 2019 as summarized in ECHA 2023a). Therefore, this study is included for completeness, but is not included in the weight of evidence.*

- ECHA 2018
  - In its assessment on the dossier for methylparaben, ECHA identified concerns for reproductive toxicity based on the previously reported reduction of normal sperm count and increased percentage of abnormal sperm in the 56-day repeated dose toxicity study from 2005. Although this effect was not dose-dependent, ECHA was concerned that both groups (1,000 and 10,000 ppm) had similar effects that were statistically significant compared to concurrent controls. In response, ECHA suggested performance of an extended one-generation reproductive toxicity study such as EU B.56 / OECD TG 443. *Although ECHA has not re-evaluated the data, ToxServices suggests this request has been fulfilled by the*

OECD TG 443 study (Unnamed 2019) summarized above, in which the effects were not reproduced.

- In its assessment on the dossier for methylparaben, ECHA identified concerns for reproductive toxicity based on the previously reported effects on serum levels of estradiol and T4, potential impact on the ovaries, and delayed vaginal opening (Vo et al. 2010). In response, ECHA suggested performance of an extended one-generation reproductive toxicity study such as EU B.56 / OECD TG 443. *Although ECHA has not re-evaluated the data, ToxServices suggests this request has been fulfilled by the OECD TG 443 study (Unnamed 2019) summarized above, in which the effects were not reproduced.*

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): L

Methylparaben is assigned a score of Low for developmental toxicity based on lack of indications of developmental toxicity in numerous prenatal developmental toxicity studies in several species, and lack of indications of developmental toxicity and/or developmental neurotoxicity an extended one-generation reproductive toxicity study performed to OECD TG 443, in rats orally exposed at up to 1,000 mg/kg/day. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high based on high quality data for the target compound.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9] (note developmental toxicity studies in zebrafish embryos are assessed under chronic aquatic toxicity)
  - Methylparaben was evaluated in a non-GLP compliant prenatal developmental toxicity study performed in a manner equivalent or similar to OECD TG 414. Dutch-belted rabbits (≥9/group) were administered 3, 14, 65, or 300 mg/kg/day methylparaben (purity not reported) via gavage on gestation days 6 through 18. On gestation day 29, animals were subject to a cesarean section and reproductive parameters and dead fetuses were evaluated. Pup body weight was recorded. Pups were evaluated for external abnormalities, visceral abnormalities, and skeletal defects. Treatment had no effect on the sex ratio or fetal body weight. The study authors found no visceral abnormalities or skeletal defects. The study authors identified a developmental NOAEL of 300 mg/kg/day (highest dose tested) (Klimisch 2, reliable with restrictions based body weights being recorded every 6 days instead of daily, and use of only 12 dams, compared to the guideline recommended minimum of 16 dams with implantation sites) (Unnamed 1973 study).
  - Methylparaben was evaluated in a pre-GLP, prenatal developmental toxicity study performed in a manner equivalent or similar to OECD TG 414. Female Wistar rats (≥ 23/dose) were administered methylparaben (purity not specified) by gavage in water at 0, 5.5, 25.5, 118, or 550 mg/kg/day, on gestation days 6 through 15. Dams were monitored daily for changes in clinical signs and mortality. Dam body weight was measured on days 0, 6, 11, 15, and 20. On day 20 all dams were subjected to a cesarean section and reproductive parameters and the number of live and dead fetuses were recorded. The urogenital tract of each dam was examined in detail for anatomical normality. All pups were weighed and evaluated for external abnormalities. One-third of the pups underwent a detailed visceral examination under 10x magnification and the remaining two-thirds of the pups were examined for skeletal defects. Treatment did not alter maternal or fetal body weight, or sex ratio. There were no treatment-related increases in skeletal findings or soft tissue

abnormalities. The study authors identified a developmental NOAEL of 550 mg/kg/day (highest dose tested) (Klimisch 2, reliable with restrictions) (Unnamed 1972 study).

- Methylparaben was evaluated in a pre-GLP, prenatal developmental toxicity study performed in a manner equivalent or similar to OECD TG 414. Female CD-1 mice (≥ 21/dose) were administered methylparaben (purity not specified) by gavage in water at 0, 5.5, 25.5, 118, or 550 mg/kg/day, on gestation days 6 through 15. Dams were monitored daily for changes in clinical signs and mortality. Dam body weight was measured on days 0, 6, 11, 15, and 17. On day 17 all dams were subjected to a cesarean section and reproductive parameters and the number of live and dead fetuses were recorded. The urogenital tract of each dam was examined in detail for anatomical normality. All pups were weighed and evaluated for external abnormalities. One-third of the pups underwent a detailed visceral examination under 10x magnification and the remaining two-thirds of the pups were examined for skeletal defects. Treatment did not alter maternal or fetal body weight, or sex ratio. There were no treatment-related increases in skeletal findings or soft tissue abnormalities. The study authors identified a developmental NOAEL of 550 mg/kg/day (highest dose tested) (Klimisch 2, reliable with restrictions) (Unnamed 1972 study).
- Methylparaben was evaluated in a pre-GLP, prenatal developmental toxicity study performed in a manner equivalent or similar to OECD TG 414. Female golden hamsters (strain not specified) (≥ 21/dose) were administered methylparaben (purity not specified) by gavage in water at 0, 3.0, 14.0, 65.0, and 300.0 mg/kg/day, on gestation days 6 through 10. Dams were monitored daily for changes in clinical signs and mortality. Dam body weight was measured on days 0, 8, 10, and 14. On day 14 all dams were subjected to a cesarean section and reproductive parameters and the number of live and dead fetuses were recorded. The genital tract of each dam was examined in detail for anatomical normality. All pups were weighed and evaluated for external abnormalities. One-third of the pups underwent a detailed visceral examination under 10x magnification and the remaining two-thirds of the pups were examined for skeletal defects. Treatment did not alter maternal or fetal body weight, or sex ratio. There were no treatment-related increases in skeletal findings or soft tissue abnormalities. The study authors identified a developmental NOAEL of 300 mg/kg/day (highest dose tested) (Klimisch 2, reliable with restrictions) (Unnamed 1972 study).
- Methylparaben was evaluated in the previously summarized GLP-compliant extended one-generation reproductive toxicity study with F2 generation and developmental neurotoxicity (Cohorts 1A, 1B with extension, 2A and 2B), performed according to OECD TG 443. Wistar rats were administered methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg. Parental (P1) males were dosed from 14 days pre-mating, through mating, and until terminal sacrifice, for a total of 10 weeks. P1 females were dosed from 14 days pre-mating, through mating, and gestation, and until weaning on PND 21, for a total of 8-10 weeks. Pups were dosed from weaning on PND 22. In addition to standard parameters, pups were assessed for developmental neurotoxicity (Cohort 2 for auditory startle, functional observational battery, motor activity, and neuropathology assessments; Cohort 4 for learning and memory on PND 38-39), and developmental immunotoxicity (Cohort 3, using a T-cell dependent antibody response assay). There were no significant findings on developmental toxicity, including developmental neurotoxicity, and developmental immunotoxicity parameters. The developmental toxicity NOAEL was assigned at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2019 study).
- Methylparaben was evaluated in the previously summarized GLP-compliant combined repeated dose toxicity study with a reproductive and developmental screening test performed

according to OECD TG 422. Wistar rats (Crl: WI(Han) (Full Barrier)) were administered methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg/day, 7 days/week (10/sex/dose). Females were exposed from 14 days pre-mating, during mating, gestation, and up to PND 12, for up to 63 days total. Males were exposed for 2 weeks pre-mating, during mating, and after mating for a total of 28 days. There were no mortalities in treated animals or their pups, and there were 2 mortalities in control pups that were considered incidental. There were no significant findings on developmental endpoints, including number of live pups, pre- and post-implantation loss, number of male and female pups, sex ratio, still births, runts, litter weight data, anogenital distance, nipple retention, and external abnormalities**.** The NOAEL for developmental toxicity was 1,000 mg/kg, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2018 study).

- CIR 2020
    - As noted previously, several parabens were assessed for reproductive and developmental effects in a non-guideline study in prepubertal rats (Vo et al. 2010). Methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, and isobutylparaben were administered to groups of prepubertal Sprague Dawley rats (8 weeks old) at 0, 62.5, 250, or 1,000 mg/kg by gavage in corn oil once per day (10/group) on PND 21 to 40. EE was used as a positive control administered at 1 mg/kg/day. All rats were sacrificed at 24 hours following the final exposure.
        - A statistically significant delay in vaginal opening was observed in rats exposed to methylparaben at 1,000 mg/kg, and to isopropylparaben at ≥ 250 mg/kg, whereas there was a statistically significant accelerated date of vaginal opening for the positive control animals. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens based on lack of reproducibility with ethylparaben, propylparaben, and butylparaben.*
        - At 1,000 mg/kg, there was a statistically significant decrease in ovary weights for rats exposed to methylparaben and isopropylparaben; decreased kidney weights in rats exposed to ethylparaben and isopropylparaben; increases in adrenal gland weights in rats exposed to methylparaben, ethylparaben, and propylparaben, and increases in thyroid gland weights in rats exposed to methylparaben. Liver weights were increased for all doses of rats exposed to butylparaben. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there was no mention of corresponding pathological effects.*
        - Decreased number of corpora lutea with increased number of cystic follicles and thinning of the follicular epithelium was observed in the ovaries of rats (test substance(s) and dose(s) not specified). Myometrial hypertrophy in the uterus was identified in rats exposed to propylparaben and isopropylparaben at 1,000 mg/kg, and in rats exposed to butylparaben and isobutylparaben at ≥62.5 mg/kg. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens.*
        - Serum estradiol concentrations were significantly reduced in rats exposed to ethylparaben and isopropylparaben at 1,000 mg/kg, and prolactin concentrations were increased in rats exposed to methylparaben at 1,000 mg/kg. *ToxServices notes the severity and biological significance of these observations relative to the negative*

and positive controls was not reported, and there does not appear to be any particular trend among the parabens.

- Serum concentrations of T4 were statistically significantly reduced in rats exposed to methylparaben at 1,000 mg/kg, propylparaben and isopropylparaben at ≥ 250 mg/kg, and isobutylparaben, propylparaben, and isopropylparaben at ≥ 62.5 mg/kg. The IC50 values for affinity to ERα and ERβ range from 2.07E-6 to 5.55E-5 in the following order: isobutylparaben > butylparaben > isopropylparaben = propylparaben > ethylparaben (the value for methylparaben was not reported); comparatively, the IC50 for 17 β-estradiol was approximately 3E-9. *ToxServices suggests these effects indicate the parabens have very weak affinity for ERα and Erβ.*

ToxServices notes the observations of delayed vaginal opening are of questionable toxicological significance particularly as the study was not guideline, and has limited reporting (e.g., severities are not reported). Furthermore, these effects have not been reproduced in the other more comprehensive guideline reproductive and developmental toxicity studies.

- Methylparaben was evaluated in a non-guideline reproductive toxicity study. Groups of nulli-parous and parous Sprague-Dawley rats were exposed to methylparaben to examine effects on the mammary glands. The start of dosing was not specified for F0 animals, but they were dosed through lactation, therefore, F1 animals were exposed through lactation. After weaning on lactation day (LD) 28, F1 offspring were divided into 2 groups – nulliparous and parous, and were exposed orally through PND 181 (10 rats/group) at 0 or 0.105 mg/kg in olive oil by gavage. Parous F1 females were mated on PND97 and exposed through pregnancy and lactation of the F2 pups. Nulliparous females were exposed through PND 181. There was a statistically significant increase in the number of pups born to treated F1 females compared to controls. **F2 pups had increased mortality at PND 7 and thereafter compared to controls.** All non-parous F1 females exhibited normal mammary tissue morphology. **In treated parous F1 females, during lactation the mammary alveoli were not always milk-filled, increase in adipose tissue was noted, and collapsed alveolar and duct structures showed residual secretory content. Microscopic examination showed decreased lobular structures in treated F1 females compared to controls.** There were no significant findings based on histopathology of treated animals compared to controls (Manservisi et al. 2015). *ToxServices notes the severity, biological, and statistical significance of these findings are not discussed in the CIR report, and this was a non-guideline study with only one dose. Therefore, while the study suggests an effect on F2 pup mortality, and effects on lactation, the study is of low reliability. Furthermore, the effects were not reproduced in the previously summarized GLP-compliant extended one-generation reproductive toxicity study with F2 generation and developmental neurotoxicity performed according to OECD TG 443 (Unnamed 2019 as summarized in ECHA 2023a). Therefore, this study is included for completeness, but is not included in the weight of evidence.*

- ECHA 2018
  - In its assessment on the dossier for methylparaben, ECHA identified concerns for developmental toxicity, specifically sexual function and fertility, based on the previously reported delayed vaginal opening in prepubertal rats (Vo et al. 2010), noting the OECD TG 414 studies, although performed in multiple species, cannot suffice to address these concerns because the exposure period is limited to a period of gestation, and does not address peri- and post-natal developmental toxicity. In response, ECHA suggested performance of an extended one-generation reproductive toxicity study such as EU B.56 / OECD TG 443. *Although ECHA has not re-evaluated the data, ToxServices suggests this request has been*

fulfilled by the OECD TG 443 study (Unnamed 2019 study) summarized above, in which the effects were not reproduced.

Endocrine Activity (E) Score (H, M, or L): *M*

Methylparaben is assigned a score of Moderate for endocrine activity based on evidence of very weak endocrine activity in some *in vitro* and *in vivo* assays with no observations of related adverse health effects. GreenScreen® criteria classify chemicals as a Moderate hazard for endocrine activity when there is evidence of endocrine activity and no corresponding adverse health effects have been identified. It may be noted that the EU – Priority Endocrine Disruptors – Category 1 and TEDX ratings correspond with high or moderate hazard ratings (CPA 2018b). The confidence in the score is low because the level of endocrine activity across numerous assays is in every case extremely weak and may not be relevant to human health.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - EU – Priority Endocrine Disruptors – Category 1 – *In vivo* evidence of Endocrine Disruption Activity
    - TEDX – Potential Endocrine Disruptors – Potential Endocrine Disruptor
- ECHA 2023a[9] (Note additional studies examining endocrine effects on fish sexual development are available in the REACH dossier but were not included in this assessment as there are no corresponding guidelines with the GHS guidance (UN 2021), or the GreenScreen® guidance document (CPA 2018b), to assign a hazard rating)
  - *In vitro:* Methylparaben was evaluated in a non-guideline competitive binding assay performed to assess competitive inhibition of ^{3H}-Estradiol binding, and expression of estrogen-regulated genes in MCF7 human breast cancer cells (GLP compliance not specified). The cell cultures were pre-treated to deplete steroid hormone levels prior to harvesting. Methylparaben (99.0% purity) showed negligible estrogenic binding activity at 10^{-4}M, compared to 17β-estradiol which binds at 10^{-10}M, a difference of 500,000-fold. Authors concluded methylparaben had negligible antagonistic effect on estrogen binding in this study (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).
  - *In vitro:* Methylparaben was evaluated in a non-guideline competitive binding assay performed to assess competitive inhibition of methylparaben on estrogen receptor cells (GLP compliance not specified). Uteri from ovariectomized Sprague-Dawley rats were used as a source of estrogen receptors. Cells were administered 10 nM to 0.1 mM methylparaben (99% purity). Binding activity was very weak compared to the positive control, 17β-estradiol. The calculated IC50 was 0.25 mM for methylparaben, compared to 0.9 nM for 17 β-estradiol (Klimisch 2, reliable with restrictions) (Unnamed 1999 study).
  - *In vitro:* Methylparaben was evaluated in a study designed to assess estrogenic activity using a yeast two-hybrid assay incorporating either human or medaka estrogen receptor α (hERα and medERα), and by using hERα competitive enzyme-linked immunosorbent assay (ER-ELISA) (GLP compliance not specified). Methylparaben did not show any estrogenic properties in the yeast two-hybrid assay at up to 10,000 nM, or in the ER-ELISA assay at up to 38,000 nM (Klimisch 2, reliable with restrictions) (Unnamed 2008 study).
  - *In vitro:* Methylparaben was assessed for estrogenic activity against estrogen receptors α and β, using three reporter cell lines (HELN, HELN ERα, and HELN ERβ) generated from human cervical epithelioid carcinoma HeLa cells. Methylparaben did not show any estrogenic activity when applied to HELN, HELN ERα, and HELN ERß cells at up to 10 µM (Klimisch 2, reliable with restrictions) (Unnamed 2004 study).

- *In vivo:* Methylparaben was evaluated in the previously summarized GLP-compliant extended one-generation reproductive toxicity study with F2 generation and developmental neurotoxicity (Cohorts 1A, 1B with extension, 2A and 2B), performed according to OECD TG 443. Wistar rats were administered methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg. Parental (P1) males were dosed from 14 days pre-mating, through mating, and until terminal sacrifice, for a total of 10 weeks. P1 females were dosed from 14 days pre-mating, through mating, and gestation, and until weaning on PND 21, for a total of 8-10 weeks. Pups were dosed from weaning on PND 22. There were no significant findings based on T4 and TSH levels in parent animals, in F1 pups on PND 4 and 21, or in Cohort 1A animals, compared to controls. There were no significant findings based on reproductive function, including estrus cycles and sperm measures (Klimisch 1, reliable without restriction) (Unnamed 2019 study).
- *In vivo:* Methylparaben was evaluated in a uterotrophic bioassay performed according to OECD TG 440 (GLP compliance not specified). Female Alpk:AP rats were administered methylparaben (>99% purity) by gavage in arachis oil at 0, 40, 400, or 800 mg/kg, or subcutaneously at 0, 40, or 80 mg/kg (5/dose/group). In the mature group, rats were ovariectomized at 6-8 weeks of age, and were maintained for 2 weeks post-operation, then were administered the test substance for 3 consecutive days, and were sacrificed and necropsied 24 hours after the last treatment. Another group of immature rats, 21-22 days old, was administered the test substance for 3 consecutive days, and were sacrificed and necropsied 24 hours after the last treatment. The vehicle control group received arachis oil, and the positive control group was administered 17β-estradiol at 0.4 mg/kg by gavage. There were no clinical indications of toxicity, no increases in uterus weights, no premature vaginal opening, and no increases in vaginal cornification in any group exposed to methylparaben compared to the negative controls. The positive control group had significantly increased uterus weights, as expected. The NOEL is reported at 800 mg/kg/day. Authors concluded the test substance was not estrogenic under the conditions of the test (Klimisch 2, reliable with restrictions) (Unnamed 1998 study).
- *In vivo:* Methylparaben was evaluated in a uterotrophic bioassay performed according to OECD TG 440 (GLP compliance not specified). Female B6D2 F1 (C57B6 X DBA2J) mice were administered methylparaben (purity not specified) by gavage in a mixture of 90% peanut oil and 10% ethanol oil, at 0, 1, 10, or 100 mg/kg, or subcutaneously at 100 mg/kg (5/dose for oral, 7/dose for subcutaneous, and 5-10 animals per control group). Ovariectomized mice were administered the test substance for 3 consecutive days, and were sacrificed and necropsied 24 hours after the last treatment. Another group of immature mice, 18-20 days old, was administered the test substance for 3 consecutive days, and were sacrificed and necropsied 24 hours after the last treatment. The positive control group was administered estradiol benzoate at 0.1 mg/kg by gavage. There were no clinical indications of toxicity, no increases in uterus weights, no premature vaginal opening, and no increases in vaginal cornification in any group exposed to methylparaben compared to the negative controls. The positive control group had significantly increased uterus weights, as expected. The NOEL is reported at 100 mg/kg/day. Authors concluded the test substance was not estrogenic under the conditions of the test (Klimisch 2, reliable with restrictions) (Unnamed 1999 study).
- *In vivo:* Methylparaben was evaluated in a uterotrophic bioassay performed according to OECD TG 440 (GLP compliance not specified). Groups of female CD-1 mice and female Wistar rats were administered methylparaben (purity not specified) subcutaneously in propylene glycol, at 0.55, 5.5, 16.5, 55, or 165 mg/kg (≥ 10 animals/dose/group). All groups were administered the test or control substance for 3 consecutive days, and were sacrificed

and necropsied 24 hours after the last treatment. The positive control group was administered estradiol at 0.010 mg. There were statistically significantly and dose-related increases in uterine weights in the immature mice exposed at ≥16.5 mg/kg/day, compared to vehicle controls ($p < 0.05$), and in immature rats exposed at ≥ 55 mg/kg/day ($p < 0.05$). Although statistically significant, the increases were 33-50% relative to that of a very low dose of estradiol (0.010 mg/kg, which is several orders of magnitude lower than the methylparaben dose), indicating weak activity. There were no significant effects on uterine weights in ovariectomized mice. Authors stated that based on the weak activity, and lack of reproducibility relative to the previous study, these results should be interpreted with caution (Klimisch 2, reliable with restrictions) (Unnamed 2003 study).

- *In vivo:* Methylparaben was evaluated in a non-GLP, non-guideline subchronic feeding study in rats. Male Crj:Wistar rats were administered methylparaben (purity 99.9%) in the diet at 0.1 or 1.0%, equivalent to 102 or 1,030 mg/kg/day, respectively, for 56 days (8/dose). At the end of 8 weeks, the rats were sacrificed and the weights of the testes, epididymides, prostates, seminal vesicles and preputial glands were determined. There were no treatment-related effects on body weights and the absolute and relative organ weights. Additionally, the test substance did not exhibit anti-spermatogenic effects or elicit changes in testosterone, LH and FSH levels. Based on this, authors established a NOEL of 1.0% (equivalent to 1,030 mg/kg/day as calculated by the study authors) for male reproductive parameters including weight of reproductive organs and sperm analysis (Klimisch 2, reliable with restrictions) (Unnamed 2004).
- *In vivo:* Methylparaben was evaluated in a GLP-compliant, non-guideline study in male Crj.(WI)BR rats. Animals were exposed to methylparaben (99.9% purity) in the diet at 0, 100, 1,000, or 10,000 ppm, equivalent to 0, 11.2, 110.0, or 1,141.1 mg/kg/day (16/dose), beginning at 21 days postpartum for at least 56 days. Animals were evaluated for clinical signs of toxicity, body weight, and food consumption. In addition, reproductive organs from all rats, as well as the liver, thyroid and pituitary glands were weighed and histological examination performed. Sperm evaluations were conducted to determine sperm concentration, motility and morphology and a detailed quantitative examination of the testes was performed, taking into account the tubular stages of the spermatogenic cycle. There were no treatment-related effects on any of the reproductive parameters measured (histopathology of reproductive organs and sperm analysis). Authors established a NOAEL of 1,141 mg/kg/day for general toxicity, including histopathology of reproductive organs and sperm analysis; which was the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2005 study).

- CIR 2020
  - Several parabens were assessed for reproductive and developmental effects in a non-guideline study in prepubertal rats (Vo et al. 2010). Methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, and isobutylparaben were administered to groups of prepubertal Sprague Dawley rats (8 weeks old) at 0, 62.5, 250, or 1,000 mg/kg by gavage in corn oil once per day (10/group) on PND 21 to 40. EE was used as a positive control administered at 1 mg/kg/day. All rats were sacrificed at 24 hours following the final exposure.
    - A statistically significant delay in vaginal opening was observed in rats exposed to methylparaben at 1,000 mg/kg, and to isopropylparaben at ≥ 250 mg/kg, whereas there was a statistically significant accelerated date of vaginal opening for the positive control animals. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there does not*

appear to be any particular trend among the parabens based on lack of reproducibility with ethylparaben, propylparaben, and butylparaben.

- At 1,000 mg/kg, there was a statistically significant decrease in ovary weights for rats exposed to methylparaben and isopropylparaben; decreased kidney weights in rats exposed to ethylparaben and isopropylparaben; increases in adrenal gland weights in rats exposed to methylparaben, ethylparaben, and propylparaben, and increases in thyroid gland weights in rats exposed to methylparaben. Liver weights were increased for all doses of rats exposed to butylparaben. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there was no mention of corresponding pathological effects.*
- Decreased number of corpora lutea with increased number of cystic follicles and thinning of the follicular epithelium was observed in the ovaries of rats (test substance(s) and dose(s) not specified). Myometrial hypertrophy in the uterus was identified in rats exposed to propylparaben and isopropylparaben at 1,000 mg/kg, and in rats exposed to butylparaben and isobutylparaben at ≥62.5 mg/kg. *ToxServices notes the severity of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens.*
- Serum estradiol concentrations were significantly reduced in rats exposed to ethylparaben and isopropylparaben at 1,000 mg/kg, and prolactin concentrations were increased in rats exposed to methylparaben at 1,000 mg/kg. *ToxServices notes the severity and biological significance of these observations relative to the negative and positive controls was not reported, and there does not appear to be any particular trend among the parabens.*
- Serum concentrations of T4 were statistically significantly reduced in rats exposed to methylparaben at 1,000 mg/kg, propylparaben and isopropylparaben at ≥ 250 mg/kg, and isobutylparaben, propylparaben, and isopropylparaben at ≥ 62.5 mg/kg. The IC50 (the concentration causing 50% inhibition activity) values for affinity to ERα and ERβ range from 2.07E-6 to 5.55E-5 in the following order: isobutylparaben > butylparaben > isopropylparaben = propylparaben > ethylparaben (the value for methylparaben was not reported); comparatively, the IC50 for 17 β-estradiol was approximately 3E-9. *ToxServices suggests these effects indicate the parabens have very weak affinity for ERα and Erβ.*

ToxServices notes the observations of myometrial hypertrophy in the uterus, and reduced concentrations of serum T4, are of questionable toxicological significance particularly as the study was not guideline, has limited reporting (e.g., severities are not reported), and there do not appear to be any corresponding pathological effects. Furthermore, these effects have not been reproduced in the other more comprehensive guideline reproductive and developmental toxicity studies.

- Zebrafish embryos were exposed to methylparaben at 0.1, 1, 10, and 100 ppb (duration not specified). Authors reported increased inhibition of acetylcholinesterase activity, and increased cortisol levels (severity and doses not specified). Additionally, there were effects on heart rate and hatching percentage in the embryos at ≥ 10 ppb, and anxiety-like behavior in the larvae at 0.1 and 1 ppb (no further details provided) (Luzeena et al. 2019). *ToxServices notes the study details are insufficient for comparison to the GreenScreen® guidance (CPA 2018b) and GHS guidance (UN 2021), therefore, this summary is included for completeness but is not included in the weight of evidence.*
- Zebrafish embryos were exposed to methylparaben at 200, 400, 800, and 1,000 µM for 96 hours post-fertilization (hpf). Authors reported observations of decreased heart rate and

hatching rate, and developmental abnormalities including pericardial edema blood cell accumulation and bent spine. The 96 hpf LC_{50} was 428 µM (0.065 mg/L) and expression of vitellogenin was significantly upregulated compared to controls at 100 µM (which was not one of the reported test substance concentrations) (no further details provided) (Dambal et al. 2017). *ToxServices notes the study details are insufficient for comparison to the GreenScreen® guidance (CPA 2018b) and GHS guidance (UN 2021), therefore, this summary is included for completeness but is not included in the weight of evidence.*

- ECHA 2018
  - In its assessment on the dossier for methylparaben, ECHA identified concerns for endocrine activity based on the previously reported effects on serum levels of estradiol and T4, potential impact on the ovaries, and delayed vaginal opening (Vo et al. 2010). In response, ECHA suggested performance of an extended one-generation reproductive toxicity study such as EU B.56 / OECD TG 443. *Although ECHA has not re-evaluated the data, ToxServices suggests this request has been fulfilled by the OECD 443 study (Unnamed 2019 study) summarized above, in which the effects were not reproduced.*
- TEDX 2015
  - Methylparaben was placed on the TEDX list of potential endocrine disruptors in 2011 based on *in vitro* evidence of endocrine activity. Abstract of studies cited by TEDX are summarized below:
    - *In vitro:* Byford et al. (2002) found evidence of estrogenic activity of parabens in MCF7 human breast cancer cells. The study authors reported that competitive inhibition of [^{3}H]estradiol binding to MCF7 cell estrogen receptors was detected at 1,000,000-fold molar excess of *n*-butylparaben (86%), *n*-propylparaben (77%), ethyl-paraben (54%), and methylparaben (21%). Parabens increased the expression of endogenous estrogen-regulated genes in MCF7 cells at concentrations $\geq 10^{-6}$ M. They also increased proliferation of cells in a monolayer culture in an estrogen receptor dependent manner.
    - *In vitro:* Chen et al. (2007) found evidence of anti-androgenic activity of parabens in an *in vitro* androgen receptor-mediated transcriptional activity assay. Methyl-, propyl- and butyl-4-hydroxybenzoate inhibited testosterone-induced transcriptional activity by 40%, 33%, and 19%, respectively. However, the major metabolite, 4-hydroxybenzoic acid had no effect on testosterone-induced transcriptional activity.
    - *In vitro:* Gomez et al. (2005) found evidence of estrogenic activity in three reporter cell lines. The parabens were found to activate the ERα and ERβ similarly.
    - *In vivo and in vitro:* Routledge et al. (1998) reported that a range of alkyl hydroxybenzoate preservatives (parabens) including methylparaben were weakly estrogenic when tested in *in vitro* (a receptor-binding assay and yeast-based estrogen assay) and *in vivo* (uterotrophic) studies with butylparaben as the most potent paraben. When administered orally to immature rats, the parabens were inactive. However, subcutaneous administration of butylparaben produced a positive uterotrophic response *in vivo* although it was approximately 100,000 times less potent than 17β-estradiol.
    - *In vitro:* Song et al. (1991) reported that parabens have potent *in vitro* spermicidal activity against human spermatozoa.
- SCCS 2011
  - Based on the results from *in vitro* and *in vivo* rodent tests, parabens can exert weak estrogenic activity as the potency values were 3 to 6 orders of magnitude lower than the potency of the positive control 17β-estradiol. In addition, the estrogenic activity of parabens

appears to increase with increasing chain length and butylparaben appears to be more potent than propyl-, ethyl- and methylparaben. As a result, the Scientific Committee on Consumer Safety (SCCS) panel concluded that methylparaben was not the subject of concern.
    - Methylparaben and ethylparaben were shown not to adversely affect the secretion of sex hormones or male reproductive function when administered orally at doses up to 1,000 mg/kg/day (Oishi 2004).
- Danish Centre on Endocrine Disruptors 2012
    - In a 2012 review of endocrine activity data on methylparaben, the following highlights are noted: A few human studies have indicated weak associations between increased paraben exposure and markers for reproductive health, however, the data are limited. Methylparaben has conflicting data suggesting possible weak estrogenic and weak anti-androgenic effects *in vitro* and *in vivo*. The *in vivo* data identified an increase in abnormal sperm and a decrease in normal sperm number, with no change in total sperm count. Additionally, there was some evidence of thyroid toxicity based on decreased T4 levels and decreased relative thyroid weight in peripubertal rats. In fish there were increased vitellogenin induction and testicular tissue changes. The Danish Centre on Endocrine Disruptors concluded methylparaben is a endocrine disrupter in category 2a (suspected).

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II* endpoints are distinguished in the v 1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen® Guidance v1.4, Annex 2 for more details.

Acute Mammalian Toxicity (AT) (Group II) Score (vH, H, M, or L): L

Methylparaben is assigned a score of Low for acute toxicity based on an oral LD_{50} > 2,000 mg/kg. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when the lowest oral LD_{50} is > 2,000 mg/kg (CPA 2018b). The confidence in the score is high based on reliable data for the target compound. Although no data were found for acute dermal toxicity, toxicokinetic data demonstrate slower and reduced absorption for dermal exposure, compared to oral exposure, and similar metabolites. Therefore, acute dermal toxicity is expected to be similarly low, or lower than that for the oral route. While no data were found for acute inhalation toxicity, there are low concerns as particle size information indicate very low fraction of respirable particle size (i.e., 3.7 +/- 0.2% < 10 µm).

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
    - *Oral:* Methylparaben was evaluated in a pre-GLP acute oral toxicity study conducted similarly to OECD TG 401. Male Sprague-Dawley rats (5/dose) were administered methylparaben (FDA 71-38, purity not reported) at single doses of 100, 500, 1,000, 2,000, 3,000 or 5,000 mg/kg via gavage in 0.85% saline. Animals were observed for 10 days. Death occurred within 24 hours in animals at 1,000 mg/kg and above (1/5 at 1,000 mg/kg, 2/5 at 2,000 mg/kg, 4/5 at 3,000 and 4,000 mg/kg, and 10/10 at 5,000 mg/kg). The oral LD_{50} was determined to be 2,100 mg/kg (Klimisch 2, reliable with restrictions) (Unnamed 1974 study).
    - *Oral:* The oral LD_{50} for methylparaben in dogs is 3,000 mg/kg (no further details provided) (Klimisch 2, reliable with restrictions) (Lewis 1999).

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score (vH, H, M, or L): *M*

Methylparaben is conservatively assigned a score of Moderate for systemic toxicity (single dose) based on a majority of notifiers in the ECHA Classification and Labeling Inventory indicating Hazard Code H335 – May cause respiratory irritation, which corresponds to GHS Category 3 classification. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when data support GHS Category 3 classification for any route of exposure (CPA 2018b). Confidence is low as no supporting inhalation data were found. Available oral data suggest low concerns for systemic effects following single exposure. No data were found for acute dermal exposure, however, as noted previously, toxicokinetic data suggest toxicity following dermal exposure will be similar or lower than that for oral exposure, based on slower and less extensive absorption.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
  - *Other*: EU – Manufacturer REACH hazard submissions – H335 – May cause respiratory irritation (unverified) [Specific target organ toxicity – single exposure; Respiratory tract irritation – Category 3]
- ECHA 2023a[9] (Note: One additional acute oral toxicity study in dogs is summarized in the REACH dossier with a Klimisch 2 rating (reliable with restrictions), however, it is insufficiently detailed to meet the Klimisch 2 criteria, therefore, ToxServices considered it unreliable and did not include it here).
  - *Oral*: Methylparaben was evaluated in a pre-GLP acute oral toxicity study conducted similarly to OECD TG 401. Male Sprague-Dawley rats (5/dose) received methylparaben (FDA 71-38, purity not reported) at single doses of 100, 500, 1,000, 2,000, 3,000 or 5,000 mg/kg via oral gavage in 0.85% saline. Animals were observed for 10 days. Death occurred within 24 hours in animals at 1,000 mg/kg and above (1/5 at 1,000 mg/kg, 2/5 at 2,000 mg/kg and 4/5 at higher doses). A reddened stomach lining and congested lung were reported for animals that died during the study, and no substance-related changes were found at necropsy of surviving animals. The oral LD_{50} is determined as 2,100 mg/kg (Klimisch 2, reliable with restrictions) (Unnamed 1974 study). No clinical signs were reported and it is unclear if they were recorded.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*) Score (H, M, or L): L

Methylparaben is assigned a score of Low for systemic toxicity (repeated dose), including immunotoxicity, based on the weight of evidence from numerous oral repeated dose toxicity studies on the target compound. Several guideline studies do not identify systemic effects up to the highest dose tested (OECD TG 408, 407, 443, and 422). One pre-GLP, pre-guideline study reported effects in two high dose animals, however the study had reduced reliability and the effects were not repeated in the later, more reliable guideline studies. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on reliable data for the target compound. One additional study suggests low concerns for systemic toxicity following repeated dermal exposure, however, the study was non-guideline and has limited reliability.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]

- *Oral:* Methylparaben was evaluated in a GLP-compliant subchronic oral toxicity study performed according to OECD TG 408. Wistar rats (Crl: WI(Han) (Full Barrier)) were exposed to methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg/day, 7 days/week for 90 days (10/sex/dose, plus an additional 5/sex/dose for a 28-day post-exposure recovery period). In addition to the standard battery, several sperm parameters were evaluated. Slight to moderate increased salivation was noted in high dose males and females, and regular moving of the bedding in all males and females of the high dose group, and one male at the mid dose were observed. As both clinical signs were in close approximation to dosing, or in anticipation thereof, investigators considered these to be indications of discomfort or a local reaction as opposed to systemic effects. Thus there were no significant findings based on clinical observations. One high dose female of the recovery group was found moribund on day 56 and was sacrificed. At necropsy, the animal had abnormal dark red color in the lungs along with multifocal alveolar hemorrhages. There were no further deaths in treated or control animals. Due to the single incidence, authors considered the finding incidental and not treatment related. There were no significant findings based on food consumption, hematology, clinical chemistry, behavior (functional observations), organ weights, gross pathology, histopathology, or the additional optional sperm parameters. Authors assigned the NOAEL at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2019 study).
- *Oral:* Methylparaben was evaluated in the previously mentioned GLP-compliant repeated dose toxicity study conducted according to OECD TG 407, EU Method B.7, and OPPTS 870.3050. Wistar rats (5/sex/dose, plus an extra 5/sex for the control and high dose groups for a 14-day recovery period) were administered methylparaben (purity > 99%) by gavage in propylene glycol at 0, 50, 250, and 1,000 mg/kg/day for 28 days. The animals were evaluated for standard parameters as well as ophthalmological examination, and functional observational battery with assessment of motor activity. Treated animals at the high dose showed several clinical signs of toxicity such as piloerection and/or hunched posture and labored respiration. One male and one female at the high dose were sacrificed for ethical reasons on Day 14 and 24, respectively, due to several clinical signs indicative of ill health. Microscopic findings examination revealed minimal/slight erosions in the stomach, correlating to the irregular surface recorded at necropsy, slight red pulp atrophy of the spleen, slight to moderate myeloid atrophy in the bone marrow of the sternum, and slight/moderate lymphoid atrophy of the thymus, correlating to the reduced size recorded at necropsy. To further investigate the cause of death, additional sections of esophagus, larynx, nasopharynx and nasal cavity were prepared and examined. For both animals the macroscopic distension with gas of the gastrointestinal tract correlated with the clinically observed abdominal swelling. The major microscopic findings of the sacrificed male included massive diffuse ulcerative inflammation of the nasopharynx and inflammatory lesions in the nasal cavity. In the sacrificed female, wispy material with erythrocytes was identified in the larynx. The alterations in the nasal cavity and larynx were suggestive of a gavage procedure (reflux)-related cause of moribundity, with secondary changes in thymus, bone marrow and/or spleen. Therefore, study authors did not consider the death of both animals to be the result of a systemic test item effect. No other treatment related effects were seen in any of the remaining animals. The NOAEL is reported at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2009 study).
- *Oral:* ToxServices notes no indications of systemic toxicity, organ toxicity, or immunotoxicity were identified in the previously summarized carcinogenicity/chronic exposure studies, reproductive toxicity studies, or developmental toxicity studies (see respective sections for details).

- ECHA 2018
    - In its assessment on the dossier for methylparaben, ECHA identified concerns for systemic toxicity following repeated exposure. The concerns were in part due to data deficiencies because of limited parameters and/or low number of animals/group in the pre-GLP, pre-guideline sub-chronic toxicity studies in rabbits, guinea pigs, and rats, and the chronic study in mongrels. Additionally, the 28-day (OECD TG 407) study in rats (Unnamed 2009 study), reported deaths in 1 male and 1 female at 1,000 mg/kg/day with several clinical signs of toxicity and microscopic findings including erosion in the stomach correlating with irregular surface at necropsy, slight red pulp in the spleen, and lymphoid atrophy of the thymus correlating with reduced thymus size at necropsy. Whereas the study investigators considered these effects unrelated to treatment, ECHA does not agree that the data are sufficient to make such a conclusion. ECHA added that there is inconsistency in the histopathology findings of the 28-day study and the two chronic toxicity studies, in that the 28-day study demonstrated clear signs of organ toxicity where the chronic studies did not have significant histopathological findings. In conclusion, ECHA suggested performance of a repeated dose toxicity study in accordance with EU Method B.26 / OECD TG 408 in rats. *Although ECHA has not re-evaluated the data, ToxServices suggests this request has been fulfilled by the OECD TG 408 study (Unnamed 2019 study) summarized above, in which no adverse effects were identified in rats exposed at up to 1,000 mg/kg/day.*
- CIR 2008
    - *Dermal:* Methylparaben and propylparaben were evaluated in numerous repeated dose toxicity studies presented in the CIR (2008) review. These studies used formulations containing methylparaben alone (up to 0.7%[12]), propylparaben alone (up to 0.3%), and product formulations containing multiple parabens (0.2% methylparaben and 0.2% propylparaben). Rats and/or rabbits were dermally exposed to the product formulation for up to 13 weeks. The studies occasionally found slight changes in hematologic and blood chemistry parameters; however, these changes were not accompanied by any significant gross or histopathological changes and were considered toxicologically insignificant. Treatment caused no changes in animal body weight or food consumption and no gross or histopathological changes were found. Treatment-related effects were limited to localized effects (i.e., mild to severe inflammation, moderate to well-defined erythema, slight edema, and slight to mild desquamation) of the treated skin. The study authors found no cumulative systemic toxic effects.
- NCI 1977
    - *Intramuscular:* Methylparaben and propylparaben were evaluated in a non-guideline antigen study in guinea pigs. Animals were injected a saline solution with 1.6 mg methylparaben and 0.4 mg propylparaben per 100 mg body weight (3/sex/treatment group and 2/sex as vehicle controls) once per day on Monday, Wednesday, and Friday of week 1, and Monday of the following week. A challenge dose was administered after a 14-day rest period directly into the heart of 6 test, and 4 control animals. Animals were observed for signs of respiratory distress and death within 1 hour post-administration. After one hour, animals were sacrificed and necropsied for gross pathological examination. One of the 6 exposed animals exhibited clonic-tonic convulsions and had bloody discharge from its mouth and nostrils, and also had massive cardiac hemorrhage and a large needle puncture wound in the heart identified at necropsy. Investigators reported the death was likely due to mechanical trauma to the heart, rather than an antigenic response. Necropsies of several control animals

[12] mg/kg/day dose cannot be calculated without information on the frequency and amount applied on the animals.

identified a few small hemorrhages on the lung, but no cardiac bleeding. Authors concluded the test substance was not antigenic under the conditions of the test.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *L*
Methylparaben is assigned a score of Low for neurotoxicity (single dose) based on the lack of indications of neurotoxicity following single exposure in rats and humans. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (single dose) when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is low as the rat data (OECD TG 401) are limited to clinical indications and necropsy and such studies do not typically assess additional neurotoxicity parameters (e.g., startle reflex, righting reflex, grip strength, etc.).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - *Oral:* Methylparaben was evaluated in the previously summarized pre-GLP acute oral toxicity study conducted similarly to OECD TG 401. Male Sprague-Dawley rats (5/dose) were administered methylparaben (FDA 71-38, purity not reported) at single doses of 100, 500, 1,000, 2,000, 3,000 or 5,000 mg/kg via oral gavage in 0.85% saline. Animals were observed for 10 days. Death occurred within 24 hours in animals at 1,000 mg/kg and above (1/5 at 1,000 mg/kg, 2/5 at 2,000 mg/kg, 4/5 at 3,000 and 4,000 mg/kg, and 10/10 at 5,000 mg/kg). Reddened stomach lining and congested lung were identified at necropsy (no further details provided) (Klimisch 2, reliable with restrictions) (Unnamed 1974 study).
  - *Oral:* The oral LD_{50} for methylparaben in dogs is 3,000 mg/kg (no further details provided) (Klimisch 2, reliable with restrictions) (Lewis 1999).
- HSDB 2017
  - *Intravenous:* Methylparaben and propylparaben were evaluated in a non-guideline human exposure study designed to investigate effects on cerebral vasodilation and intracranial pressure. Healthy humans were administered intravenous injections of methylparaben and propylparaben, and Cerebral blood flow (CBF) and cerebral blood flow velocity (CBFV) were measured with inhaled ^{133}-Xenon and transcranial Doppler. There were no significant changes in CBF or CBFV identified for either test substance.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): L
Methylparaben is assigned a score of Low for neurotoxicity (repeated dose) based on lack of indications of neurotoxicity in several repeated dose toxicity studies at doses above the GHS classification cutoffs. GreenScreen® criteria classify chemicals as a low hazard for neurotoxicity (repeated dose) when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on measured data for the target compound.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9] (Note developmental neurotoxicity (OECD TG 443) is assessed under the developmental toxicity endpoint whereas this section includes other types of neurotoxicity).
  - *Oral*: Methylparaben was evaluated in the previously summarized GLP-compliant subchronic oral toxicity study performed according to OECD TG 408. Wistar rats (Crl: WI(Han) (Full Barrier)) were exposed to methylparaben (99.8% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg/day, 7 days/week for 90 days (10/sex/dose, plus an additional 5/sex/dose for a 28-day post-exposure recovery period.

There were no significant findings based on clinical observations, behavior (functional observations), or other effects based on necropsy that would suggest an effect on the nervous system (Klimisch 1, reliable without restriction) (Unnamed 2019 study). *ToxServices identified a NOAEL of 1,000 mg/kg/day for neurotoxicity for this study.*

- *Oral*: Methylparaben was evaluated in the previously described GLP-compliant repeated dose toxicity study conducted according to OECD TG 407. Wistar rats (5/sex/dose) received methylparaben (purity > 99%) in propylene glycol at doses of 50, 250 and 1,000 mg/kg/day by oral gavage daily for 28 days. Animals were evaluated for neurobehavioral endpoints (Functional Observation Battery tested: hearing ability, pupillary reflex, static righting reflex, grip strength and motor activity). No treatment related effects were seen in any of these parameters (Klimisch 1, reliable without restriction) (Unnamed 2009 study). *ToxServices identified a NOAEL of 1,000 mg/kg/day for neurotoxicity. According to GHS criteria, this NOAEL is above the duration adjusted GHS Guidance value for Category 2 of 321 mg/kg/day for a 28-day study and therefore, methylparaben is not classified per GHS.*

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): L

Methylparaben is assigned a score of Low for skin sensitization based on measured data for the target substance and a strong surrogate. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on measured data for the target substance and a strong surrogate. It may be noted that no data were found to support the New Zealand's GHS classification to Category 1, therefore ToxServices discounted the New Zealand classification in the weight of evidence.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – New Zealand – Skin sensitisation Category 1
- ECHA 2023a[9]
  - Methylparaben was evaluated in a pre-GLP guinea pig sensitization study conducted according to Maurer Optimization Test Method (similar to OECD TG 406). Male and female Pirbright guinea pigs (10/sex) were intradermally induced with 0.1 % methylparaben (purity not specified) (10 injections) and challenged with 0.1% (intradermal) 14 days after the last induction application, and re-challenged with 5% methylparaben (epidermal) in soft white petrolatum after another 10-day rest. Treatment induced allergic reactions in a few animals (3/20 (15%) in 0.1% dose group, and 4/20 (20%) in 5% group), but was not considered statistically significant. Positive and negative controls provided the anticipated results and the study was considered valid (Klimisch 2, reliable with restrictions) (Unnamed 1980 study). *According to GHS criteria for Category 1A, a positive response of ≥ 30% is required at an intradermal induction dose of ≤ 0.1%, or ≥ 60% response at a dose > 0.1 and ≤ 1%. Therefore, response rates of 15% at 0.1% concentration, and 20% at 5% concentration, do not warrant GHS classification for skin sensitization.*
  - Methylparaben was evaluated in a pre-GLP, non-guideline guinea pig sensitization study conducted in a manner similar to the Maurer Optimization Test Method and similar to OECD TG 406. Guinea pigs (strain not specified) (10/sex) were induced intradermally with 0.1% methylparaben in physiological saline (3 times per week for 10 injections) and challenged 2 weeks after the 10th injection with 0.1% (intradermal) methylparaben in physiological saline. Animals were observed for 48 hours and there were no allergic responses in any of the exposed animals (Klimisch 2, reliable with restrictions) (Unnamed 1952 study).

- CIR 2020
    - Methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzoparaben were evaluated for skin irritation and skin sensitization in a non-guideline *in vitro* study using cocultured human keratinocytes and peripheral blood mononuclear cells (PBMCs). The co-cultures were exposed to the parabens at unspecified concentrations in DMSO, and were incubated for 48 hours. Sensitization was assessed based on CD86 expression, compared to vehicle controls. EC_{50} values for CD86 expression for extreme, strong, moderate, and non-sensitizing substances are ≤ 12.5 µM, > 12.5 to ≤ 50 µM, > 50 to ≤ 100 µM, and > 100 µM, respectively. Methylparaben, ethylparaben, propylparaben, and isopropylparaben were weak sensitizers, and butylparaben, isobutylparaben, and benzoparaben were strong sensitizers (Sonnenburg et al. 2015). *ToxServices notes this non-guideline study does not appear to reflect a validated method, and is considered low reliability. Additionally, the scoring system and results are not suitable for comparison to the GHS guidance. However, it does suggest the sensitization potential of methylparaben, ethylparaben, and propylparaben are similar.*
- CIR 2008
    - The CIR Expert Panel presented multiple clinical studies which found evidence that patients sensitive to one paraben show cross-reactivity to another paraben. They indicated that evidence of paraben sensitization was reported in case literature, but it primarily occurred when the exposure involved damaged or broken skin. Patch-testing data indicated that in patients with chronic dermatitis less than 4% of individuals were sensitive to parabens. Additionally, patch testing data over the past 20 years showed no significant change in the incidence of dermatitis patients that tested positive for parabens.
- HSDB 2017
    - In a repeated insult patch test, each paraben (methylparaben, ethylparaben, propylparaben, and butylparaben) were administered to the skin of 50 subjects (25/sex) for 4 to 8 hours every other day for 3 weeks (10 applications), followed by a 3-week rest period. The test substance was then reapplied and observations were recorded at 24 and 48 hours post exposure. There were no indications of sensitization in any subjects at 24 or 48 hours post-challenge.
- ECHA 2023c[9]
    - <u>*Surrogate Propylparaben:*</u> Propylparaben was not sensitizing in a mouse local lymph node assay conducted in a manner equivalent or similar to OECD TG 429 using (GLP compliance not specified). CBA/Ca mice (4/group) were dermally administered 25 µL of 5, 10, or 25% propylparaben (98% purity) in acetone/olive oil (4:1 v/v) on the dorsal surface of each ear for 3 consecutive days. Following the final application, the animals were sacrificed and the lymph nodes isolated to perform the proliferation assay. The stimulation indices for the 5, 10, and 25% doses were 1.3, 1.6, and 1.3, respectively. As all of the stimulation indices for the applied doses were less than 3, propylparaben was not sensitizing to the skin of mice in this study (Klimisch 2, reliable with restrictions) (Basketter and Scholes 1992).
    - <u>*Surrogate Propylparaben:*</u> Propylparaben was not sensitizing in a guinea pig maximization assay conducted according to OECD TG 406 (GLP compliance not specified). Dunkin-Hartley guinea pigs induced with propylparaben (> 98% purity) in physiological saline at 0.5% by intradermal injection, and 25% in acetone/polyethylene glycol 400 (70:30 v/v) by epicutaneous administration. The challenge was performed with 10% propylparaben in acetone/PEG 400 (70:30 v/v) by epicutaneous administration. No skin reactions were seen in any of the exposed animals at the 24 and 48 hours readings. 2-Mercaptobenzothiazole was the positive control substance and provided the expected results. Study authors

concluded the test substance was not sensitizing by EU criteria (Klimisch 2, reliable with restrictions) (Basketter and Scholes 1992).

- *Surrogate Propylparaben:* Propylparaben was not sensitizing in a mouse local lymph node assay conducted in a manner equivalent or similar to OECD TG 429 using (GLP compliance not specified). CBA/Ca mice (4/group) were dermally administered 25 µL of 5, 10, or 25% propylparaben (98% purity) in acetone/olive oil (4:1 v/v) on the dorsal surface of each ear for 3 consecutive days. Following the final application, the animals were sacrificed and the lymph nodes isolated to perform the proliferation assay. The stimulation indices for the 5, 10, and 25% doses were 1.4, 1, and 1.3, respectively. As all of the stimulation indices for the applied doses were less than 3, propylparaben was not sensitizing to the skin of mice in this study (Klimisch 2, reliable with restrictions) (Basketter et al. 1994).
- *Surrogate Propylparaben:* Propylparaben was not sensitizing in a pre-GLP, pre-guideline guinea pig maximization assay conducted in a manner equivalent or similar to OECD TG 406. Hartley strain and Hartley-English short hair cross-strain guinea pigs (n=23) were induced with propylparaben (purity not specified) by intradermal injection at 3% (vehicle not specified), every other day for 10 injections. The challenge was performed by intradermal injection at 3% (vehicle not specified) and by epicutaneous administration at 3% (vehicle not specified) on day 34. There were no positive reactions in any exposed animals after the challenge. The substance is reported as not sensitizing (no further details provided) (Klimisch 2, reliable with restrictions) (Marzulli et al. 1968).

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *L*

Methylparaben is assigned a score of Low for respiratory sensitization in accordance with the guidance from ECHA (2017). Specifically, methylparaben has low concerns for respiratory sensitization based on extrapolation from negative skin sensitization data, lack of structural alerts for respiratory sensitization, and lack of indications of respiratory sensitization in the public literature despite long historical and widespread use. GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is low as this evaluation does not include non-immunologic mechanisms of respiratory sensitization, and no specific data are available for respiratory sensitization.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2022
  - Methylparaben does not contain any structural alerts for respiratory sensitization (Appendix D)
- ECHA 2017
  - The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization. ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). As methylparaben was not sensitizing to the skin (see skin sensitization section above), a literature search did not find any human evidence of respiratory sensitization by methylparaben, and as methylparaben does not contain any structural alerts for respiratory sensitization (OECD 2022, Appendix D), methylparaben is not expected to be a respiratory sensitizer.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): L

Methylparaben is assigned a score of Low for skin irritation/corrosivity based on the weight of evidence. Two reliable studies in rabbits indicate methylparaben and the surrogate ethylparaben were not irritating when tested undiluted. In humans exposed to lower concentrations of parabens, data suggest negligible skin irritation. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on reliable data for the target compound and a close structural surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - Methylparaben was not irritating to the skin of rabbits in a dermal irritation study conducted according to a modified Draize method. Nine albino rabbits (male and female) received an application of 0.5 mL (undiluted) test substance to intact sites on the skin for 24 hours under occlusive conditions. The primary dermal irritation index (PDII) was 0.67/4 in test rabbits and 0.44/4 in control animals. It was concluded that methylparaben was not classifiable as a skin irritant (Klimisch 2, reliable with restrictions) (Unnamed 1976 study as summarized in CIR 2006).
- CIR 2020
  - Methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzoparaben were evaluated for skin irritation and skin sensitization in a non-guideline *in vitro* study using cocultured human keratinocytes and peripheral blood mononuclear cells (PBMCs). The co-cultures were exposed to the parabens at unspecified concentrations in DMSO, and were incubated for 48 hours. Irritancy was assessed based on cell death and the corresponding EC_{50} value. EC_{50} values for irritating, weakly irritating, and non-irritating are ≤ 50 µM, > 50 to ≤ 1,000 µM, and > 1,000 µM, respectively. Methylparaben and ethylparaben were not irritating, and propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzoparaben were weakly irritating (Sonnenburg et al. 2015). *ToxServices notes this non-guideline study does not appear to reflect a validated method, and is of low reliability. Additionally, the scoring system and results are not suitable for comparison to the GHS guidance. However, it does suggest the skin irritation potential of methylparaben and ethylparaben are similar and less irritating than longer chain parabens.*
- HSDB 2017
  - Methylparaben, ethylparaben, propylparaben, and butylparaben were each applied to the backs of 50 volunteers at concentrations of 5, 7, 10, 12, and 15% in propylene glycol for 5 days under occlusive patches. The no effect levels for skin irritation of methylparaben, ethylparaben, propylparaben, and butylparaben were 5%, 7%, 12%, and 5%, respectively (no further details provided). *Although not stated as such, ToxServices notes this study summary implies methylparaben was irritating at ≥ 5%. However, due to lack of additional study details, ToxServices considered this study of low reliability.*
- CIR 2008
  - Methylparaben, butylparaben, and propylparaben were evaluated in a clinical 21-day cumulative irritancy study. Product formulations containing mixtures of methylparaben (0.2%), butylparaben (0.1%), or propylparaben (0.2%) produced no irritation to slight irritation. Volunteers were treated with the product formulation for 23 hours under occlusive conditions for 21 consecutive days.

  - Methylparaben and propylparaben were evaluated in a clinical controlled use test (4 weeks). An eye makeup formulation containing 0.2% methylparaben and 0.1% propylparaben caused no irritation.
  - Methylparaben or propylparaben were evaluated in a skin irritation study. A paste containing hydrophilic ointment and either 10% methylparaben or propylparaben was applied to the shaved backs of albino rabbits (number not reported) for 48 hours. The study summary did not indicate if treatment occurred under occlusive, semi-occlusive, or non-occlusive conditions. Treatment produced no irritation. No further details were provided.
  - Methylparaben and propylparaben were evaluated in a skin irritation study in rabbits. A product formulation containing 0.2% methylparaben and 0.1% propylparaben produced minimal irritation in rabbits, with a primary irritation index of 0.5. No further details were provided.
- ECHA 2023d[9]
  - *Surrogate Ethylparaben:* Ethylparaben was evaluated in a dermal irritation test conducted similarly to OECD TG 404 (GLP compliance not specified). Three male HC:NWZ rabbits were administered topical applications of 500 mg ethylparaben (purity not reported) moistened with water to clipped skin under semi occlusive dressing for 4 hours. An observation period of 7 days followed the exposure period. No edema or erythema was seen. The overall irritation score at 72 hours was 0 for both edema and erythema. The study authors concluded that ethylparaben was not irritating to the skin in this study (Klimisch 1, reliable without restriction) (Unnamed 1983 study).
- ECHA 2023c[9] (no data for skin irritation of propylparaben was identified in the dossier)

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): L

Methylparaben is assigned a score of Low for eye irritation/corrosivity based on high quality measured data for the target substance which was tested undiluted. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on measured data for the target compound. Although New Zealand classifies methylparaben as a Category 2 eye irritant, and some notifiers in REACH classify it as Category 1 or 2A, no supporting data are found, therefore these classifications are discounted.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – New Zealand – Eye irritation category 2
- ECHA 2023a[9]
  - Methylparaben was evaluated in a non-GLP compliant ocular irritation study conducted according to modified Draize test. Six New Zealand White rabbits (male and female) received a single instillation of 0.1 mL of methylparaben (purity 100%) into the eye for 24 hours without rinsing. Examinations for injuries were made at 24, 48, and 72 hours, and at four and seven days. Treatment produced slight transient irritation with an eye irritation score of 1/110, and effects were fully reversible within 48 hours (Klimisch 2, reliable with restrictions) (1976 study as summarized in CIR 2006).

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): M

Methylparaben is assigned a score of Moderate for acute aquatic toxicity based on an EC_{50} of 11.2 mg/L in the most sensitive species, Daphnia. GreenScreen® criteria classify chemicals as a Moderate hazard for acute aquatic toxicity when the most conservative LC/EC_{50} value is in the range of 10-100 mg/L (CPA 2018b). The confidence in the score is high based on high quality data for the target compound for all three trophic levels. It may be noted that Japan's classification to H402/Category 3 is also consistent with a Moderate hazard rating.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan – H402 – Harmful to aquatic life [Hazardous to the aquatic environment (acute) – Category 3]
- ECHA 2023a[9]
  - 96-hour LC_{50} (*Oryzias latipes,* Japanese rice fish) = 59.5 mg/L (nominal) under semi-static conditions (OECD TG 203 and GLP). Measured concentrations were within +/- 20% of nominal (Klimisch 1, reliable without restriction) (Unnamed 2000 study).
  - 48-hour EC_{50} (*Daphnia magna,* invertebrate) = 11.2 mg/L (nominal) under static conditions (ISO Guideline 6341 15, GLP not specified). Measured concentrations were within +/- 20% of nominal (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).
  - 48-hour EC_{50} (*D. magna,* invertebrate) = 41.1 mg/L (nominal) under static conditions (similar to OECD TG 202, GLP not specified). Measured concentrations were within +/- 20% of nominal (Klimisch 2, reliable with restrictions) (Unnamed 2005 study).
  - 72-hour EC_{50} and 72-hour NOEC (*Raphidocelis subcapitata*, previously *Pseudokirchneriella subcapitata*, green algae) were 91 mg/L and 20 mg/L (nominal), respectively under static conditions, based on growth rate (ISO Guideline 8692, GLP not specified). Measured concentrations were within +/- 20% of nominal (Klimisch 2, reliable with restrictions) (Unnamed study published by Madsen in 2001).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *H*

Methylparaben is assigned a score of High for chronic aquatic toxicity based on the most sensitive trophic level available, a NOEC of 0.20 mg/L in Daphnia from a 21-day reproduction test (OECD TG 211, GLP-compliant). GreenScreen® criteria classify chemicals as a High hazard for chronic aquatic toxicity when the most conservative chronic toxicity value is in the range of > 0.1 to 1.0 mg/L (CPA 2018b). The confidence in the score is reduced due to lack of reliable data for the fish trophic level.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9] (Note additional studies examining endocrine effects on fish sexual development are available in the REACH dossier but were not included in this assessment as there are no corresponding guidelines with the GHS guidance (UN 2021), or the GreenScreen® guidance document (CPA 2018b), to assign a hazard rating)
  - *D. magna* were exposed to methylparaben in a 21-day reproduction test (OECD TG 211, GLP compliant). The 21-day EC_{50}, NOEC, and LOEC were 5.32, 0.20, and 0.81 mg/L, respectively, based on reproduction. The 21-day-EC_{50} for parental mortality was 0.89 mg/L (Klimisch 1, reliable without restriction) (Unnamed 2000 study).

  - As summarized previously the 72-hour NOEC (*R. subcapitata*, green algae) was 20 mg/L (nominal), under static conditions, based on growth rate (ISO Guideline 8692, GLP not specified). Measured concentrations were within +/- 20% of nominal (Klimisch 2, reliable with restrictions) (Unnamed study published by Madsen in 2001).
- CIR 2020
  - Zebrafish embryos were exposed to methylparaben at 0.1, 1, 10, and 100 ppb (duration not specified). Authors reported observations of increased inhibition of acetylcholinesterase activity, and increased cortisol levels (severity and doses not specified). Additionally, there were effects on heart rate and hatching percentage in the embryos at ≥ 10 ppb, and anxiety-like behavior in the larvae at 0.1 and 1 ppb (no further details provided) (Luzeena et al. 2019). *ToxServices notes the study details are insufficient for comparison to the GreenScreen guidance (CPA 2018b) and GHS guidance (UN 2021), therefore, this summary is included for completeness but is not included in the weight of evidence.*
  - Zebrafish embryos were exposed to methylparaben at 200, 400, 800, and 1,000 µM for 96 hours post-fertilization (hpf). Authors reported observations of decreased heart rate and hatching rate, and developmental abnormalities including pericardial edema blood cell accumulation and bent spine. The 96 hpf LC_{50} was 428 µM (0.065 mg/L) and expression of vitellogenin was significantly upregulated compared to controls at 100 µM (which was not one of the reported test substance concentrations) (no further details provided) (Dambal et al. 2017). *ToxServices notes the study details are insufficient for comparison to the GreenScreen guidance (CPA 2018b) and GHS guidance (UN 2021), therefore, this summary is included for completeness but is not included in the weight of evidence.*
- HSDB 2017
  - Chronic toxicity was evaluated in non-guideline study with *Ceriodaphnia dubia* exposed to various parabens for 7 days under static conditions. The range of EC_{50} values for mortality, offspring number, and first brood production were 0.30-3.1, 0.047-12, and 1.3-6.3 mg/L, respectively. The NOEC and LOEC values for the number of neonates ranged from 0.63 to 10 mg/L, and 1.2 to 19 mg/L, respectively. The **NOEC for methylparaben**, benzoparaben, and dichlorinated benzoparaben was **1.3**, 0.04, and 0.63 mg/L, respectively. NOEC and LOEC values could not be determined for propylparaben, chlorinated propylparaben, isopropylparaben, and chlorinated isopropylparaben as these compounds exhibited nonmonotonic concentration-dependent responses (no further details provided).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vL

Methylparaben is assigned a score of Very Low for persistence based on measured data indicating ready biodegradability (>60% in 28 days), and it meets the 10-day window. Additionally, methylparaben is predicted to partition to soil with a half-life of 30 days. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when soil is the dominant compartment and the substances is readily biodegradable and meets the 10-day window (CPA 2018b). The confidence in the score is high based on measured data for the target compound.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - In a GLP-compliant ready biodegradability test conducted according to OECD TG 301 B (Ready Biodegradability: CO2 Evolution Test), domestic non-adapted activated sludge was

exposed to methylparaben (purity not reported) at a concentration of 20 mg/L for 28 days. The reference substance was sodium acetate, which provided the expected results. Methylparaben reached > 73 % degradation in 10 days, and reached 89% by day 28. The substance was considered readily biodegradable, and met the 10-day window (Klimisch 1, reliable without restriction) (Unnamed 2004 study).

- In a ready biodegradability test conducted according to OECD TG 301F (manometric respiratory), methylparaben was found to be readily biodegradable. The inoculum (not specified) was exposed to the test substance (20 mg/L) under aerobic conditions for 28 days. Biodegradation was measured based on oxygen consumption. The test substance reached 63% biodegradation on day 6, and 92% by day 28. The reference substance was sodium benzoate, which provided the expected results. The substance was readily biodegradable, based on > 60% in 28 days, and met the 10-day window based on > 60% by day 6 (Klimisch 2, reliable with restrictions) (Madsen et al. 2001).
- In an inherent biodegradability test conducted according to OECD TG 302 B (Zahn-Wellens/EMPA Test) and DIN 38.412, industrial non-adapted activated sludge was exposed to methylparaben (purity not reported) at a concentration of > 50 - < 400 mg/L for 6 days. A degradation rate of 100% was achieved at the end of the exposure period. The substance was considered inherently biodegradable (Klimisch 2, reliable with restrictions) (Wellens 1990).
- In an anaerobic screening test conducted according to the method as described by ISO 11734, methylparaben attained 40% of the theoretical gas production in 90 days. The substance was considered inherently biodegradable under anaerobic conditions. The reference substance was sodium acetate, which provided the expected results (Klimisch 2, reliable with restrictions) (Madsen et al. 2001).

- U.S. EPA 2017
  - The Level III Fugacity model (MCI method) predicts methylparaben will partition primarily to soil at 79.6% with a half-life of 30 days, 20% will partition to water with a half-life of 15 days, 0.106% will partition to sediment with a half-life of 135 days, and 0.0401% will partition to air with a half-life of 23.2 hours (Appendix E).

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL

Methylparaben is assigned a score of Very Low for bioaccumulation based on measured data indicating a log K_{ow} of 1.98 and the most conservative predicted BCF of 9.406. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the log K_{ow} is ≤ 4 and the BCF is ≤ 100 (CPA 2018b). The confidence in the score is high based on a measured log K_{ow} and a conservatively modeled BCF.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - Methylparaben has a measured log K_{ow} of 1.98 at 20°C obtained from a shake flask method similar to OECD TG 107.
- U.S. EPA 2017
  - BCFBAF predicts a BCF of 9.406 L/kg wet-weight, using the regression based model based on a measured log K_{ow} of 1.98, and a BCF of 4.007 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix E).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): L

Methylparaben is assigned a score of Low for reactivity based on measured data demonstrating it is not self-igniting or oxidizing, and based on its molecular structure with lacks reactive functional groups associated with explosivity. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on high quality measured data and physico-chemical properties. It may be noted that no data were found regarding corrosivity to metal.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - Methylparaben was evaluated for self-ignition in a GLP-compliant study performed according to EU Method A.16. The test substance (≥ 99% purity) melted in the range of 125-160°C, and could not be ignited up to 403 °C, therefore the self-ignition temperature of the test substance was > 403°C (Klimisch 1, reliable without restriction) (Unnamed 2009 study).
  - Methylparaben was evaluated in a GLP-compliant study performed according to EU Method A.17, oxidising properties (solids). The test substance (⩾ 99% purity) was mixed with powdered cellulose. The maximum burning rate under aerobic conditions was 3.64 mm/s at a 60% mixture in cellulose. As the burning rate of the test substance was faster than the reference substance, a follow-up test was performed in which the test item mixtures were tested with silica gel. In the second test, no independent burning of the test item occurred with 50-70% mixtures. Authors concluded the test substance is not oxidizing (Klimisch 1, reliable without restriction) (Unnamed 2009 study).
- No measured data were identified for explosivity. Therefore, screening procedures were used here to estimate the reactivity property of methylparaben. These procedures are listed in the GHS (UN 2021).
  - Based on the structure of its components or moieties, methylparaben is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix F).

Flammability (F) Score (vH, H, M, or L): L

Methylparaben is assigned a score of Low for flammability based on measured data indicating the substance is not flammable in a guideline test. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score was high based on measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a[9]
  - Methylparaben was evaluated in a GLP-compliant study for the flammability of solids, according to EU Method A.10. The test substance (≥ 99% purity) did not ignite on contact with air. In the course of the preliminary test, the item could not be ignited, but melted. Authors of the REACH dossier concluded the test substance is not flammable (no further details provided) (Klimisch 1, reliable without restriction) (Unnamed 2009 study).

Use of New Approach Methodologies (NAMs)[13] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* testing for mutagenicity, endocrine activity, and skin irritation, and *in silico* modeling for respiratory sensitization and bioaccumulation. NAMs are non-animal alternatives that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

As shown in Table 4, Type I (input data) uncertainties in methylparaben's NAMs dataset include lack of experimental data and lack of validated methods for assessing respiratory sensitization. Methylparaben's Type II (extrapolation output) uncertainties include reliance on *in vitro* data in which the exogenous metabolic activation does not entirely mimic *in vivo* conditions and extrapolation of skin sensitization data to respiratory sensitization which is incomplete in that it does not account for non-immunologic mechanisms of respiratory sensitization. Some of methylparaben's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses

Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Respiratory sensitization**: No experimental data are available and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD TG 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[14]. The mammalian cell gene mutation assay (as defined in OECD TG 476) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[15]

[13] NAMs refers to any non-animal technology, methodology, approach, or combination thereof that inform chemical hazard and risk assessments. NAMs include *in silico*/computational tools, *in vitro* biological profiling (e.g., cell cultures, 2,3-D organotypic culture systems, genomics/transcriptomics, organs on a chip), and frameworks (i.e., adverse outcome pathways (AOPs), defined approaches (DA), integrated approaches to testing and assessment (IATA).
[14] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[15] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE

	The *in vitro* chromosome aberration assay (OECD TG 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[16]. **Endocrine activity:** The exogenous metabolic activation system does not entirely mimic *in vivo* conditions. The relevance of available data to human health (e.g., weak endocrine activity *in vitro*) is not known. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.

Endpoint	NAMs Data Available and Evaluated? (Y/N)	Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)
Carcinogenicity	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* tests for estrogen receptor binding
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	N	
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	Y	*In vitro* skin irritation study
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

[16] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

References

Byford, J.R., L.E. Shaw, M.G. Drew, G.S. Pope, M.J. Sauer, and P.D. Darbre. 2002. Oestrogenic activity of parabens in MCF7 human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 80(1):49-60. [Abstract Only].

Chemical Carcinogenesis Research Information System (CCRIS). 1992. CAS #99-76-3. United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/substance/?source=CCRIS&sourceid=99-76-3

Chemical Entities of Biological Interest (ChEBI). 2017. Entry for methylparaben. Available: methylparaben (CHEBI:31835) (Accessed March 21, 2023).

Chen, J.G., K.C. Ahn, N.A. Gee, S.J. Gee, B.D. Hammock, and B.L. Lasley. 2007. Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. *Toxicol. Appl. Pharmacol*. 221(3):278-284. [Abstract Only]. Available: http://www.ncbi.nlm.nih.gov/pubmed/17481686

Clean Production Action (CPA). 2018a. GreenScreen® Assessment Expiration Policy. October 2, 2018.

Clean Production Action (CPA). 2018b. The GreenScreen® for Safer Chemicals Guidance. Version 1.4 Guidance. Dated January, 2018. Available: https://www.greenscreenchemicals.org/static/ee_images/uploads/resources/GreenScreen_Guidance_v1_4_2018_01_Final.pdf

Cosmetic Ingredient Review (CIR). 2008. Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products. *Int. J. Toxicol*. 27 (Suppl. 4): 1-82. Available: http://www.cir-safety.org/sites/default/files/PR427.pdf

Cosmetic Ingredient Review (CIR). 2020. Amended safety assessment of parabens as used in cosmetics. *Int. J. Toxicol*. 39(Suppl. 1):5S-97S.

Danish Centre on Endocrine Disrupters. 2012. Evaluation of tebuconazole, triclosan, methylparaben and ethylparaben according to the Danish proposal for criteria for endocrine disrupters. May 2012. Available: https://mst.dk/media/mst/9106715/chemicalsreportandannex.pdf

Darbre, P.D., A. Aljarrah, W.R. Miller, N.G. Coldham, M.J. Sauer, and G.S. Pope. 2004. Concentrations of parabens in human breast tumors. *J. Applied Toxicol.* 24: 5-13. [As cited in Darbre and Harvey 2008].

Darbre, P.D., and P.W. Harvey. 2008. Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. *J. Applied Toxicol.*. DOI: 10.1002/jat.1358.

European Chemicals Agency (ECHA). 2017. Guidance on information requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. Dated: July 2017.

Available:
https://echa.europa.eu/documents/10162/17224/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2567f?t=1500286622893

European Chemicals Agency (ECHA). 2018. Decision on a compliance check. Decision number CCH-D-2114412038-60-01/F. Methyl 4-hydroxybenzoate (CAS #99-76-3). Available: https://echa.europa.eu/documents/10162/f84aa19d-601d-51e6-fc53-d77133ae5912

European Chemicals Agency (ECHA). 2023a. REACH Dossier for Methylparaben (CAS #99-76-3). Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/14310/1

European Chemicals Agency (ECHA). 2023b. Classification and Labeling Inventory (C &L). Entry for Methylparaben (CAS #99-76-3). Available: https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/97250

European Chemicals Agency (ECHA). 2023c. REACH Dossier for Propylparaben (CAS #94-13-3). Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/13890

European Chemicals Agency (ECHA). 2023d. REACH Dossier for Ethylparaben (CAS #120-47-8). Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/13843

FooDB. 2023. FooDB Version 1.0. Entry for methylparaben. Available: https://foodb.ca/compounds/FDB010509 (Accessed March 21, 2023).

Gomez, E, A. Pillon, H. Fenet, D. Rosain, M.J. Duchesne, J.C. Nicolas, P. Balaguer, and C. Casellas. 2005. Estrogenic activity of cosmetic components in reporter cell lines: parabens, UV screens, and musks. *J. Toxicol. Environ. Health A* 68(4):239-251. [Abstract Only]. Available: http://www.ncbi.nlm.nih.gov/pubmed/15799449

Hazardous Substances Data Bank (HSDB). 2017. Entry for methylparaben, CAS #99-76-3. Available: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/1184 (Accessed March 21, 2023).

Health Canada (HC). 2020. Draft screening assessment, parabens group. Available: https://www.canada.ca/content/dam/eccc/documents/pdf/pded/parabens/Draft-screening-assessment-parabens-group.pdf

Madden, J.C., S.J. Enoch, A. Paini, and M.T.D. Cronin. 2020. A review of *in silico* tools as alternatives to animal testing: principles, resources, and applications. *Alt. Lab. Animals.* 1-27. Available: https://journals.sagepub.com/doi/pdf/10.1177/0261192920965977

McHugh. B. 2022. Wide-scope target and suspect screening of emerging contaminants and their transformation products in marine biota samples from the North-East Atlantic. In : OSPAR, 2023 : The 2023 Quality Status Report for the Northeast Atlantic. OSPAR Commission, London. Available: https://oap.ospar.org/en/ospar-assessments/quality-status-reports/qsr-2023/other-assessments/connect-study

National Cancer Institute (NCI). 1977. Toxicity of 4:1 mixture of methyl :propyl parabens in saline following 24-hour IV infusion in Beagle Dogs. Final Report. Available : www.toxplanet.com

Oishi, S. 2004. Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats. *Food Chem. Toxicol*. 42(11), 1845-1849. [Abstract Only].

Organisation for Economic Co-operation and Development (OECD). 2020. Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA), Series on Testing and Assessment, No. 329, Environment, Health and Safety, Environment Directorate. Available: http://www.oecd.org/chemicalsafety/risk-assessment/concepts-and-available-guidance-related-to-integrated-approaches-to-testing-and-assessment.pdf

Organisation for Economic Co-operation and Development (OECD). 2022. OECD QSAR Toolbox for Grouping Chemicals into Categories Version 4.5. SP1. Available: http://toolbox.oasis-lmc.org/?section=download&version=latest.

Pharos. 2023. Pharos chemical and material library entry for methylparaben (CAS #99-76-3). Available: http://www.pharosproject.net/material/.

Prival, M.J., V.F. Simmon, and K.E. Mortelmans. 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. *Mutat. Res*. 260(4):321-329. (Secondary reference as cited in CCRIS 1992).

PubChem. 2023. Methylparaben (CAS #99-76-3). United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/compound/7456

Routledge, E.J., J. Parker, J. Odum, J. Ashby, and J.P. Sumpter. 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Appl. Pharmacol* 153(1):12-19. [Abstract Only]. Available: https://www.sciencedirect.com/science/article/pii/S0041008X98985441

Scientific Committee on Consumer Products (SCCP). 2005a. Extended Opinion on the Safety Evaluation of Parabens. Adopted by the SCCP by written procedure on 28 January 2005. Available: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_019.pdf.

Scientific Committee on Consumer Products (SCCP). 2005b. Extended Opinion of Parabens, underarm cosmetics and breast cancer. Adopted by the SCCP as written procedure on 28 January 2005. Available: http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_00d.pdf.

Scientific Committee on Consumer Safety (SCCS). 2011. Opinion on Parabens. COLIPA no P82. Available: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_041.pdf

Scientific Committee on Consumer Safety (SCCS). 2013. Opinion on Parabens. COLIPA nº P82. 3 May 2013. The SCCS adopted this opinion at its 9th plenary on 14 December 2010. Available: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_132.pdf

Song, B.L., D.R. Peng, H.Y. Li, G.H. Zhang, J. Zhang, K.L. Li, and Y.Q. Zhao. 1991. Evaluation of the effect of butyl p-hydroxybenzoate on the proteolytic activity and membrane function of human spermatozoa. *J. Reprod. Fertil*, 91:435-440. [Abstract Only]. Available: http://www.ncbi.nlm.nih.gov/pubmed/2013872

The Endocrine Disruption Exchange (TEDX). 2015. TEDX List of Potential Endocrine Disruptors. Methylparaben (CAS #99-76-3). Available: https://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/search-the-tedx-list?sname=&x=0&y=0&action=search&sall=1&searchfor=any&scas=94-13-3&searchcats=all#sname=99-76-3&searchfor=any&sortby=chemname&action=search&searchcats=all&sortby=chemname

ToxServices. 2021. SOP 1.37: GreenScreen® Hazard Assessments. Dated: May 24, 2021.

United Nations (UN). 2021. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Ninth revised edition.

United States Department of Transportation (U.S. DOT). 2008a. Chemicals Listed with Classification. 49 CFR § 172.101. Available: http://www.gpo.gov/fdsys/pkg/CFR-2008-title49-vol2/pdf/CFR-2008-title49-vol2-sec172-101.pdf.

United States Department of Transportation (U.S. DOT). 2008b. Classification Criteria. 49 CFR § 173. Available: http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&tpl=/ecfrbrowse/Title49/49cfr173_main_02.tpl

United States Environmental Protection Agency (U.S. EPA). 2015. Safer Choice Standard. Available: https://www.epa.gov/saferchoice/standard

United States Environmental Protection Agency (U.S. EPA). 2017. Estimation Programs Interface (EPI) Suite™ Web, v4.11, Washington, DC, USA. Available: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm.

United States Environmental Protection Agency (U.S. EPA). 2020. New Approach Methods Workplan. Office of Research and Development. Office of Chemical Safety and Pollution Prevention. EPA 615B20001. June 2020. Available: https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf

United States Environmental Protection Agency (U.S. EPA). 2023. Safer Chemical Ingredients List (SCIL). Available: https://www.epa.gov/saferchoice/safer-ingredients

United States Food and Drug Administration (U.S. FDA). 2022. Substances Added to Food (formerly EAFUS). Available: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances

United States Food and Drug Administration (U.S. FDA). 2023. Inactive ingredients database. Available: https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm

APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for Methylparaben (CAS #99-76-3)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

	Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
	Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
							S	R *	S	R *	*	*								
Inorganic Chemical?	Chemical Name	CAS#	C	M	R	D	E	AT	STs	STr	Ns	Nr	SNS*	SNR*	IrS	IrE	AA	CA	P	B	Rx	F
No	Methylparaben	99-76-3	L	L	L	L	*M*	L	*M*	L	*L*	L	L	*L*	L	L	M	*H*	vL	vL	L	L

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	g
1	No	No	No	No	No		
2	No	No	No	No	Yes	No	No
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Methylparaben	2

Note: Chemical has not undergone a data gap assessment. Not a Final GreenScreen™ Score

Table 6

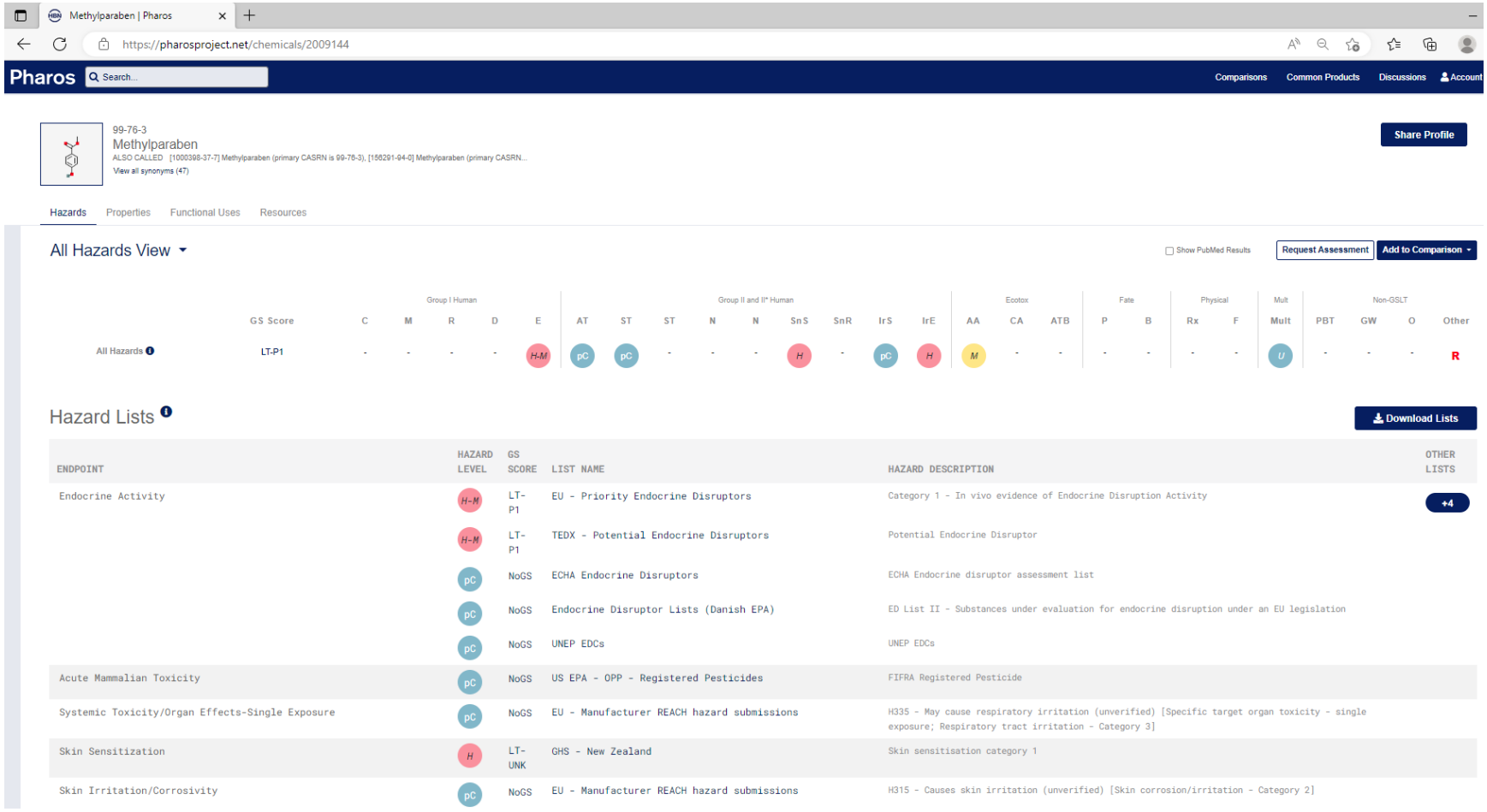
Chemical Name	Final GreenScreen® Benchmark Score
Methylparaben	2

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												
2	Yes	Yes	Yes	Yes	Yes							2
3												
4												

APPENDIX C: Pharos Output for Methylparaben (CAS #99-76-3)



Methylparaben | Pharos

https://pharosproject.net/chemicals/2009144

Pharos | Search... | Comparisons | Common Products | Discussions | Account

99-76-3
Methylparaben
ALSO CALLED [1000398-37-7] Methylparaben (primary CASRN is 99-76-3), [156291-94-0] Methylparaben (primary CASRN...
View all synonyms (47)

Share Profile

Hazards | Properties | Functional Uses | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

		Group I Human					Group II and II* Human								Ecotox			Fate		Physical		Mult	Non-GSLT			
	GS Score	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
All Hazards	LT-P1	-	-	-	-	H-M	pC	pC	-	-	-	H	-	pC	H	M	-	-	-	-	-	-	U	-	-	-	R

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Endocrine Activity	H-M	LT-P1	EU - Priority Endocrine Disruptors	Category 1 - In vivo evidence of Endocrine Disruption Activity	+4
	H-M	LT-P1	TEDX - Potential Endocrine Disruptors	Potential Endocrine Disruptor	
	pC	NoGS	ECHA Endocrine Disruptors	ECHA Endocrine disruptor assessment list	
	pC	NoGS	Endocrine Disruptor Lists (Danish EPA)	ED List II - Substances under evaluation for endocrine disruption under an EU legislation	
	pC	NoGS	UNEP EDCs	UNEP EDCs	
Acute Mammalian Toxicity	pC	NoGS	US EPA - OPP - Registered Pesticides	FIFRA Registered Pesticide	
Systemic Toxicity/Organ Effects-Single Exposure	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
Skin Sensitization	H	LT-UNK	GHS - New Zealand	Skin sensitisation category 1	
Skin Irritation/Corrosivity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	

Eye Irritation/Corrosivity	H	LT-UNK	GHS - New Zealand	Eye irritation category 2	+2
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H318 - Causes serious eye damage (unverified) [Serious eye damage/eye irritation - Category 1]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H319 - Causes serious eye irritation (unverified) [Serious eye damage/eye irritation - Category 2A]	
Acute Aquatic Toxicity	M	LT-UNK	GHS - Japan	H402 - Harmful to aquatic life [Hazardous to the aquatic environment (acute) - Category 3]	
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-UNK	GHS - Japan	H412 - Harmful to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 3]	+2
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H411 - Toxic to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 2]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H412 - Harmful to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 3]	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-UNK	German FEA - Substances Hazardous to Waters	Class 1 - Low Hazard to Waters	

Restricted Substance Lists (14)

- CA SCP - Candidate Chemicals: Candidate Chemical List
- Credo Beauty's Restricted Substance List: Prohibited Chemicals
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- GreenScreen Certified Standard for Food Service Ware RSL: Ortho-Phthalates
- GreenScreen Certified Standard for Food Service Ware RSL: Parabens
- HEL LIST - Chemicals Prohibited by the Protect Land + Sea Certification: Prohibited Chemicals
- MDH - Chemicals of High Concern and Priority Chemicals: Chemicals of High Concern
- ME DEP - Chemicals of High Concern and Priority Chemicals: Chemicals of High Concern
- SCHF - Hazardous 100: Chemicals of high concern
- Sephora - High Priority Chemicals: High priority chemicals
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Chemical Substance Inventory - Active
- Vermont Chemicals of High Concern to Children: Chemicals of High Concern to Children
- WA DoE - Chemicals of High Concern to Children: Chemicals of High Concern to Children

Positive Lists (4)

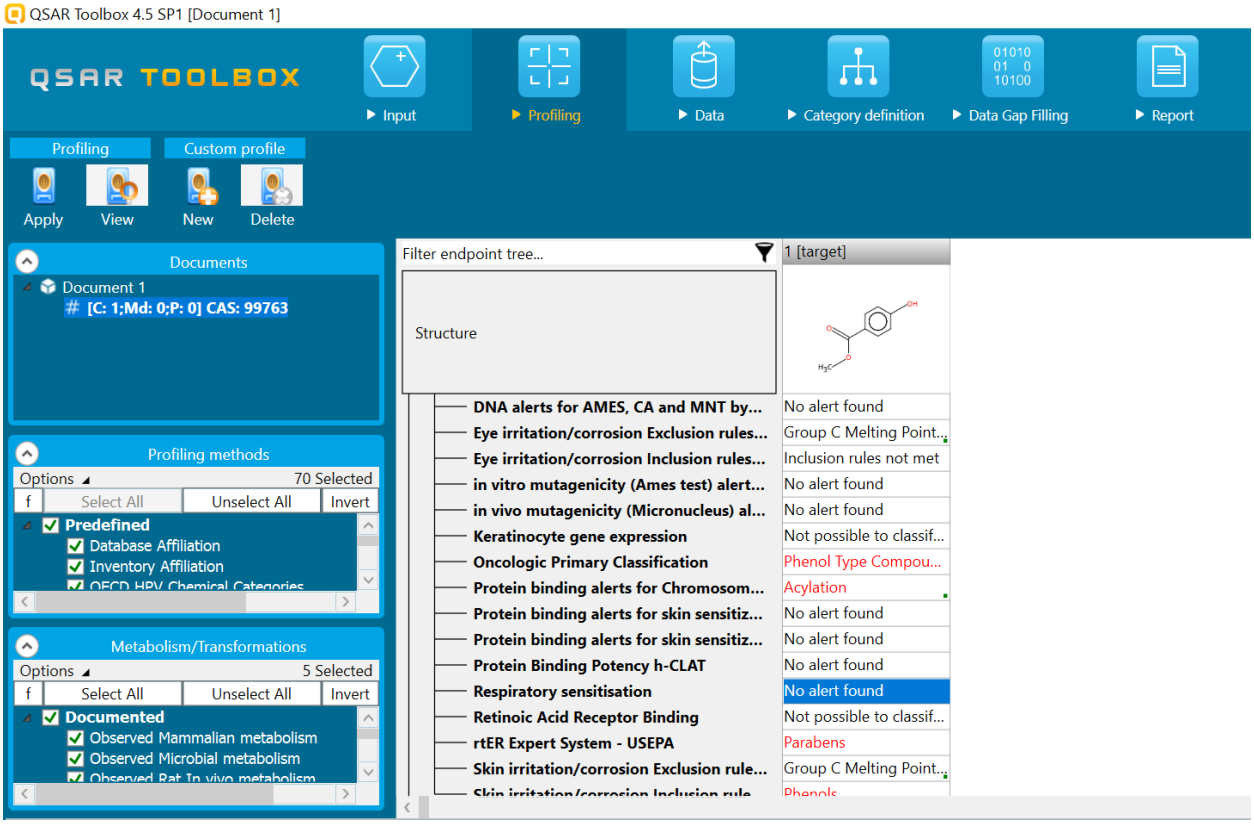
- Cosmetic Ingredient Review (CIR): Safe with Qualifications
- EU - Cosmetics Regulation: Annex V - Preservatives Allowed
- GB 9685 National Food Safety Standard (2016): GB 9685 National Food Safety Standard (2016)
- Inventory of Existing Cosmetic Ingredients in China (IECIC 2015): Cosmetic Ingredients

Discussions

No discussions have been posted yet.

Ask a question about this chemical in the forums >

APPENDIX D: OECD Toolbox Profiling Results for Methylparaben (CAS #99-76-3)



APPENDIX E: EPI Suite™ Modeling Results for Methylparaben (CAS #99-76-3)

(Estimated values included in the GreenScreen® are highlighted)

```
CAS Number: 99-76-3
SMILES : O=C(OC)c(ccc(O)c1)c1
CHEM   : Benzoic acid, 4-hydroxy-, methyl ester
MOL FOR: C8 H8 O3
MOL WT : 152.15
---------------------------- EPI SUMMARY (v4.11) --------------------------
 Physical Property Inputs:
    Log Kow (octanol-water):   1.98
    Boiling Point (deg C)  :   ------
    Melting Point (deg C)  :   125.00
    Vapor Pressure (mm Hg) :   ------
    Water Solubility (mg/L):   1880
    Henry LC (atm-m3/mole) :   ------

 Log Octanol-Water Partition Coef (SRC):
    Log Kow (KOWWIN v1.69 estimate) =  2.00
    Log Kow (Exper. database match) =  1.96
       Exper. Ref:  HANSCH,C ET AL. (1995)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
    Boiling Pt (deg C):  252.18  (Adapted Stein & Brown method)
    Melting Pt (deg C):  51.47  (Mean or Weighted MP)
    VP(mm Hg,25 deg C):  0.000994  (Modified Grain method)
    VP (Pa, 25 deg C) :  0.132  (Modified Grain method)
    MP  (exp database):  131 deg C
    BP  (exp database):  275 dec deg C
    Subcooled liquid VP: 0.00986 mm Hg (25 deg C, Mod-Grain method)
                       : 1.31 Pa (25 deg C, Mod-Grain method)

 Water Solubility Estimate from Log Kow (WSKOW v1.42):
    Water Solubility at 25 deg C (mg/L):  3449
       log Kow used: 1.98 (user entered)
       melt pt used: 125.00 deg C
     Water Sol (Exper. database match) =  2500 mg/L (25 deg C)
        Exper. Ref:  YALKOWSKY,SH & HE,Y (2003)

 Water Sol Estimate from Fragments:
    Wat Sol (v1.01 est) =  4250.6 mg/L

 ECOSAR Class Program (ECOSAR v1.11):
    Class(es) found:
       Esters
       Phenols

 Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
   Bond Method :   3.61E-009  atm-m3/mole  (3.66E-004 Pa-m3/mole)
   Group Method:   2.23E-009  atm-m3/mole  (2.26E-004 Pa-m3/mole)
 For Henry LC Comparison Purposes:
   User-Entered Henry LC:  not entered
   Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
      HLC:  1.058E-007 atm-m3/mole  (1.073E-002 Pa-m3/mole)
      VP:   0.000994 mm Hg (source: MPBPVP)
      WS:   1.88E+003 mg/L (source: User-Entered)

 Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
  Log Kow used:  1.98  (user entered)
  Log Kaw used:  -6.831  (HenryWin est)
      Log Koa (KOAWIN v1.10 estimate):  8.811
      Log Koa (experimental database):  8.570

 Probability of Rapid Biodegradation (BIOWIN v4.10):
   Biowin1 (Linear Model)         :   0.9651
   Biowin2 (Non-Linear Model)     :   0.9971
 Expert Survey Biodegradation Results:
   Biowin3 (Ultimate Survey Model):   3.0595  (weeks       )
   Biowin4 (Primary Survey Model) :   3.8969  (days        )
 MITI Biodegradation Probability:
   Biowin5 (MITI Linear Model)    :   0.6261
```

```
  Biowin6 (MITI Non-Linear Model):   0.7786
 Anaerobic Biodegradation Probability:
  Biowin7 (Anaerobic Linear Model):  0.6274
 Ready Biodegradability Prediction:   YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
    Structure incompatible with current estimation method!

 Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
  Vapor pressure (liquid/subcooled):  1.31 Pa (0.00986 mm Hg)
  Log Koa (Exp database): 8.570
   Kp (particle/gas partition coef. (m3/ug)):
       Mackay model           :  2.28E-006
       Octanol/air (Koa) model:  9.12E-005
   Fraction sorbed to airborne particulates (phi):
       Junge-Pankow model     :  8.24E-005
       Mackay model           :  0.000183
       Octanol/air (Koa) model:  0.00724

 Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
   Hydroxyl Radicals Reaction:
      OVERALL OH Rate Constant =  11.0649 E-12 cm3/molecule-sec
      Half-Life =     0.967 Days (12-hr day; 1.5E6 OH/cm3)
      Half-Life =    11.600 Hrs
   Ozone Reaction:
      No Ozone Reaction Estimation
   Reaction With Nitrate Radicals May Be Important!
   Fraction sorbed to airborne particulates (phi):
      0.000132 (Junge-Pankow, Mackay avg)
      0.00724 (Koa method)
    Note: the sorbed fraction may be resistant to atmospheric oxidation

 Soil Adsorption Coefficient (KOCWIN v2.00):
      Koc    :  86.29  L/kg (MCI method)
      Log Koc:  1.936       (MCI method)
      Koc    :  132.3  L/kg (Kow method)
      Log Koc:  2.122       (Kow method)

 Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
  Total Kb for pH > 8 at 25 deg C :  6.365E-003  L/mol-sec
  Kb Half-Life at pH 8:       3.451  years
  Kb Half-Life at pH 7:      34.506  years
    (Total Kb applies only to esters, carbmates, alkyl halides)

 Bioaccumulation Estimates (BCFBAF v3.01):
   Log BCF from regression-based method = 0.973 (BCF = 9.406 L/kg wet-wt)
   Log Biotransformation Half-life (HL) = -1.6691 days (HL = 0.02143 days)
   Log BCF Arnot-Gobas method (upper trophic) = 0.603 (BCF = 4.007)
   Log BAF Arnot-Gobas method (upper trophic) = 0.603 (BAF = 4.007)
       log Kow used: 1.98 (user entered)

 Volatilization from Water:
    Henry LC:  2.23E-009 atm-m3/mole  (estimated by Group SAR Method)
    Half-Life from Model River: 3.239E+005  hours   (1.349E+004 days)
    Half-Life from Model Lake : 3.533E+006  hours   (1.472E+005 days)

 Removal In Wastewater Treatment:
    Total removal:               2.23  percent
    Total biodegradation:        0.10  percent
    Total sludge adsorption:     2.13  percent
    Total to Air:                0.00  percent
      (using 10000 hr Bio P,A,S)

 Level III Fugacity Model: (MCI Method)
           Mass Amount    Half-Life    Emissions
            (percent)       (hr)       (kg/hr)
   Air       0.0401         23.2         1000
   Water     20             360          1000
```

Soil	79.9	720	1000
Sediment	0.106	3.24e+003	0

Persistence Time: 730 hr

Level III Fugacity Model: (MCI Method with Water percents)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0401	23.2	1000
Water	20	360	1000
water	(20)		
biota	(9.54e-005)		
suspended sediment	(0.00259)		
Soil	79.9	720	1000
Sediment	0.106	3.24e+003	0

Persistence Time: 730 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.042	23.2	1000
Water	23.2	360	1000
water	(23.2)		
biota	(0.000111)		
suspended sediment	(0.00136)		
Soil	76.7	720	1000
Sediment	0.0801	3.24e+003	0

Persistence Time: 698 hr

```
CAS Number: 99-76-3
SMILES : O=C(OC)c(ccc(O)c1)c1
CHEM   : Benzoic acid, 4-hydroxy-, methyl ester
MOL FOR: C8 H8 O3
MOL WT : 152.15
------------------------------ EPI SUMMARY (v4.11) --------------------------
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    Vapor Pressure (mm Hg) :   ------
    Water Solubility (mg/L):   1880
    Henry LC (atm-m3/mole) :   ------

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       Phenols

 Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
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   Biowin4 (Primary Survey Model) :   3.8969  (days        )
 MITI Biodegradation Probability:
   Biowin5 (MITI Linear Model)    :   0.6261
```
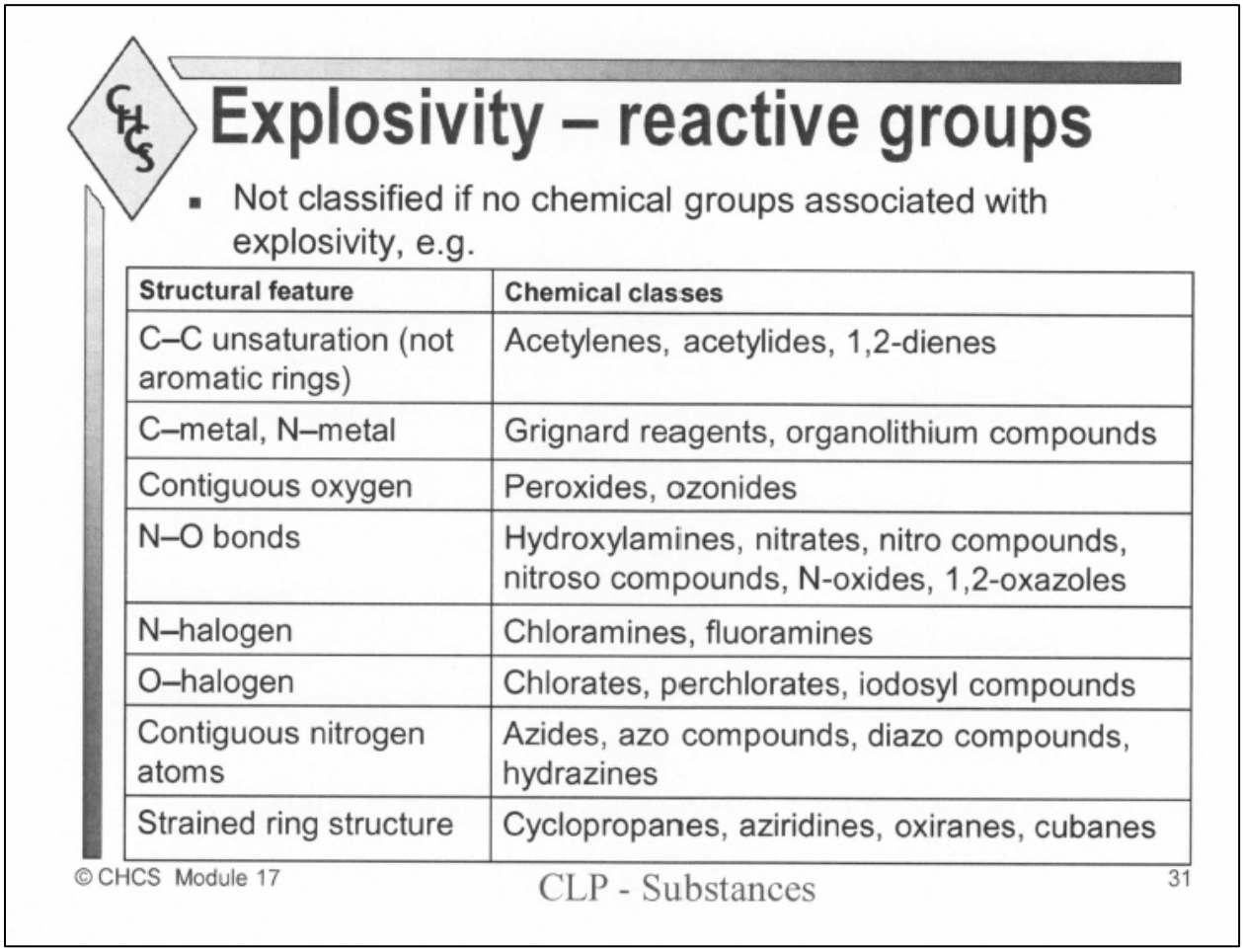

APPENDIX F: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



Explosivity – reactive groups

- Not classified if no chemical groups associated with explosivity, e.g.

Structural feature	Chemical classes
C–C unsaturation (not aromatic rings)	Acetylenes, acetylides, 1,2-dienes
C–metal, N–metal	Grignard reagents, organolithium compounds
Contiguous oxygen	Peroxides, ozonides
N–O bonds	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
N–halogen	Chloramines, fluoramines
O–halogen	Chlorates, perchlorates, iodosyl compounds
Contiguous nitrogen atoms	Azides, azo compounds, diazo compounds, hydrazines
Strained ring structure	Cyclopropanes, aziridines, oxiranes, cubanes

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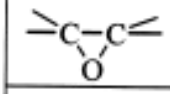
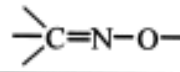
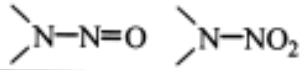
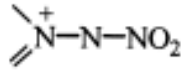
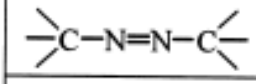
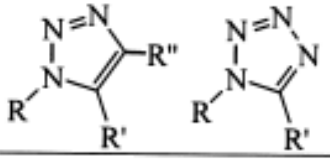
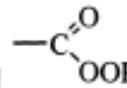
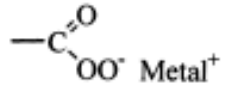
Explosivity – Full List

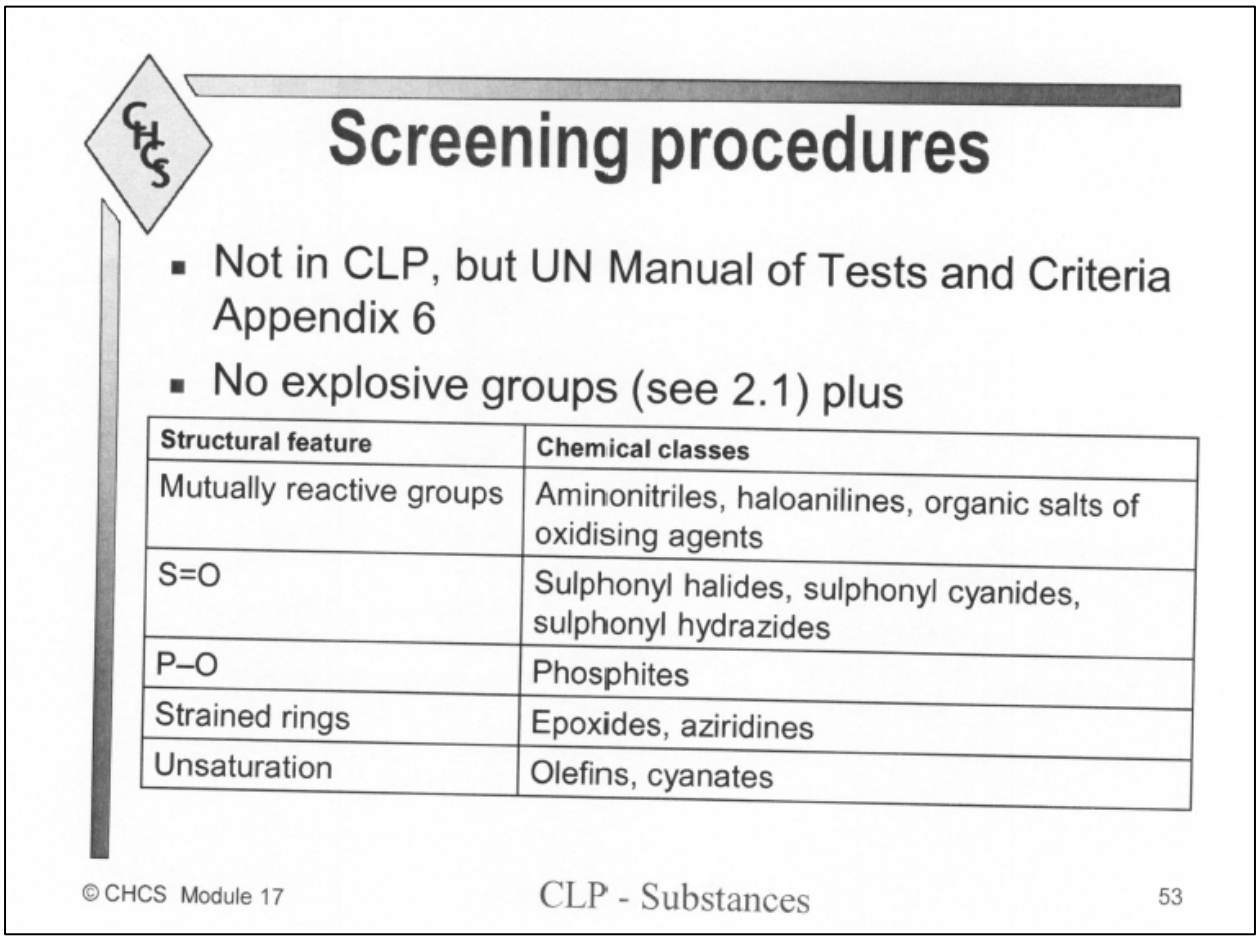
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	Chemical Class
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
	Diazo Compounds
-N=O -NO_2	Nitroso and Nitro Compounds,
R-O-N=O R-O-NO_2	Acyl or Alkyl Nitrites and Nitrates
	1,2-Epoxides
	Metal Fulminates or *aci*-Nitro Salts
	N-Metal Derivatives (especially heavy metals)
	N-Nitroso and N-Nitro Compounds
	N-Azolium Nitroimidates
	Azo Compounds
Ar-N=N-O-Ar	Arene Diazoates
$(ArN=N)_2O$, $(ArN=N)_2S$	Bis-Arenediazo Oxides and Sulfides
RN=N-NR'R''	Triazines
	High-nitrogen Compounds: e.g. Triazoles, Tetrazoles

Chemical group	Chemical Class
[1] ROOR', [2] 	Peroxy Compounds: [1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic); [2] Peroxo acids (R'=H), Peroxyesters (R'=organic)
[1] ROOMetal, [2] 	Metal peroxides, Peroxoacids salts
$-N_3$	Azides e.g. PbN_6, CH_3N_3
$^{-}O-C-N_2^{+}$	Arenediazonium oxides i.e. inner diazonium salts in which the counter ion is an oxide
Ar-N=N-S- Ar-N=N-S-Ar	Diazonium sulfides and derivatives, Arenediazo Aryl Sulfides
XO_n	Halogen Oxide: e.g. percholrates, bromates, etc
NX_3 e.g. $NC1_3$, $RNC1_2$	N-Halogen Compounds

Adapted from Bretherick (Bretherick's Handbook of Reactive Chemical Hazards 6th Ed., 1999, Butterworths, London)

Self-Reactive Substances



APPENDIX G: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for methylparaben. The GreenScreen® Benchmark Score for methylparaben has not changed over time. The original GreenScreen® assessment was performed in 2018 under version 1.4 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. The BM-2 score was maintained in the current version 1.4 update. Several new studies were identified in the public literature and are incorporated herein. These studies add to the weight of evidence for numerous endpoints and fulfill the previously identified data gap for reproductive toxicity.

Table 5: Change in GreenScreen® Benchmark™ for Methylparaben

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
July 12, 2018	BM-2	v. 1.4	Original GreenScreen® assessment.
April 11, 2023	BM-2	v. 1.4	Data gap for reproductive toxicity fulfilled. Several new studies in the public literature have been incorporated and add to the weight of evidence for multiple endpoints. These changes do not affect the final Benchmark score.
June 21, 2023	BM-2	v. 1.4	Minor changes to skin sensitization are incorporated based on Washington Ecology's feedback. These changes do not affect the final Benchmark score.

Licensed GreenScreen® Profilers

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