# ETHYLPARABEN

## (CAS #120-47-8)

## GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

**Prepared by:**

**ToxServices LLC**

**Assessment Date: June 21, 2023**

**Expiration Date: June 21, 2028**

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING
1367 Connecticut Ave., N.W., Suite 300
Washington, D.C. 20036

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## GreenScreen® Executive Summary for Ethylparaben (CAS #120-47-8)

Ethylparaben is a commonly used preservative in pharmaceuticals and cosmetic products. It is a crystalline powder at room temperature, and is not explosive, oxidizing, or flammable. If released to the environment, ethylparaben is expected to partition to soil and water. Ethylparaben 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).

Ethylparaben 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)

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* testing for genotoxicity, endocrine activity, and skin irritation, and *in silico* modeling for respiratory sensitization, chronic aquatic toxicity, 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 ethylparaben's NAMs dataset include lack of experimental data and validated methods for assessing respiratory sensitization. Ethylparaben'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 ethylparaben'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 Ethylparaben

| 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** | **L** | **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 Ethylparaben (CAS #120-47-8)

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

**GreenScreen® Assessment (v.1.4) Prepared By:**
Name: Nancy Linde, M.S.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: March 30, 2023; June 8, 2023

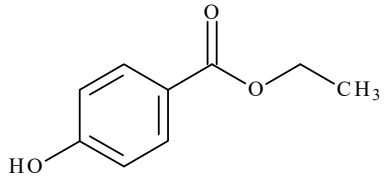
**Quality Control Performed By:**
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: April 17, 2023; June 21, 2023

Expiration Date: June 21, 2028[2]

**Chemical Name:** Ethylparaben

**CAS Number:** 120-47-8

**Chemical Structure(s):**

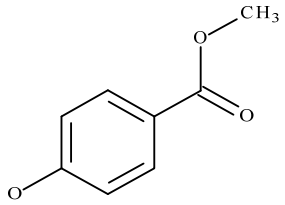



**Also called:** Ethyl 4-hydroxybenzoate; ethyl p-hydroxybenzoate; ethyl parahydroxybenzoate; 4-hydroxybenzoic acid ethyl ester; p-hydroxybenzoic acid ethyl ester; benzoic acid, 4-hydroxy-, ethyl ester; p-carbethoxyphenol; p-oxybenzoesaeureaethylester; 4-carbethoxyphenol; p-hydroxybenzoate ethyl ester; EC 204-399-4; HSDB 938; EINECS 204-399-4 (PubChem 2023)

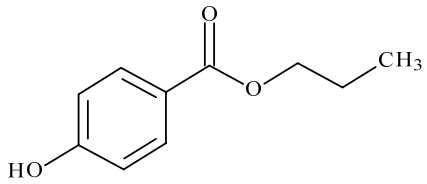
**Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):**
Ethylparaben has a relatively limited dataset. In its REACH registration dossier, as well as assessments by Health Canada (2020), Cosmetic Ingredient Review (CIR) (2020), and the Scientific Committee on Consumer Safety (SCCS) (2011), methylparaben (CAS# 99-76-3), propylparaben (CAS# 94-13-3), isopropylparaben (CAS #4191-73-5), butylparaben (CAS# 94-26-8), and isobutylparaben (CAS #4247-02-3) were used as surrogates to either fill data gaps or add supporting evidence. As discussed in the toxicokinetic section below, some data suggest toxicity of parabens increases with increasing alkyl chain length, and other data demonstrate metabolism of ethylparaben is faster and more similar to that of methylparaben. Therefore, as methylparaben and propylparaben each differ from ethylparaben by only 1 methyl group each, both are considered strong surrogates. In some cases where data are insufficient or

[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).

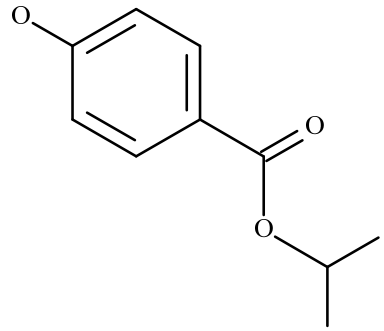
weak for ethylparaben and propylparaben, data for the other short-chained parabens are also used in the weight of evidence.



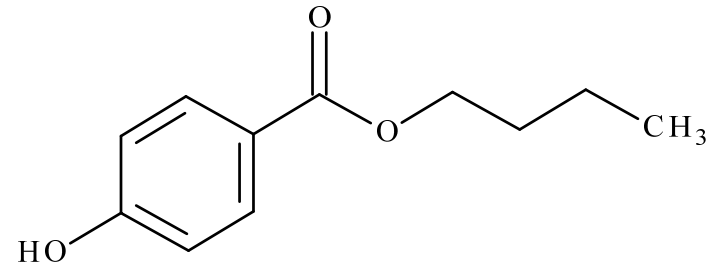
Methylparaben (CAS #99-76-3)



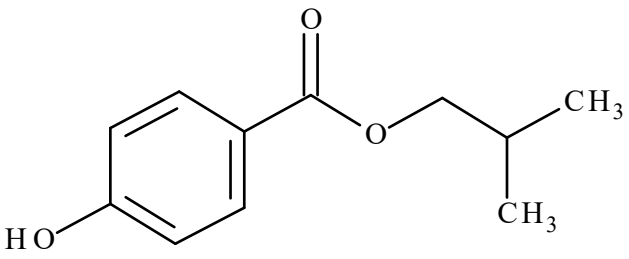
Propylparaben (CAS #94-13-3)



Isopropylparaben (CAS #4191-73-5)



Butylparaben (CAS #94-26-8)




Isobutylparaben (CAS #4247-02-3)

**Identify Applications/Functional Uses:**
A preservative in cosmetics and numerous consumer and industrial products. Reported maximum use levels in cosmetics include 0.5% in rinse-off products (e.g., shampoo), 0.65% in leave-on products, 0.65% in products used near the eye (e.g., mascara), 0.1% bath oils, tablets, and salts, 0.032% in baby lotions, oils, and creams (CIR 2020). Ethylparaben is approved for use as an excipient (inactive ingredient) in pharmaceuticals (e.g., up to 250 mg in nasal solutions, 800 mg in elixir, 1.8 mg in oral tablets) (U.S. FDA 2023). Contrary to methylparaben and propylparaben, ethylparaben does not have any approved food uses in the United States (U.S. FDA 2022).

**Known Impurities[3]:**
p-Hydroxybutanoic acid is a commonly specified impurity for the parabens 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 ethylparaben, as well as a functional group, and primary metabolite. This GreenScreen®, however, is performed on the theoretical pure substance.

**GreenScreen® Summary Rating for Ethylparaben[4,5 6,7]:** Ethylparaben was 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 Ethylparaben**

| 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 | L | L | *L* | L | L | *L* | L | L | M | *H* | vL | vL | *L* | L |

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

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 ethylparaben 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 ethylparaben in 26% of mollusks and 3% of fish samples collected over OSPAR Regions I to IV (primarily western European coastal regions of the Atlantic Ocean), with a maximum concentration of 26.2 µg/kg wet weight (Appendix 3, McHugh 2022). 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.

## Introduction

Ethylparaben is an ester of p-hydroxybenzoate that is used as an antimicrobial preservative in drugs and cosmetics for over 50 years. It is produced by the *n*-propanol esterification of *p*-hydroxybenzoic acid in the presence of sulfuric acid followed by distillation (HSDB 2017). In 2012, the European Food Safety Authority (EFSA) Scientific Panel on Food Additives, Flavorings, Processing Aids and Materials in Contact with Food evaluated the safety of ethylparaben in food at up to 1,800 µg/person/day and concluded it did not give rise to safety concerns at its current dietary intake (EFSA 2012).

ToxServices assessed ethylparaben 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 2023a). 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).

Ethylparaben 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

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

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 ethylparaben can be found in Appendix C.

- Ethylparaben is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Ethylparaben is not listed on the U.S. DOT list.
- Ethylparaben is on the following GreenScreen®-specified list for multiple endpoints:
  - 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 ethylparaben, and the majority of notifiers as well as its REACH registration dossier authors indicate it is not classified, as shown in Table 1 (ECHA 2023b). 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 Ethylparaben (CAS #120-47-8) (ECHA 2023a,b)**

| H Statement | H Statement Details |
|---|---|
| No harmonized GHS H statements are reported by the European Chemicals Agency (ECHA). According to the notifications provided by companies to ECHA, no hazards have been classified. | |

**Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Ethylparaben (CAS #120-47-8)**

| Personal Protective Equipment (PPE) | Reference | Occupational Exposure Limits (OEL) | Reference |
|---|---|---|---|
| Respiratory protection – short term – filter apparatus, Filter P2; wear chemical resistant gloves (according to category III of DIN EN 374), safety goggles, and protective clothing | ECHA 2023a | None | |

**Physicochemical Properties of Ethylparaben**

Ethylparaben is a white or colorless crystalline powder under standard temperature and pressure. Its calculated water solubility indicates that it is moderately soluble in water. It has negligible vapor pressure and is therefore not a volatile organic compound (VOC). Inhalation exposure to dust or aerosol particles is possible, however it is not known to have respirable particle sizes (i.e., < 10 µm) based on 90% having aerodynamic diameter of ≥ 50 µm. It is expected to have a low potential for bioaccumulation based on its measured log $K_{ow}$ of 2.3.

**Table 3: Physical and Chemical Properties of Ethylparaben (CAS #120-47-8)**

| Property | Value | Reference |
|---|---|---|
| Molecular formula | $C_9H_{10}O_3$ | PubChem 2023 |
| SMILES Notation | CCOC(=O)C1=CC=C(C=C1)O | PubChem 2023 |
| Molecular weight | 166.17 | PubChem 2023 |

| Table 3: Physical and Chemical Properties of Ethylparaben (CAS #120-47-8) | | |
|---|---|---|
| **Property** | **Value** | **Reference** |
| Physical state | Solid | ECHA 2023a |
| Appearance | Colorless crystals or white powder | ECHA 2023a |
| Melting point | 117°C | ECHA 2023a |
| Boiling point | 297°C (substance is reported to decompose during boiling) | ECHA 2023a |
| Vapor pressure | 9.56E-5 mmHg at 25°C | U.S. EPA 2017a |
| Water solubility | 750-855 mg/L | ECHA 2023a |
| Dissociation constant | 8.4 at 20°C | ECHA 2023a |
| Density/specific gravity | 1.291 g/cm$^3$ | ECHA 2023a |
| Partition coefficient | 2.3 | ECHA 2023a |
| Particle size distribution | D10: 50 µm<br>D50: 307.5 µm<br>D90: 770.6 µm | ECHA 2023a |

**Toxicokinetics**

Ethylparaben 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).

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 radiolabeled dose of 100 mg/kg ethylparaben orally administered to rats, and a single intravenous dose of 50 mg/kg administered to dogs, was not detected in plasma at any time, and para-hydroxybutanoic acid (PHBA) (the primary metabolite) was detected within 1 hour. The majority of the applied dose (64%) was excreted within 24 hours as PHBA and other (unspecified) metabolites, and only 0.034% was of the parent compound was excreted. At sacrifice, small amounts of ethylparaben were detected in the brain and pancreas (Jones et al. 1956 as cited in HC 2020). While chronic exposure studies indicate that parabens do not accumulate in the body (CIR 2020), ethylparaben has been detected at low levels in tumorous breast tissue, human adipose tissue, and the brain (Barr et al. 2012, Wang et al. 2015, and van der Meer 2017, 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. In liver and skin subcellular fractions, hydrolysis of ethylparaben is 2 to 10 times faster than that of propylparaben and butylparaben (HC 2020). Hydrolysis in human liver cells is rapid based on a half-life of 35 minutes, which is significantly faster than in skin cells, and both are more efficient than plasma (HC 2020). *In vitro* data suggest rat skin and liver cells hydrolyze ethylparaben about 2 to 3 time faster than human skin and liver cells (HC 2020). In rats, but not humans, hydrolysis in the skin as faster with increasing alkyl chain lengths (HC 2020).

## Hazard Classification Summary

### Group I Human Health Effects (Group I Human)

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

Ethylparaben is assigned a score of Low for carcinogenicity based on surrogate data. There are no indications of carcinogenicity in non-standard carcinogenicity studies for multiple 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 structurally close surrogates with multiple routes of exposure.

- 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 2023c[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: Surrogate Methylparaben:* 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: Surrogate Methylparaben:* 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

[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.

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, 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 2023d[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 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.

[10] 1,000 mg/kg/day * 6 days/7 days = 857 mg/kg/day
[11] Values reported in the ECHA REACH Dossier.

  - *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 2020 – no new data were identified.
- CIR 2008
  - "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."
- 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**

Ethylparaben is assigned a score of Low for mutagenicity/genotoxicity based on a negative bacterial reverse mutation assay for the target compound, and numerous *in vitro* and *in vivo* studies for structurally close surrogates that demonstrate lack of mutagenicity or 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 target compound and structurally close surrogates.

- 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:* Ethylparaben was not mutagenic in a GLP-compliant Ames assay conducted according to OECD Test Guideline (TG) 471, EU Method B.13/14, and EPA OPPTS 870.5100. *Salmonella typhimurium* tester strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to the ethylparaben (purity not specified) in DMSO at concentration up to 5,000 µg/plate with and without metabolic activation, using the plate incorporation and preincubation methods. No increase in the mutation frequency was observed in the presence or absence of metabolic activation in any strain, with either method. Testing in each strain was performed up to cytotoxicity limits, or the recommended dose limit, and controls performed as expected (Klimisch 1, reliable without restriction) (Unnamed 2012 study).
- ECHA 2018
  - In its evaluation of the REACH dossier for ethylparaben, ECHA identified data gaps for mutagenicity and clastogenicity. To address the mutagenicity data gap, ECHA recommended performance of an *in vitro* study in mammalian cells, such as OECD TG 476 or 490. To address the clastogenicity data gap, ECHA recommended performance of an *in vitro* study in mammalian cells such as OECD TG 473 or 487. *ToxServices suggests both data gaps are fulfilled by read-across from the surrogates methylparaben and propylparaben, as summarized below.*
- ECHA 2023c[9]
  - *In vitro:* <u>*Surrogate Methylparaben:*</u> Methylparaben was not mutagenic in numerous bacterial reverse mutation assays (Ames assays) at concentration ranging from 50 µg/plate to 10 mg/plate using *S. 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:* <u>*Surrogate Methylparaben:*</u> 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:* <u>*Surrogate Methylparaben:*</u> 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:* *Surrogate Methylparaben:* 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:* *Surrogate Methylparaben:* 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).

- Prival et al. 1991, as cited in CCRIS 1992
  - *In vitro:* *Surrogate Methylparaben:* 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:* *Surrogate Methylparaben:* 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.*
    - 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.*
- ECHA 2023d[9]
    - *In vitro:* <u>*Surrogate Propylparaben:*</u> Propylparaben was not mutagenic when tested in a GLP-compliant bacterial reverse mutation assay conducted according to OECD TG 471. *S. typhimurium* tester strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to propylparaben (99.7% purity) in DMSO at concentrations up to 1 mg/plate, with and without exogenous metabolic activation, using the both the plate incorporation and pre-incubation methods. The positive controls were 2-nitrofluorene, sodium azide and 9-aminoacridine, and mitomycin C for trials without activation, and 2-aminoanthracene for trials with activation. Controls performed as expected. The highest concentration was based on cytotoxicity determined in a preliminary test. There were no increases in the mutation

frequency in any of the tested strains, at any concentration, in the presence or absence of metabolic activation (Klimisch 1, reliable without restriction) (Unnamed 2018 study).

- *In vitro:* Surrogate Propylparaben: Propylparaben was not mutagenic when tested in non-GLP-compliant bacterial reverse mutation assay conducted in a manner equivalent or similar to OECD TG 471. *S. typhimurium* tester strains TA1535, and TA1537 were exposed to propylparaben (purity not specified) in DMSO at concentrations up to 0.075%, with and without exogenous metabolic activation from mice, rats, and primates, using the both the plate incorporation and pre-incubation methods. The positive controls were dimethylnitrosamine 2-acetylaminofluorene with activation, and ethyl methanesulfonate, 2-nitrofluorene, and quinacrine mustard without activation. Controls performed as expected. The highest concentration was based on cytotoxicity determined in a preliminary test. There were no increases in the mutation frequency in any of the tested strains, at any concentration, in the presence or absence of metabolic activation from any of the three species (Klimisch 2, reliable with restrictions) (Unnamed 1975 study).
- *In vitro:* Surrogate Propylparaben: Propylparaben was not clastogenic or aneugenic in a GLP-compliant *in vitro* mammalian cell micronucleus test performed according to OECD TG 487. Human lymphocytes were obtained from male and female donors, 21-33 years of age. The test substance (99.7% purity) was added to the cell cultures at 2 mg/mL, in DMSO, with and without activation. Cells were exposed short term (3 to 6 hours) with and without activation, and long term (20-24 hours) without activation. Cytochalasin B was used for the cytokinesis block, and cytotoxicity was determined based on the cytokinesis-block proliferation index (CBPI). Positive controls were cyclophosphamide, mitomycin C, and colchicine. There were no significant increases in the number of micronuclei in treated cells, in the presence or absence of metabolic activation, at any concentration, compared to vehicle controls. Authors concluded the test substance was not clastogenic and/or aneugenic under the conditions of the test (Klimisch 1, reliable without restriction) (Unnamed 2018 study).
- *In vitro:* Surrogate Propylparaben: Propylparaben was evaluated in a GLP-compliant *in vitro* mammalian cell gene mutation test conducted according to OECD TG 476 and EU Method B.17. Chinese hamster lung fibroblasts (V79) were exposed to propylparaben (purity not reported) in dimethyl sulfoxide (DMSO) at concentrations up to 112.0 µg/L without activation, and up to 448.0 µg/mL with activation for 4 hours in Experiment 1. In Experiment II, cells were exposed up to 224.0 µg/mL without activation for 24 hours, and up to 448.0 µg/mL with activation for 4 hours. Ethylmethanesulfonate and 7,12-dimethylbenzanthracene were the positive control substances and each provided the expected results. The highest concentrations were based on cytotoxicity. There were no significant increases in mutations at the HPRT locus in treated cells compared to vehicle controls at any concentration, with or without activation, in either experiment. Authors concluded the test substance was not mutagenic under the conditions of the test (Klimisch 1, reliable without restriction) (Unnamed 2012 study).

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

Ethylparaben is assigned a score of Low for reproductive toxicity based on the lack of reproductive toxicity in multiple studies for the target substance and two structurally close surrogates. 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 and two structurally close surrogates.

- 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:* Ethylparaben was evaluated in a non-guideline subchronic feeding study (GLP not specified) was performed in rats to examine effects on the reproductive system. Male Crj:Wistar rats were administered ethylparaben (purity 99.9%) in the diet at 0.1 or 1.0%, equivalent to 103 or 1,043 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, epididymis, ventral prostates, seminal vesicles, and preputial glands were measured, as well as sperm count in epididymides, enumeration of cauda epididymal sperm reserve, and sperm morphology, and concentrations of testosterone, LH, and FSH in serum. There were no treatment-related effects on body weights, food consumption, organ weights, sperm parameters, or concentrations of measured hormones. The NOEL is reported at 1.0% (equivalent to 1,043 mg/kg/day as calculated by the study authors) (Klimisch 2, reliable with restrictions) (Oishi 2004).
- ECHA 2018
  - In its assessment on the dossier for ethylparaben, ECHA identified insufficient data for reproductive and developmental toxicity and requested performance of a study such as OECD TG 421/422 in rats, for the oral route of exposure. *ToxServices notes that while no such study has been identified for ethylparaben, read-across from the surrogates methylparaben and propylparaben, as summarized below (particularly the OECD TG 443 studies), fulfill this data gap.*
- ECHA 2023c[9]
  - *Oral: <u>Surrogate Methylparaben:</u>* 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: <u>Surrogate Methylparaben:</u>* 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 a 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:* *Surrogate Methylparaben:* 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:* *Surrogate Methylparaben:* 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:* *Surrogate Methylparaben:* 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 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 (the concentration causing 50% inhibition activity) 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: Surrogate Methylparaben:* 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 2023c). Therefore, this study is included for completeness, but is not included in the weight of evidence.*

- ECHA 2023d[9]
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a GLP-compliant combined repeated dose toxicity study with the reproduction/developmental toxicity screening test conducted according to OECD TG 422 and EPA OPPTS 870.3650. Wistar rats (11/sex/dose) were exposed to propylparaben (purity not specified) in the diet at 0, 1,500, 4,500, or 15,000 ppm (equivalent to 98.0, 305.1, and 980.0 mg/kg/day in males (pre-

pairing); 59.3, 178.3, and 605.0 mg/kg/day in males (after pairing); 116.0, 341.9, and 1,076.4 mg/kg/day in females (pre-pairing); 121.6, 349.2, and 1,124.6 mg/kg/day in females (gestation); and 137.3, 431.8, and 1,380.0 mg/kg/day in females (lactation) (values were reported in the ECHA REACH Dossier)). Male rats were treated for 2 weeks prior to mating and throughout the mating period (a minimum of 28 days). Females were treated for 2 weeks prior to mating, through pregnancy, and then to postpartum day 4 (approximately 7 weeks). The parental animals were evaluated for clinical signs of toxicity, food consumption, body and organ weights, estrous cyclicity, sperm parameters, fertility indices, post-implantation losses, mean litter size, functional observational battery, gross pathology and histopathology. Hematology and blood serum chemistry were only evaluated in parental animals. No treatment-related changes in clinical signs were reported in parental animals. High-dose parental males had slightly reduced body weight gain which occasionally reached statistical significance. No body weight changes were found in females. There were no changes in sperm parameters or estrous cycles. There were no treatment-related effects on any of the fertility or reproductive indices measured. The study authors identified a reproductive toxicity NOAEL of 15,000 ppm (corresponding to 1,124.6 mg/kg/day), which was the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2012 study).

- *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a GLP-compliant extended one-generation reproductive toxicity study with both developmental neuro- and immunotoxicity (Cohorts 1A, 1B without extension, 2A, 2B, and 3) performed according to OECD TG 443. Wistar rats were administered propylparaben (99.7% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg (25/sex/dose, plus an additional 5/sex/dose for the control and high dose groups). 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 until sacrifice of the respective cohort. There were no significant findings based on clinical observations, mortality, body weight and weight changes, food consumption, hematology, clinical chemistry, urinalysis, behavior (functional findings), organ weights, or histopathology for any generation. There were no significant findings based on reproductive function, including estrus cycles and sperm measures. The reproductive toxicity NOAEL is reported at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2021 study).
- *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a non-GLP compliant reproductive and developmental toxicity screening test performed in a manner equivalent or similar to OECD TG 421. Wistar rats were administered the test substance (purity not specified) by gavage (vehicle not specified) at 0, 500, or 1,000 mg/kg (5/sex/dose). Males were exposed for 21 days pre-mating, and a maximum of 14 days of mating, for a total of 35 days. Females were exposed for 21 days or pre-mating, and 14 days of mating. One dam per group was additionally exposed through gestation to gestational day (GD) 20, and the others were exposed through gestation and up through PND 21. Pups from 3 litters (one per group) were exposed from PND 13 to PND21, in accordance with the treatment group of the dam. The final administration for each animal was given 30 +/- 10 minutes prior to sacrifice and necropsy. There were no significant findings based on clinical signs, mortality, body weight and weight changes, food consumption, reproductive function and performance (including copulation, viability, and delivery indices), for any group compared to controls. The NOAEL for reproductive toxicity was reported at 1,000 mg/kg, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2018 study).

- *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a reproductive toxicity study (guideline and GLP compliance not specified). Male and female Sprague-Dawley pups were administered the test substance (99.7% purity) by gavage in 1% hydroxyethylcellulose at 0, 10, 100, or 1,000 mg/kg/day. Phase 1: rats were administered the test substance on PND 4 to 90 (25/sex/group with 10/sex/group necropsied at end of dosing, and 15/sex/group assessed for reproduction/recovery). Phase 2: rats were administered the test substance on PND 4 through 21 (5/sex for controls, and 15-30/sex for treatment groups). A separate uterotrophic assay was conducted in immature female rats to measure estrogenic activity *in vivo*. Propylparaben was administered to immature female pups by oral gavage at 0, 10, 100, or 1,000 mg/kg on PND 21 through 23 (6/dose). A positive control group (n=6 female pups) was administered 17α-ethinyl estradiol (E2) at 1 µg/kg subcutaneously. Rats were evaluated daily for survival, clinical observations, and body weight. On PND 24, rats were examined for vaginal patency and were euthanized, and uteri were excised without the ovaries. For the group in which treated males were paired with untreated females, there were no significant effects on estrus cycles, mating and fertility, gestation length, sex ratios, number of live births, or viability at PND 4. For the group in which treated females were paired with untreated males, there were no effects on the number of corpora lutea, implantation sites, number of live embryos, pre-implantation loss, post-implantation loss, or early resorptions. For the pups exposed on PND 4 to 90, there were no effects on estrus cycles or uterine weights in treated females at necropsy on PND 91. Authors concluded there was no evidence of estrogenic activity at any dose, and no effects on reproductive organs or function. The NOAEL was assigned at 1,000 mg/kg/day, the highest dose tested (Klimisch 2, reliable with restrictions) (Sivaramana et al. 2018).
- Note: the following studies have Klimisch ratings of 3 – not reliable, and 4 – not assignable – in the REACH dossier. However, as they are considered key studies by World Health Organization (WHO) 2007 and SCCS 2013, they are included in the weight of evidence:
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a reproductive toxicity study conducted by Oishi (2002), groups of eight male Wistar rats aged 3 weeks were given diets containing 0, 0.01, 0.1, or 1% propylparaben for 4 weeks. The study authors estimated approximate intakes of 10, 100, and 1,000 mg/kg/day propylparaben, respectively. Following the 4-week treatment, rats were sacrificed, blood was collected for hormone assays, testes, epididymides, prostate, seminal vesicles, and preputial glands were weighed, and sperm counts in testes and epididymis were determined. Treatment had no effect on the weight of the reproductive organs. The authors found a significant decrease in cauda epididymal sperm reserves and concentrations in rats treated with 100 and 1,000 mg/kg/day. Daily sperm production and its efficiency in the testes were also significantly decreased in all treatment groups compared to controls. Daily sperm production was approximately 70% of control values in all treated groups; however, there was no dose-response relationship (Klimisch 3 – not reliable) (Oishi 2002, as cited in ECHA 2023d, WHO 2007, and SCCS 2013). *ToxServices identified a LOAEL of 10 mg/kg/day (lowest dose tested) based on decreased daily sperm production and efficiency in the testes.*
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a GLP-compliant reproductive toxicity study (guideline not specified). Male Wistar rats (20/dose) received 0, 3, 10, 100, or 1,000 mg/kg/day propylparaben (purity = 100%) via gavage at a dose volume of 10 ml/kg. Each group was divided into two subgroups of 10 animals: subgroup 1 was necropsied at the end of an 8-week treatment period

and subgroup 2 was necropsied after a 26-week washout period. Dosing began on PND21 continued through sexual maturation, and up to 11 weeks of age (8-week treatment period). The treatment period covers juvenile (PND 21-35), peri-pubertal (PND 35-55), pubertal (PND 55-70), and early adult stages of the male rats. Animals were examined for clinical signs and weighed twice weekly during the 8-week treatment period, and then weekly during the washout period. On PND38, animals were examined to determine the day of balano preputial separation. At the end of the treatment period, animals were euthanized and examined for gross lesions, testes and epididymides were weighed separately, and the seminal vesicles and prostate were weighed together. Histopathological examination was performed on the right testis and epididymis. The study authors performed a testicular spermatid count and epididymal sperm analysis. High-dose animals experienced hypersalivation through the end of the treatment period. No other treatment-related clinical signs were observed. Treatment had no effect on mean body weight gain or sexual maturation. At the end of the 8-week treatment period there were no significant differences in the weight of the reproductive organs (epididymis, prostate and seminal vesicle, and testis). At the end of the recovery period, no consistent histopathological changes were found. The study authors found no changes in the mean testicular spermatid counts, epididymal sperm counts, or mean motility parameters in any group at the end of the treatment or recovery phase. Study authors concluded that propylparaben was not a reproductive toxicant and identified a NOAEL of 1,000 mg/kg/day; which was the highest dose tested (Klimisch 4 – not assignable) (Gazin et al. 2013).

- SCCS 2013
    - *Surrogate Propylparaben:* The SCCS (2013) concluded the study by Gazin et al. (2013) was well conducted and provided sufficient information to refute the findings of Oishi (2002) who found effects of sperm parameters and plasma testosterone concentrations of juvenile male Wistar and at doses of 100 mg/kg/day and above.

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

Ethylparaben is assigned a score of Low for developmental toxicity based on surrogate data. Numerous guideline and near-guideline studies for the surrogates methylparaben and propylparaben, covering numerous critical periods of development from conception through maturity, do not identify adverse effects on the developing organism in several species. 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 strong surrogates.

- 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 2018
    - As noted previously, in its assessment on the dossier for ethylparaben, ECHA identified insufficient data for reproductive and developmental toxicity and requested performance of a study such as OECD TG 421/422 in rats, for the oral route of exposure. *ToxServices notes while no such study has been identified for ethylparaben, read-across from the surrogates methylparaben and propylparaben, as summarized above (particularly the OECD TG 443 studies), fulfill this data gap.*

  - ECHA also identified a data gap for prenatal developmental toxicity and requested a study similar to OECD TG 414 in rats or rabbits, for the oral route of exposure. Specifically, older OECD TG 414 studies for the surrogate methylparaben had multiple deficiencies such as low number of animals and/or reduced duration of exposure. *ToxServices notes that while no such study has been identified for ethylparaben, read-across from several newer studies (OECD TG 443, 422, 440) on the surrogates methylparaben and propylparaben, as summarized below, fulfill this data gap.*
- ECHA 2023c[9]
  - *Oral: Surrogate Methylparaben:* 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 on 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).
  - *Oral: Surrogate Methylparaben:* 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).
  - *Oral: Surrogate Methylparaben:* 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).

- *Oral:* *Surrogate Methylparaben:* 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).
- *Oral:* *Surrogate Methylparaben:* 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).
- *Oral:* *Surrogate Methylparaben:* 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.*

- *Oral: Surrogate Methylparaben:* 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 study as summarized in ECHA 2023c). Therefore, this study is included for completeness, but is not included in the weight of evidence.*

- ECHA 2023d[9]
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a GLP-compliant prenatal developmental toxicity study performed according to OECD TG 414. Pregnant Wistar rats were administered the test substance (99.7% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg once daily on GD 5 through 19 (25 animals/group). Caesarean sections were performed on GD 20, the day prior to the expected day of delivery. There were no significant findings based on clinical observations, mortality, body weight and weight changes, food consumption, ovary and uterine content, gross pathology, number of abortions, pre- and post-implantation loss, number of total, early, or late resorptions, number of dead fetuses, duration of pregnancy, number of pregnant dams, fetal body weights, number of live offspring, litter size and weights, or external, skeletal, and visceral malformations. Authors assigned the NOAEL for developmental toxicity at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2018 study).
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a previously described GLP-compliant combined repeated dose toxicity study with a reproduction/developmental toxicity screening test conducted according to OECD TG 422. Male and female Wistar rats (11/sex/dose) were exposed to 0, 1,500, 4,500, or 15,000 ppm propylparaben in their feed (equivalent to 98.0, 305.1, and 980.0 mg/kg/day in males (pre-pairing); 59.3, 178.3, and 605.0 mg/kg/day in males (after pairing); 116.0, 341.9, and 1,076.4 mg/kg/day in females (pre-pairing); 121.6, 349.2, and 1,124.6 mg/kg/day in females (gestation); and 137.3, 431.8, and 1,380.0 mg/kg/day in females (lactation) (values were reported in the ECHA REACH Dossier)). Male rats were treated for 2 weeks prior to mating and throughout the mating

period (a minimum of 28 days). Females were treated for 2 weeks prior to mating, through pregnancy, and then to postpartum day 4 (approximately 7 weeks). The parental animals were evaluated for clinical signs of toxicity, food consumption, body and organ weights, estrous cycle, sperm parameters, fertility indices, post-implantation losses, mean litter size, gross pathology and histopathology. Hematology and blood serum chemistry were only evaluated in parental animals. Offspring were evaluated for survival, number and sex of pups, body weight, and external and internal abnormalities. There were no treatment-related effects on any of the developmental indices measured. The study authors identified a developmental toxicity NOAEL of 15,000 ppm (corresponding to 1,124.6 mg/kg/day), which was the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2012 study).

- *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a previously summarized GLP-compliant extended one-generation reproductive toxicity study with both developmental neuro- and immunotoxicity (Cohorts 1A, 1B without extension, 2A, 2B, and 3) performed according to OECD TG 443. Wistar rats were administered propylparaben (99.7% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg (25/sex/dose, plus an additional 5/sex/dose for the control and high dose groups). 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 until sacrifice of the respective cohort. For all generations, there were no significant findings based on clinical observations, mortality, body weight and weight changes, food consumption, hematology, clinical chemistry, urinalysis, behavior (functional findings), organ weights, or histopathology. There were no significant findings on developmental toxicity, including developmental neurotoxicity, and developmental immunotoxicity parameters. The developmental toxicity NOAEL was 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2021 study).
- *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a previously summarized non-GLP compliant reproductive and developmental toxicity screening test performed in a manner equivalent or similar to OECD TG 421. Wistar rats were administered the test substance (purity not specified) by gavage (vehicle not specified) at 0, 500, or 1,000 mg/kg (5/sex/dose). Males were exposed for 21 days pre-mating, and a maximum of 14 days of mating, for a total of 35 days. Females were exposed for 21 days or pre-mating, and 14 days of mating. One dam per group was additionally exposed through gestation to GD 20, and the others were exposed through gestation and up through PND 21. Pups from 3 litters (one per group) were exposed from PND 13 to PND21, in accordance with the treatment group of the dam. The final administration for each animal was given 30 +/- 10 minutes prior to sacrifice and necropsy. There were no significant findings based on clinical signs, mortality, body weight and weight changes, food consumption, reproductive function and performance, or developmental toxicity parameters, for any group compared to controls. The NOAEL for developmental toxicity was reported at 1,000 mg/kg, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2018 study).

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

Ethylparaben was assigned a score of Moderate for endocrine activity based on evidence of very weak endocrine activity in numerous *in vitro* and *in vivo* assays with no observations of corresponding 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]
  - *In vivo:* Ethylparaben was evaluated in a non-guideline study performed to examine endocrine effects (GLP not specified). Female Wistar rats were administered ethylparaben (99% purity) by subcutaneous injection in peanut oil at 400 mg/kg on GD 7 to 21 (10 treated and 18 vehicle controls). Animals were sacrificed 90 minutes after the final dose. Animals were observed daily for signs of toxicity and changes in body weight. Caesarian sections were performed immediately after sacrifice of the dams, and the trunk blood from all fetuses was collected for hormone analyses using one pool per litter for males and females, respectively, and plasma levels of the dams were analyzed for progesterone, 17α-hydroprogesterone, triiodothyronine (T3), and thyroxine (T4), and several endocrine-related genes were evaluated for expression in dams and fetuses. Fetuses were evaluated for body weight and anogenital distance. Fetal adrenals, ovaries, and testes were excised and sampled for hormone analyses, histopathology, and gene expression studies. Dam thyroids were also assessed for histopathology. One of 10 dams in the test substance group showed black amniotic fluid and gave rise to 5% late resorptions, and there were no other indications of toxicity in the dams. There were no significant findings in any of the examined parameters in the dams or fetuses in treated animals compared to vehicle controls (Klimisch 2, reliable with restrictions) (Taxvig et al. 2008).
  - *In vivo:* Ethylparaben was evaluated in a uterotrophic bioassay performed according to OECD TG 440 (GLP compliance not specified). Female B6D2F1 mice were administered ethylparaben (purity not specified) by gavage in a mixture of 10% ethanol in peanut oil, at 0, or 1,000 mg/kg, once daily, for 3 days (9 test animals, and 5 animals/control group). Estradiol benzoate was used as the positive control substance and was administered subcutaneously at 0.1 mg/kg. There were no increases in uterus wet weight or uterine to body weight ratios in treated animals compared to vehicle controls. The positive control performed as expected. Authors concluded the test substance was not estrogenic under the conditions of the test (Klimisch 2, reliable with restrictions) (Hossaini et al. 2000).
  - *In vitro:* Ethylparaben was evaluated in a non-guideline study performed to examine estrogen binding effects. Uteri from ovariectomized Sprague-Dawley rats were used as a source of estrogen receptors. Ethylparaben (99% purity) in ethanol was administered to the test tubes with uterine cytosol at concentrations of 1nM to 100 µM. All doses were tested in duplicate. The binding affinity of the test substance to ER was compared to that of [$^3$H]-17β-Estradiol, the positive control, to estimate a relative binding affinity (RBA). Ethylparaben exhibited weak binding to ER based on an RBA of 0.0006% (Klimisch 2, reliable with restrictions) (Blair et al. 2000).
  - *In vitro:* Ethylparaben was evaluated in a non-guideline cell proliferation and competitive binding assay using MCF7 human breast cancer cells (GLP compliance not specified). The binding affinity of the test substance for human ERα and ERβ was investigated in a competition assay in the presence of excess 17β-estradiol (10 nM). Inhibition rate of binding

to receptor ERα and ERβ were calculated from the absorbance in the presence of 1 µM diethylstilbestrol (DES) (100%) and its absence (0%). Cells were exposed to ethylparaben in ethanol for 6 days at concentrations from 0.01 to 200 µM. In the cellular proliferation assay, the test substance is suggested to be estrogenic, because its effects were completely inhibited by the pure estrogen antagonist. However, the test substance was considered to be a weak estrogen, since there was a low proliferative effect of the test substance observed (EC50 value of 3.2 µM) when compared to DES (EC50 value of >= 0.001 µM) and 17β-estradiol (EC50 value of 0.0000016 µM). In the competitive binding assay, the IC50 values of the test substance were 270 and 240 µM for ERα and ERβ, respectively, compared to the control substance DES for which the IC50 values for ERα and ERβ were 0.03 and 0.026 µM, respectively. Therefore, the relative binding affinities of the test substance for ERα and ERβ were estimated to be 0.011% of that of DES (Klimisch 2, reliable with restrictions) (Okubo et al. 2001).

- *In vitro:* Ethylparaben was evaluated in a non-guideline study designed to assess ER binding, cellular proliferation in stably transfected ERE-CAT genes, and pS2 gene expression using MCF7 human breast cancer cells (GLP compliance not specified). Methylparaben (98% purity) was added to the cells at concentrations ranging from 1.6 nM to 1.6 mM. For the competitive binding assay, cells were pre-treated to deplete steroid hormone levels. Competition was assayed between the binding of [2,4,6,7-$^3$H]Estradiol at 16x10(exp)-10 M and 1 to 1,000,000-fold molar excess of unlabeled ethylparaben. About 54% inhibition of [$^3$H]-estradiol binding was detected at 1.6 mM of the test substance, which authors reported is many orders of magnitude higher than that of the natural estrogen 17β-estradiol (2.5 × 10–12) (Watanabe et al.2013), i.e., the commonly used positive control in *in vitro* estrogen receptor transactivation studies (Soni et al. 2005;Watanabe et al. 2013; US EPA 2015).
  - In the assay of stably transfected ERE-CAT reporter gene, incubation of the MCF7 cells with the test substance at 10 µM for 24 h resulted in a 1.3-fold increase in CAT gene expression; and after an incubation period of 7 d, a 1.4-fold increase in CAT gene expression was noted at 100 µM. In comparison, after 24 h and 7 d incubation periods with 0.01 µM 17β-Estradiol, 2.6- and 2.9-fold increases of CAT gene expression were observed.
  - In the northern blot of pS2 mRNA from ethylparaben treated cells, an increase in expression of pS2 mRNA was observed at 100 µM (no interpretation on the significance of this result is reported in the dossier).
  - In the cellular proliferation assay, at 100 µM, the test substance stimulated the cell proliferation to the same extent as 17β-Estradiol at 0.0001 µM. The proliferation increase by 100 µM of the test substance was inhibited by the pure antiestrogen ICI 182,780, suggesting that effects were ER-mediated. Control experiments using the MDA-MB-231 human breast cancer cell line, which lacks ERα, was unresponsive to 17β-Estradiol for proliferation. Proliferation of these cells was also unaffected by presence of up to 100 µM ethylparaben.

Authors concluded all receptor effects only occurred at concentrations that were many orders of magnitude higher than that of the natural estrogen 17β-estradiol (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).

- TEDX 2013
  - Ethylparaben 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%), ethylparaben (54%), and methylparaben (21%). Parabens increased the expression of endogenous estrogen-regulated genes in MCF7 cells at concentrations ≥ $10^{-6}$ M. They also increased proliferation of cells in a monolayer culture in an estrogen receptor dependent manner.
- *In vitro:* Gomez et al. (2005) found evidence of estrogenic activity in three reporter cell lines. The parabens were found to activate the estrogen receptor-α (ERα) and ERβ similarly.
- *In vivo:* Lemini et al. (2004) treated ovariectomized CD1 mice with methylparaben, ethylparaben, propylparaben, butylparaben, or estradiol (positive control) subcutaneously daily for three days with two different equimolar doses (362 and 1,086 mmol/kg). For ethylparaben, the doses are equivalent to 60 and 180 mg/kg, respectively. Propylene glycol served as the vehicle. The uteri were harvested on the 4$^{th}$ day for examination of weight, luminal epithelium heights (LEH), glandular epithelium heights (GEH), and myometrium widths (MW). For ethylparaben, the relative uterotrophic potency was 0.03 and 0.004% of estradiol at 60 and 180 mg/kg, respectively. LEH, GEH, and MW were also significantly changed. Study authors concluded that parabens at the doses tested were estrogenic in mice.
- *In vitro:* Song et al. (1989) reported that parabens have potent *in vitro* spermicidal activity against human spermatozoa.
- *In vitro and in vivo:* Taxvig et al. (2008) exposed pregnant Wistar rats to ethylparaben and butylparaben on GD7-21, and examined both parabens *in vitro* in the H295R steroidogenesis assay and in the T-screen assay for thyroid hormone receptor binding activity. There were no treatment related changes in the *in vivo* study on testosterone production, anogenital distance, or testicular histopathology for ethylparaben. Ethylparaben significantly increased progesterone formation in the adrenal H295R steroidogenesis assay, but did not affect thyroid hormone receptor binding in the T-Screen assay. Study authors concluded that while only butyl paraben may have an endocrine disruption potential in the steroidogenesis pathway, neither paraben displayed endocrine-disrupting effects in the *in vivo* study.

- 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 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 ethylparaben, 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. Ethylparaben has conflicting data suggesting possible weak estrogenic and weak anti-androgenic effects *in*

*vitro* and *in vivo*. The *in vivo* data identified estrogenic effects in immature and ovariectomized mice. A study on fetal exposure to ethylparaben did not affect anogenital distance or fetal testosterone production, but changes in gene expression levels suggested subtle endocrine disrupting effects. Another study in young male rats found no adverse effects, but tendencies to lower sperm counts. Ethylparaben increased vitellogenin induction in fish. The Danish Centre on Endocrine Disruptors concluded ethylparaben is a Suspected endocrine disrupter in category 2a.

**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**

Ethylparaben was assigned a score of Low for acute toxicity based on an oral $LD_{50}$ of > 3,100 mg/kg. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when the oral $LD_{50}$ is > 2,000 mg/kg (CPA 2018b). The confidence in the score is high based on measured data for the target compound. No data were found for the dermal or inhalation routes of exposure.

- 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:* Ethylparaben was evaluated in a non-GLP compliant acute oral toxicity study performed according to OECD TG 401. Wistar rats were administered ethylparaben (purity not specified) by gavage in Lutrol at 1,000, 3,100, and 5,000 mg/kg (5/sex/dose) and were observed for 14 days post-administration. Mortality occurred in 4/10 animals at 3,100 mg/kg within the first 8 hours, and in 4/10 animals at 5,000 mg/kg in the first hour. The oral $LD_{50}$ was assigned at > 3,100 mg/kg (Klimisch 2, reliable with restrictions) (Unnamed 1982 study).
  - *Oral:* Ethylparaben was evaluated in a non-GLP compliant acute oral toxicity study performed in a manner equivalent or similar to OECD TG 401. Female Wistar rats were administered ethylparaben (purity not specified) by gavage in 1% tragacanth fluid at 0.1, 1, or 10% (equivalent to 2, 20, and 200 mg/kg according to ECHA record) (4/dose) and were observed for 7 days post-administration. There were no mortalities and the $LD_{50}$ was assigned at > 200 mg/kg (Klimisch 2, reliable with restrictions) (Moriyama et al. 1975).

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

Ethylparaben was assigned a score of Low for systemic toxicity (single dose) based on lack of indications of systemic toxicity at sub-lethal doses two acute oral toxicity studies. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (single dose) when adequate data exist and GHS classification is not warranted (CPA 2018b). The confidence in the score is high based on high quality data for the target compound. It may be noted that a minority of EU notifiers suggest hazard classification H335 – May cause respiratory irritation (Pharos 2023), however, as the majority of notifiers suggest it is not classified, and there were no supporting data identified, the notification is considered unreliable and is not included in the weight of evidence. Similarly, the notified aspiration hazard (Pharos 2023) does not apply because the substance is a solid. No data were found for the dermal and inhalation routes of exposure.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any authoritative lists for this endpoint.
- ECHA 2023a[9]
    - *Oral*: Ethylparaben was evaluated in a non-GLP compliant acute oral toxicity study performed according to OECD TG 401. Wistar rats were administered ethylparaben (purity not specified) by gavage in Lutrol at 1,000, 3,100, and 5,000 mg/kg (5/sex/dose) and were observed for 14 days post-administration. Mortality occurred in 4/10 animals at 3,100 mg/kg within the first 8 hours, and in 4/10 animals at 5,000 mg/kg in the first hour. There were no deaths or clinical signs of toxicity in animals exposed at 1,000 mg/kg. Clinical signs in the mid- and high-dose groups are reported as decreased general condition, laying down on side or prone position, and sedation. The were no significant findings at necropsy for animals sacrificed at study termination (Klimisch 2, reliable with restrictions) (Unnamed 1982 study).
    - *Oral*: Ethylparaben was evaluated in a non-GLP compliant acute oral toxicity study performed in a manner equivalent or similar to OECD TG 401. Female Wistar rats were administered ethylparaben (purity not specified) by gavage in 1% tragacanth fluid at 0.1, 1, or 10% (equivalent to 2, 20, and 200 mg/kg according to ECHA record) (4/dose) and were observed for 7 days post-administration. There were no mortalities and no clinical signs of toxicity observed during the study period (Klimisch 2, reliable with restrictions) (Moriyama et al. 1975).

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

Ethylparaben 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 and two structurally close surrogates. 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 for the surrogate methylparaben 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:* Ethylparaben was evaluated in a pre-GLP, pre-guideline oral feeding study performed in male and female Wistar rats. Animals were administered ethylparaben in the diet at 0, 2, or 8%, equivalent to 0, 900-1,200, and 5,500-5,900 mg/kg/day, respectively (12/sex/dose) for 12 weeks. Food intake and body weights were examined every 2 weeks. At study termination, all animals were sacrificed and necropsied for gross pathology, and histopathology with a focus on the kidney, liver, heart, lung, spleen, and pancreas. Clinical observations included depression, decreased motor activity, and deaths. Decreased body weight gain was reported in the high-dose group. There were no significant findings based on gross pathology or histopathology. Animals that died during the study were reported to

have extensive consolidation of the lung and pneumonia, which were not significantly different from controls, and therefore the findings were considered incidental. The NOAEL is reported at ≥ 900 and ≤ 1,200 mg/kg, corresponding to 2% in the diet (no further details provided) (Klimisch 2, reliable with restrictions) (Matthews et al. 1956). *The LOAEL is implied to be 5,500 mg/kg, and the critical effects appear to be decreased body weight gain and clinical signs of toxicity.*

- *Oral:* Ethylparaben was evaluated in a pre-GLP, pre-guideline subchronic toxicity study performed in male and female SD-JCL rats. Animals were administered ethylparaben in the diet at 0, 0.2, 1.0, and 2.0%, equivalent to approximately 0, 120, 600, and 1,200 mg/kg/day, respectively (5/sex/dose and 12 controls) for 25 weeks, according to the ECHA record. Animals were evaluated based on clinical observations, mortality, body weights, food consumption, hematology, clinical chemistry, gross pathology, histopathology. Decreased body weight gain was recorded for males at the mid- and high-doses (severity and statistical significance not specified). Alkaline phosphatase was significantly, but not dose-dependently increased in all treated males. There were no significant findings based on the remaining parameters that were evaluated. The NOAEL is reported at 2%, or 1,200 mg/kg, the highest dose tested (no further details provided) (Klimisch 2, reliable with restrictions) (Sado 1973, Liebert 1984).

- ECHA 2018
  - In its evaluation of the REACH dossier for ethylparaben, ECHA identified a data gap for subchronic toxicity via the oral route of exposure, and recommended performance of a study such as OECD TG 408 in rats. *ToxServices suggests this data gap is fulfilled by read-across for the surrogates methylparaben and propylparaben, as summarized below.*
- ECHA 2023c[9]
  - *Oral: <u>Surrogate Methylparaben:</u>* 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: <u>Surrogate Methylparaben:</u>* 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 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).

- CIR 2008
    - *Dermal: Surrogate Methylparaben:* 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: Surrogate Methylparaben:* 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

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

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 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.

- ECHA 2023d[9]
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in a GLP-compliant subchronic oral toxicity study performed according to OECD TG 408. Wistar rats were administered the test substance (99.7% purity) by gavage in 1% hydroxyethylcellulose at 0, 100, 300, or 1,000 mg/kg/day for 90 days (10/sex/group, plus an additional 5/sex for the control and high dose groups as recovery animals). Mid- and high-dose animals demonstrated slight-to-moderate salivation and moving the bedding, but as the timing was close to the test substance administration, investigators considered the effect to be local and not an indication of systemic toxicity. There were no further significant findings based on clinical observations. There were no significant findings based on mortality, body weight and weight changes, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, behavior (functional findings), immunology, organ weights, gross pathology, neuropathology, or histopathology. The NOAEL is reported at 1,000 mg/kg/day, the highest dose tested (Klimisch 1, reliable without restriction) (Unnamed 2019 study).
- ToxServices notes no indications of systemic toxicity were observed in the previously summarized chronic exposure, reproductive, and developmental toxicity studies for ethylparaben or the surrogates methylparaben and propylparaben.

**Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L):** *L*

Ethylparaben was assigned a score of Low for neurotoxicity (single dose) based on the lack of indications of neurotoxic effects at sub-lethal doses in two acute oral toxicity studies. 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 data are limited to reported findings from the clinical observations and necropsy, which did not include assessments of functional behaviors or motor activity.

- 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:* Ethylparaben was evaluated in the previously summarized non-GLP compliant acute oral toxicity study performed according to OECD TG 401. Wistar rats were administered ethylparaben (purity not specified) by gavage in Lutrol at 1,000, 3,100, and 5,000 mg/kg (5/sex/dose) and were observed for 14 days post-administration. Mortality occurred in 4/10 animals at 3,100 mg/kg within the first 8 hours, and in 4/10 animals at 5,000 mg/kg in the first hour. There were no deaths or clinical signs of toxicity in animals exposed at 1,000 mg/kg. Clinical signs in the mid- and high-dose groups are reported as decreased general condition, laying down on side or prone position, and sedation. The were no significant findings at necropsy for animals sacrificed at study termination (Klimisch 2, reliable with restrictions) (Unnamed 1982 study).
  - *Oral*: Ethylparaben was evaluated in a non-GLP compliant acute oral toxicity study performed in a manner equivalent or similar to OECD TG 401. Female Wistar rats were administered ethylparaben (purity not specified) by gavage in 1% tragacanth fluid at 0.1, 1,

or 10% (equivalent to 2, 20, and 200 mg/kg) (4/dose) and were observed for 7 days post-administration. There were no mortalities and no clinical signs of toxicity observed during the study period (Klimisch 2, reliable with restrictions) (Moriyama et al. 1975).

**Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): L**

Ethylparaben was assigned a score of Low for neurotoxicity (repeated dose) based on surrogate data indicating a lack 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 two strong surrogates.

- 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 2023c[9]
  - *Oral: Surrogate Methylparaben:* 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: Surrogate Methylparaben:* 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.*
- ECHA 2023d[9]
  - *Oral: Surrogate Propylparaben:* Propylparaben was evaluated in the previously described GLP-compliant combined repeated dose toxicity study with the reproduction/developmental toxicity screening test conducted according to OECD TG 422. Male and female Wistar rats (11/sex/dose) were exposed to 0, 1,500, 4,500, or 15,000 ppm propylparaben in their feed (equivalent to 98.0, 305.1, and 980.0 mg/kg/day in males (pre-pairing); 59.3, 178.3, and 605.0 mg/kg/day in males (after pairing); 116.0, 341.9, and 1,076.4 mg/kg/day in females (pre-pairing); 121.6, 349.2, and 1,124.6 mg/kg/day in females (gestation); and 137.3, 431.8, and 1,380.0 mg/kg/day in females (lactation) (values were reported in the ECHA REACH Dossier)). Male rats were treated for 2 weeks prior to mating and throughout the mating period (a minimum of 28 days). Females were treated for 2 weeks prior to mating, through pregnancy, and then to postpartum day 4 (approximately 7 weeks). A functional observational battery was performed in males (5/group) shortly before the scheduled sacrifice and in females (5/group) on post-partum day 3. The study authors performed cage-side

observations and evaluated the quantity of feces and urine, posture, and resistance to removal. Hand-held observations were conducted and evaluated animals for muscle tone, pupil size, palpebral closure, lacrimation, salivation, reaction to handling, and general abnormalities. Open-field observations were conducted and evaluated animals for their level of ambulatory activity including rearing (one minute evaluation), unusual body movements (e.g., spasms and convulsions), gait, behavior, coat, respiration, and quantity of feces and urine. Evaluation of animal reflexes including assessment of blinking, palpebral closure, pinna reflex, extensor thrust response, paw pinch, responsiveness to sharp noise, righting reflex, and hearing ability. Rat hind limb and fore limb grip strength was measured, and rectal temperature was taken. Locomotor activity was also quantitatively measured. No treatment-related effects were reported. The study authors reported that the mean body temperature of high dose males was statistically significantly lower than control animals. However, the change was minor and it was within the range of historical controls; therefore, the study authors considered the change to be a result of biological variability and did not consider it to be treatment-related (Klimisch 1, reliable without restriction) (Unnamed 2012 study). *ToxServices identified a neurotoxicity NOAEL of 15,000 ppm propylparaben (corresponding to 1,124.6 mg/kg/day) (highest dose tested).*

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

Ethylparaben is assigned a score of Low for skin sensitization based on measured data for the target substance and two strong surrogates. 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 two strong surrogates. It may be noted that no data were found to support New Zealand's GHS classification or the notified classification of Category 1 for REACH (Pharos 2023), therefore ToxServices considered these ratings unreliable and discounted them 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
- 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 indicate 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 Methylparaben*</u>*:* 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.*
    - <u>*Surrogate Methylparaben*</u>*:* 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 10$^{th}$ 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).
- ECHA 2023d[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*

Ethylparaben was assigned a score of Low for respiratory sensitization in accordance with the guidance from ECHA (2017). Specifically, ethylparaben 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
  - Ethylparaben does not contain any structural alerts for respiratory sensitization (Appendix D)
- The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). 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 ethylparaben is not a skin sensitizer (see skin sensitization section above), a literature search did not find any human evidence of respiratory sensitization by ethylparaben, and

as ethylparaben does not contain any structural alerts for respiratory sensitization (OECD 2022), it is not expected to be a respiratory sensitizer.

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

Ethylparaben was assigned a score of Low for skin irritation/corrosivity as it was not irritating to the skin in an *in vivo* study in rabbits exposed to the undiluted test substance (OECD TG 404). 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 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]
  - 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).
- 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 $\leq 50$ µM, $>50$ to $\leq 1{,}000$ 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 ethylparaben was irritating at ≥ 7%. However, due to lack of additional study details, ToxServices considered this study of low reliability.*

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

Ethylparaben was assigned a score of Low for eye irritation/corrosivity as it was not irritating in an *in vivo* study in rabbits exposed to the undiluted test substance (OECD TG 405). 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.

- 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]
  - Ethylparaben was evaluated in an acute eye irritation study performed in a manner equivalent or similar to OECD TG 405 (GLP not specified). Three female HC:NZW rabbits were administered ethylparaben (Solbrol A, purity not specified) powder at 0.1 mL into one eye each (no vehicle). The eyes were rinsed after 24 hours. Observations were recorded at 1, 24, 48, and 72 hours, and 7 days post-exposure. For one animal, the mean score for conjunctivae at 24, 48, and 72 hours was 1 out of 3, and the effects were fully reversible within 72 hours. The mean scores for conjunctivae for the other two animals, and the means scores for cornea opacity, iris, and chemosis for all animals, at 24, 48, and 72 hours were all 0. Authors concluded the test substance was not irritating under the conditions of the test (Klimisch 1, reliable without restriction) (Unnamed 1983 study).

**Ecotoxicity (Ecotox)**

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

Ethylparaben was assigned a score of Moderate for acute aquatic toxicity based on the most conservative LC/$EC_{50}$ of 15, >10 and <20, and 18 mg/L in fish, daphnia, and algae, respectively. GreenScreen® criteria classify chemicals as a Moderate hazard for acute aquatic toxicity when the most sensitive trophic level has LC/$EC_{50}$ values in the range of > 10 to 100 mg/L (CPA 2018b). The confidence in the score is high based on measured data for the target compound for all three trophic levels.

- 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]
  - 96h $LC_{50}$ = 15 mg/L (measured) (*Danio rerio*, fish) under static conditions (GLP, OECD TG 203 and EU Method C.1) (Klimisch 1, reliable without restriction) (Unnamed 2012 study).
  - 48h $EC_{50}$ (mobility) = > 10 and < 20 mg/L (*Daphnia magna,* daphnia) (static or semi-static not specified) (ISO 6341) (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).
  - 72h $EC_{50}$ (growth rate) = 37 mg/L (nominal) (*Raphidocelis subcapitata,* algae) under static conditions (GLP, OECD TG 201, EU Method C.3) (Klimisch 1, reliable without restriction) (Unnamed 2001 study).
  - 72h $EC_{50}$ = 18 mg/L (*R. subcapitata,* algae) (ISO 8692) (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).

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

Ethylparaben was assigned a score of Moderate for chronic aquatic toxicity based on an estimated chronic value of 0.673 mg/L in daphnia. 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 low due to lack of measured data and reliance on modeling for the fish and aquatic invertebrate trophic levels.

- 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]
  - 72h NOEC (growth rate) = 2.1 mg/L (*R. subcapitata,* algae) under static conditions (GLP, OECD 201, EU Method C.3) (Klimisch 1, reliable without restriction) (Unnamed 2001 study).
  - 72h NOEC = 5 mg/L (*R. subcapitata,* algae) (ISO 8692) (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).
- U.S. EPA 2017b
  - Ethylparaben belongs to the Esters and Phenols ECOSAR chemical categories. The most conservative predicted chronic values (ChVs) are 0.783 mg/L in fish, 0.673 mg/L in daphnia, and 2.24 mg/L in green algae (Appendix E).

**Environmental Fate (Fate)**

**Persistence (P) Score (vH, H, M, L, or vL): vL**
Ethylparaben was assigned a score of Very Low for persistence based on >60% degradation in 28 days, and it met the 10-day window (OECD TG 301F). GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when the dominant compartment is soil, and the substance 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]
  - Ethylparaben was evaluated in a ready biodegradability test conducted according to OECD TG 301F, manometric respirometry test (GLP compliance not specified). The inoculum (source 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 61.2% biodegradation on day 6, and 88.4% 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% degradation by day 6 (Klimisch 2, reliable with restrictions) (Unnamed 2001 study).
- U.S. EPA 2017a
  - The Level III Fugacity model (MCI method) predicts 81.3% will partition to soil with a half-life of 30 days, 18.5% will partition to water with a half-life of 15 days, and <1% will partition to air and sediment (Appendix F).

**Bioaccumulation (B) Score (vH, H, M, L, or vL): vL**
Ethylparaben was assigned a score of Very Low for bioaccumulation based on measured data indicating a log $K_{ow}$ of 2.3 and the most conservative predicted BCF of 15.29. 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
    - Methylparaben has a measured log $K_{ow}$ of 2.3.
- U.S. EPA 2017a
    - BCFBAF predicts a BCF of 15.29 L/kg wet-weight using the regression based model based on a measured log $K_{ow}$ of 2.30, and a BCF of 6.418 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix F).

**Physical Hazards (Physical)**

**Reactivity (Rx) Score (vH, H, M, or L):** *L*
Ethylparaben was assigned a score of Low for reactivity based on lack of reactive functional groups in its molecular structure. 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 was low due to lack of 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.
- No measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of ethylparaben. These procedures are listed in the GHS (UN 2021).
    - Based on the structure of its components or moieties, ethylparaben is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix G).
    - Based on the structure of its components or moieties, ethylparaben is not considered to have oxidizing properties as it does not contain any structural groups known to be correlated with a tendency to react exothermally with combustible materials. Specifically, organic substances which contain oxygen, fluorine, or chlorine where these elements are chemically bonded only to carbon or hydrogen, classification as an oxidizing liquid need not be applied. Therefore, as the molecular structure of ethylparaben has three oxygens, which are all bonded only to carbon and hydrogen, classification is not warranted.

**Flammability (F) Score (vH, H, M, or L): L**
Ethylparaben was assigned a score of Low for flammability based on lack of flammability in a guideline test (EU Method A.10). 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 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
    - Ethylparaben was evaluated in a study for the flammability of solids, according to EU Method A.10 (non-GLP compliant). The test substance (purity not specified) 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 was not flammable (no further details provided) (Klimisch 1, reliable without restriction) (Unnamed 2011 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 genotoxicity, endocrine activity, and skin irritation, and *in silico* modeling for respiratory sensitization, chronic aquatic toxicity, 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 ethylparaben's NAMs dataset include lack of experimental data and validated methods for assessing respiratory sensitization. Ethylparaben'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 ethylparaben'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.<br>**Chronic aquatic toxicity:** No experimental data are available for fish and invertebrate trophic levels. |
| **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].<br><br>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].<br>**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.<br>**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 | Y | *In silico* modeling: ECOSAR |
| 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

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## 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 Ethylparaben (CAS #120-47-8)

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 |
| **Table 2: Chemical Details** | | | | | | | 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 / Ethylparaben / 120-47-8 | L | L | L | L | *M* | L | L | 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 |
|---|---|
| Ethylparaben | 2 |

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

**Table 6**

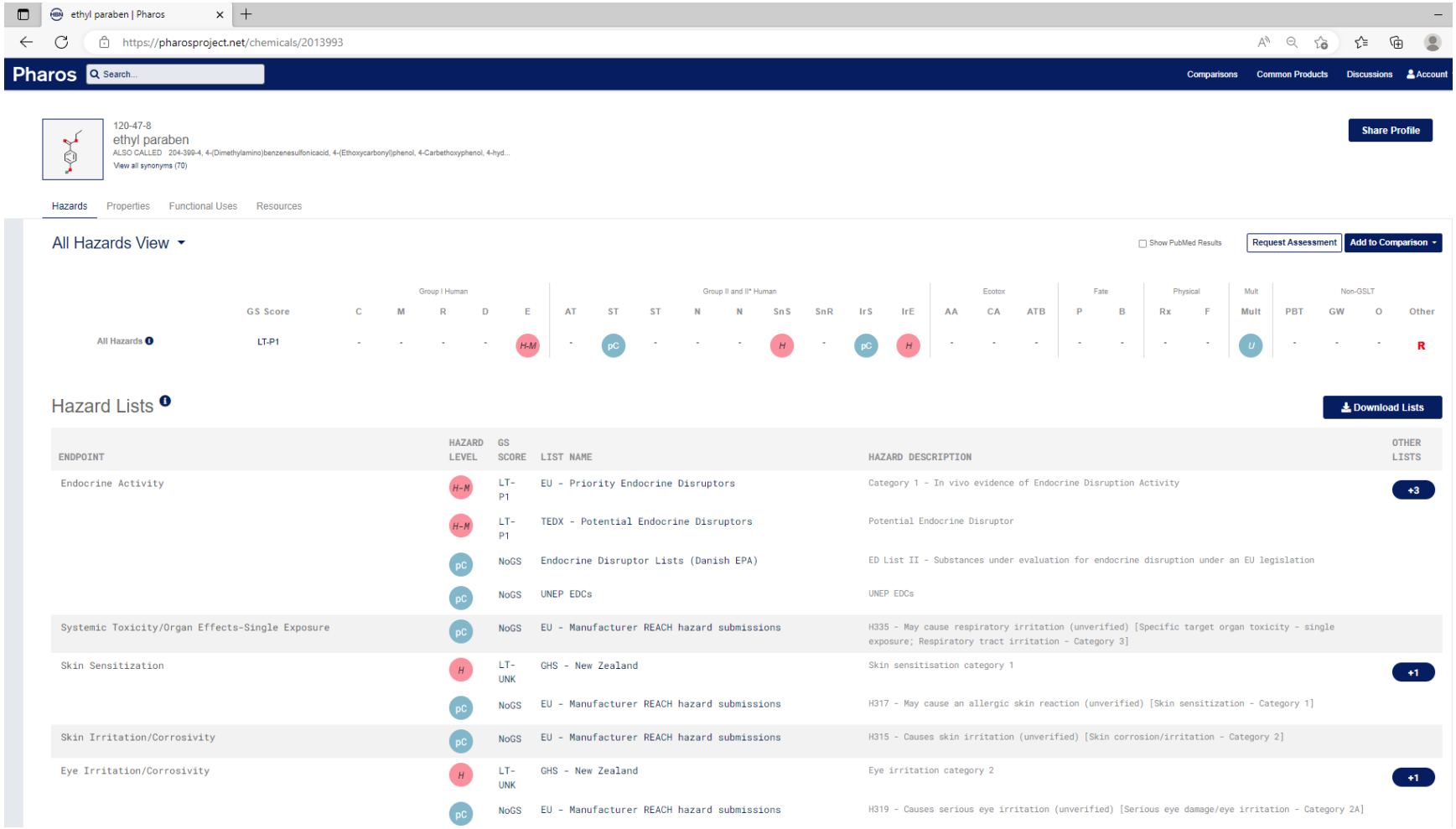
| Chemical Name | Final GreenScreen® Benchmark Score |
|---|---|
| Ethylparaben | 2 |

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 Ethylparaben (CAS #120-47-8)



ethyl paraben | Pharos

https://pharosproject.net/chemicals/2013993

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

120-47-8
ethyl paraben
ALSO CALLED 204-399-4, 4-(Dimethylamino)benzenesulfonicacid, 4-(Ethoxycarbonyl)phenol, 4-Carbethoxyphenol, 4-hyd...
View all synonyms (70)

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 | - | - | - | H | - | pC | H | - | - | - | - | - | - | - | 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 | +3 |
| | H-M | LT-P1 | TEDX - Potential Endocrine Disruptors | Potential Endocrine Disruptor | |
| | 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 | |
| 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 | +1 |
| | pC | NoGS | EU - Manufacturer REACH hazard submissions | H317 - May cause an allergic skin reaction (unverified) [Skin sensitization - 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 | +1 |
| | pC | NoGS | EU - Manufacturer REACH hazard submissions | H319 - Causes serious eye irritation (unverified) [Serious eye damage/eye irritation - Category 2A] | |

| | | | | |
|---|---|---|---|---|
| Systemic Toxicity/Organ Effects (Single Exposure - Aspiration Hazard) | pC | NoGS | EU - Manufacturer REACH hazard submissions | H304 - May be fatal if swallowed and enters airways (unverified) [Aspiration hazard - Category 1] |
| 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 (16)

- CA SCP - Candidate Chemicals: Candidate Chemical List
- Campaign for Safe Cosmetics' Red List of Chemicals of Concern: Chemicals of Concern
- 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
- GSPI - Six Classes of Problematic Chemicals: Antimicrobials
- 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 (3)

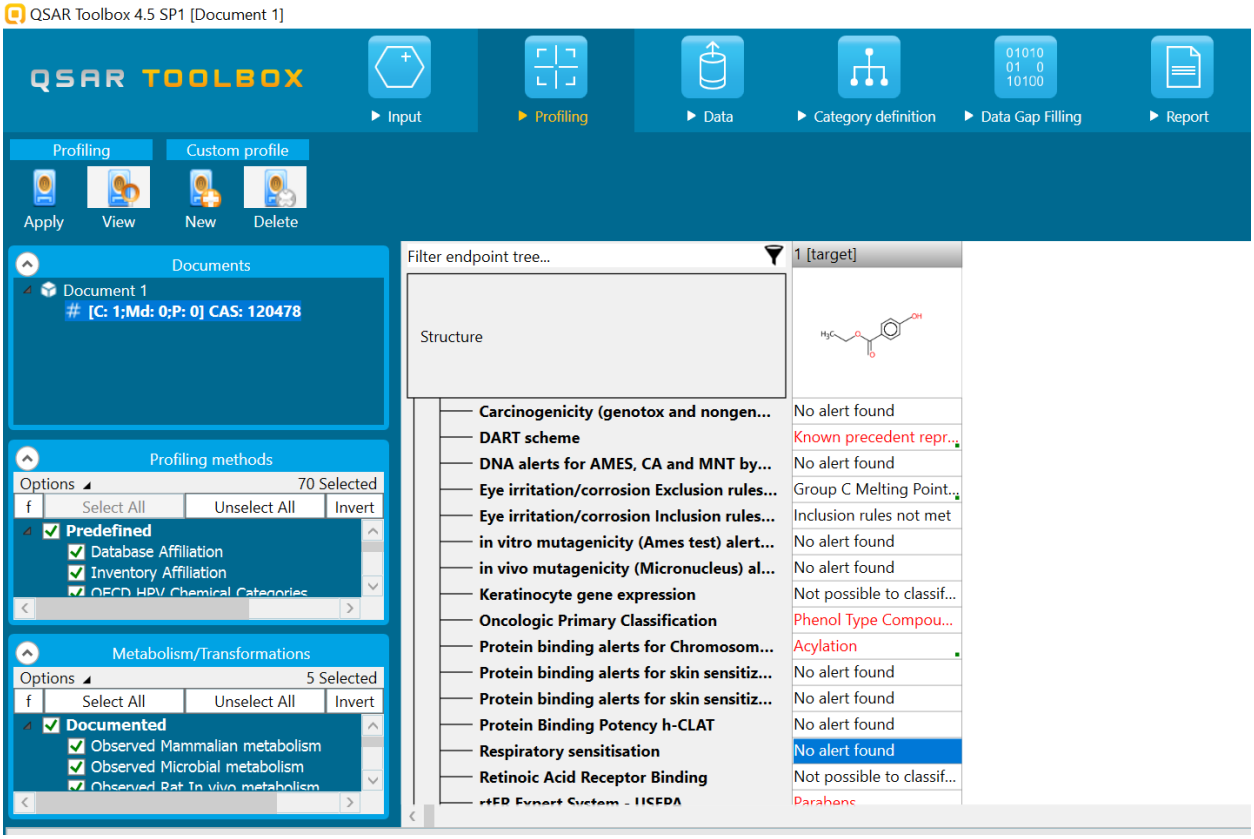
- Cosmetic Ingredient Review (CIR): Safe with Qualifications
- EU - Cosmetics Regulation: Annex V - Preservatives Allowed
- Inventory of Existing Cosmetic Ingredients in China (IECIC 2015): Cosmetic Ingredients

### Discussions

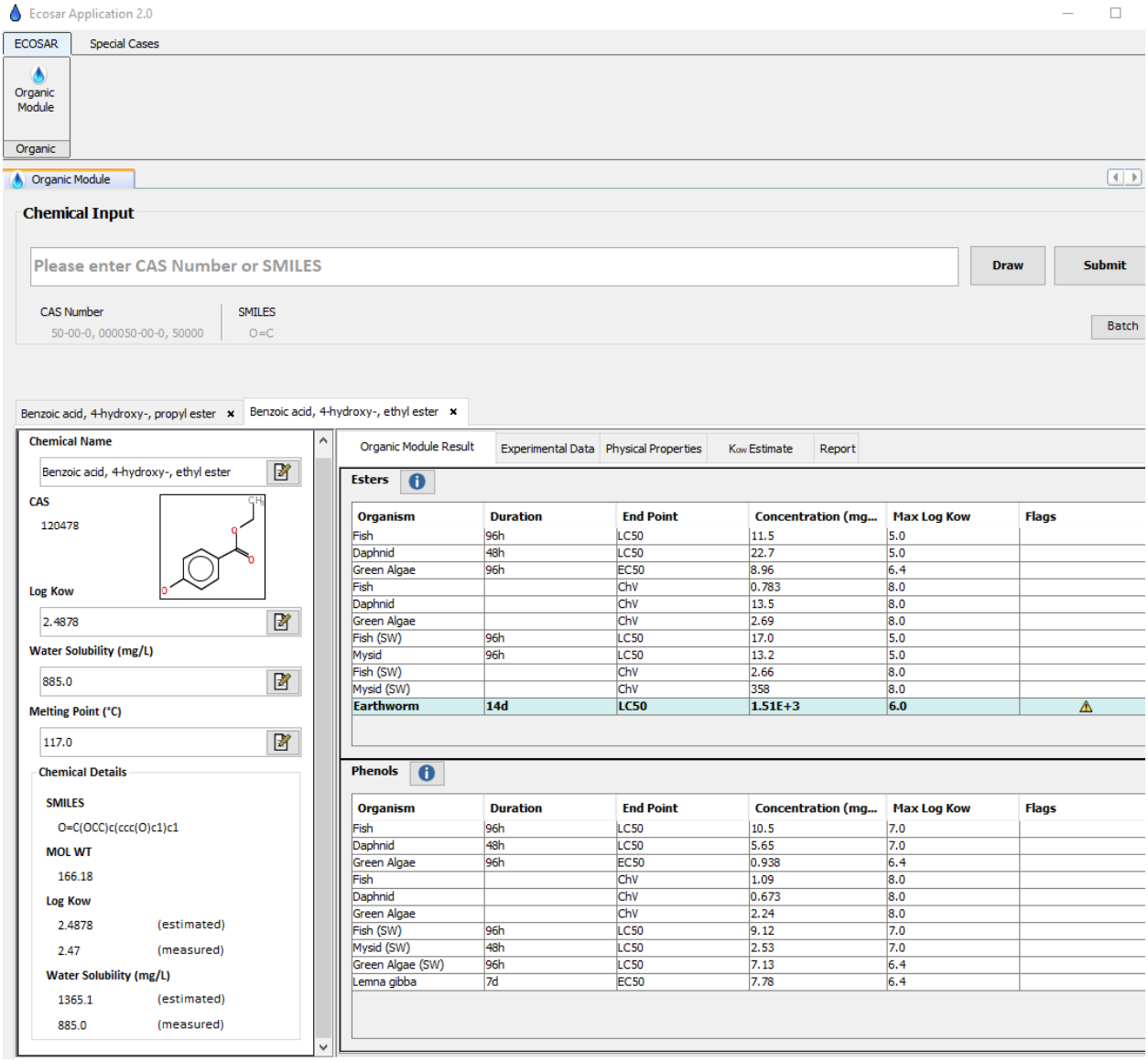
No discussions have been posted yet.

Ask a question about this chemical in the forums >

## APPENDIX D: OECD Toolbox Profiling Results for Ethylparaben (CAS #120-47-8)



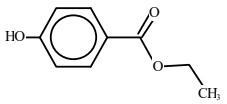
# APPENDIX E: ECOSAR Modeling Results for Ethylparaben (CAS #120-47-8)



## APPENDIX F: EPI Suite™ Modeling Results for Ethylparaben (CAS #120-47-8)

(Estimated values included in the GreenScreen® are highlighted and bolded)

EPI Suite Results For CAS 120-47-8




```
SMILES : O=C(OCC)c(ccc(O)c1)c1
CHEM   : Benzoic acid, 4-hydroxy-, ethyl ester
MOL FOR: C9 H10 O3
MOL WT : 166.18
------------------------------ EPI SUMMARY (v4.11) -------------------------
Physical Property Inputs:
Log Kow (octanol-water):   2.30
Boiling Point (deg C)  :   297.00
Melting Point (deg C)  :   117.00
Vapor Pressure (mm Hg) :   ------
Water Solubility (mg/L):   ------
Henry LC (atm-m3/mole) :   ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) =  2.49
Log Kow (Exper. database match) =  2.47
Exper. Ref:  HANSCH,C ET AL. (1995)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C):  269.11  (Adapted Stein & Brown method)
Melting Pt (deg C):  61.81  (Mean or Weighted MP)
VP(mm Hg,25 deg C):  9.56E-005  (Modified Grain method)
VP (Pa, 25 deg C) :  0.0127  (Modified Grain method)
MP  (exp database):  117 deg C
BP  (exp database):  297.5 deg C
Subcooled liquid VP: 0.000777 mm Hg (25 deg C, Mod-Grain method)
: 0.104 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L):  1988
log Kow used: 2.30 (user entered)
melt pt used: 117.00 deg C
Water Sol (Exper. database match) =  885 mg/L (25 deg C)
Exper. Ref:  YALKOWSKY,SH & HE,Y (2003)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) =  1348.1 mg/L

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

```
Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method :   4.79E-009  atm-m3/mole  (4.86E-004 Pa-m3/mole)
Group Method:   3.01E-009  atm-m3/mole  (3.05E-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:  1.051E-008 atm-m3/mole  (1.065E-003 Pa-m3/mole)
VP:   9.56E-005 mm Hg (source: MPBPVP)
WS:   1.99E+003 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used:  2.30  (user entered)
Log Kaw used:  -6.708  (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):  9.008
Log Koa (experimental database):  None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model)         :   0.9584
Biowin2 (Non-Linear Model)     :   0.9964
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model):   3.0285  (weeks       )
Biowin4 (Primary Survey Model) :   3.8766  (days        )
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model)    :   0.6295
Biowin6 (MITI Non-Linear Model):   0.7772
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model):  0.6534
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):  0.104 Pa (0.000777 mm Hg)
Log Koa (Koawin est  ): 9.008
Kp (particle/gas partition coef. (m3/ug)):
Mackay model           :  2.9E-005
Octanol/air (Koa) model:  0.00025
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model     :  0.00104
Mackay model           :  0.00231
Octanol/air (Koa) model:  0.0196

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant =  12.5753 E-12 cm3/molecule-sec
Half-Life =     0.851 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =    10.207 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Reaction With Nitrate Radicals May Be Important!
Fraction sorbed to airborne particulates (phi):
0.00168 (Junge-Pankow, Mackay avg)
0.0196 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation
```

```
Soil Adsorption Coefficient (KOCWIN v2.00):
Koc    :  157.3  L/kg (MCI method)
Log Koc:  2.197       (MCI method)
Koc    :  198.9  L/kg (Kow method)
Log Koc:  2.299       (Kow method)
Experimental Log Koc:  2.21  (database)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C :  6.012E-003  L/mol-sec
Kb Half-Life at pH 8:       3.653  years
Kb Half-Life at pH 7:      36.534  years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 1.185 (BCF = 15.29 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.5825 days (HL = 0.02615 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.807 (BCF = 6.418)
Log BAF Arnot-Gobas method (upper trophic) = 0.807 (BAF = 6.418)
log Kow used: 2.30 (user entered)

Volatilization from Water:
Henry LC:  3.01E-009 atm-m3/mole  (estimated by Group SAR Method)
Half-Life from Model River: 2.507E+005  hours   (1.045E+004 days)
Half-Life from Model Lake : 2.736E+006  hours   (1.14E+005 days)

Removal In Wastewater Treatment:
Total removal:               2.64  percent
Total biodegradation:        0.10  percent
Total sludge adsorption:     2.54  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       0.0523           20.4         1000
Water     18.5             360          1000
Soil      81.3             720          1000
Sediment  0.148            3.24e+003    0
Persistence Time: 742 hr

Level III Fugacity Model: (MCI Method with Water percents)
Mass Amount    Half-Life    Emissions
(percent)        (hr)       (kg/hr)
Air       0.0523           20.4         1000
Water     18.5             360          1000
water     (18.5)
biota     (0.000185)
suspended sediment (0.00437)
Soil      81.3             720          1000
Sediment  0.148            3.24e+003    0
Persistence Time: 742 hr

Level III Fugacity Model: (EQC Default)
Mass Amount    Half-Life    Emissions
(percent)        (hr)       (kg/hr)
Air       0.0537           20.4         1000
```

```
Water     20.2            360          1000
water     (20.2)
biota     (0.000202)
suspended sediment (0.00248)
Soil      79.6            720          1000
Sediment  0.104           3.24e+003    0
Persistence Time: 723 hr
```

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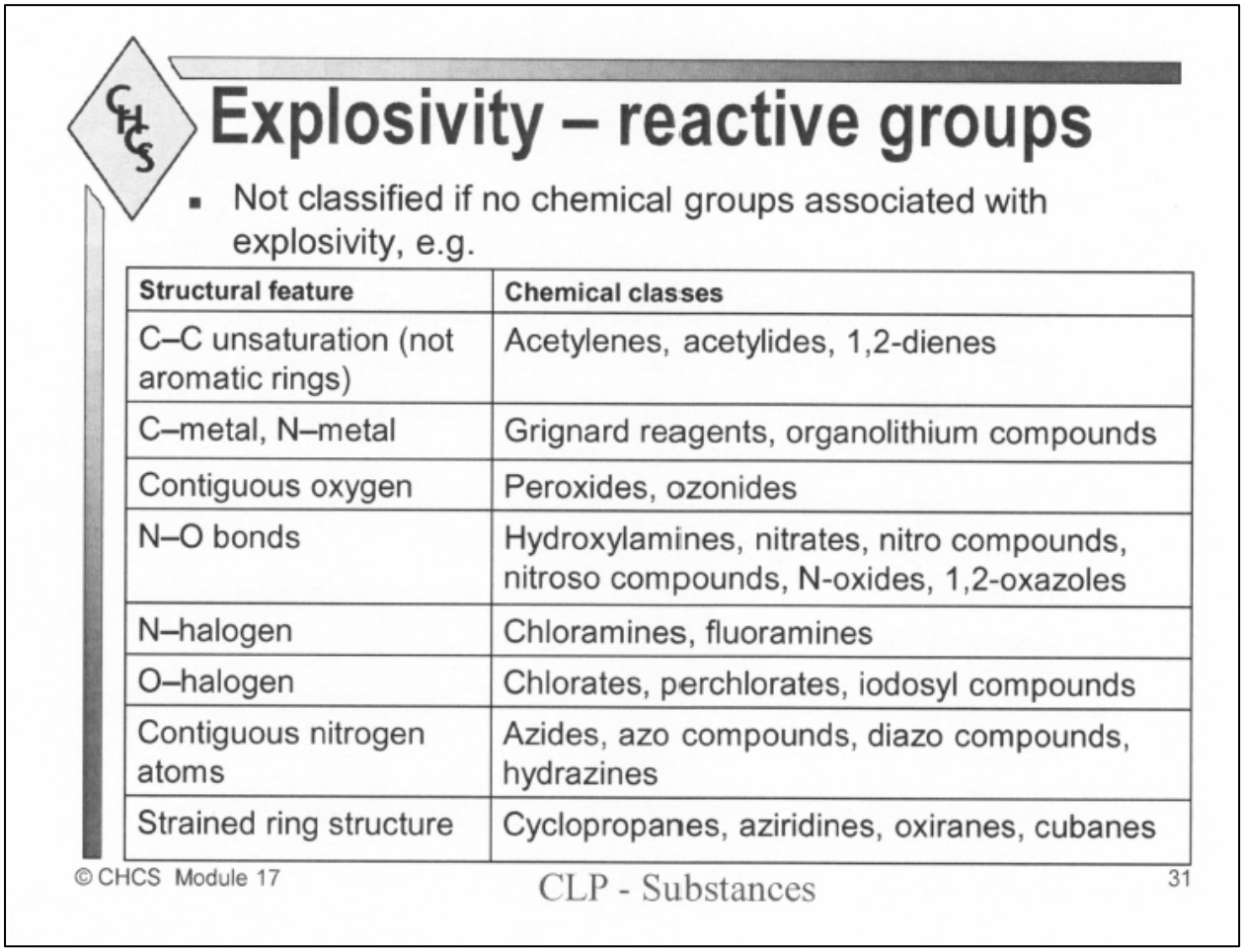
## APPENDIX G: Known Structural Alerts for Reactivity

### 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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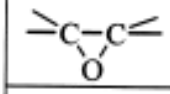
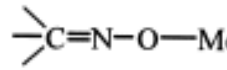
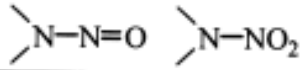
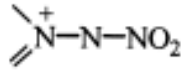
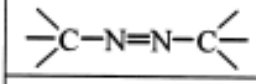
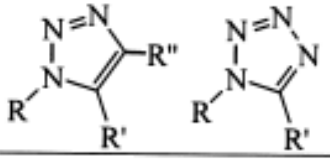
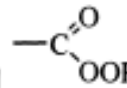
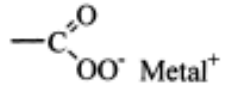
## Explosivity – Full List

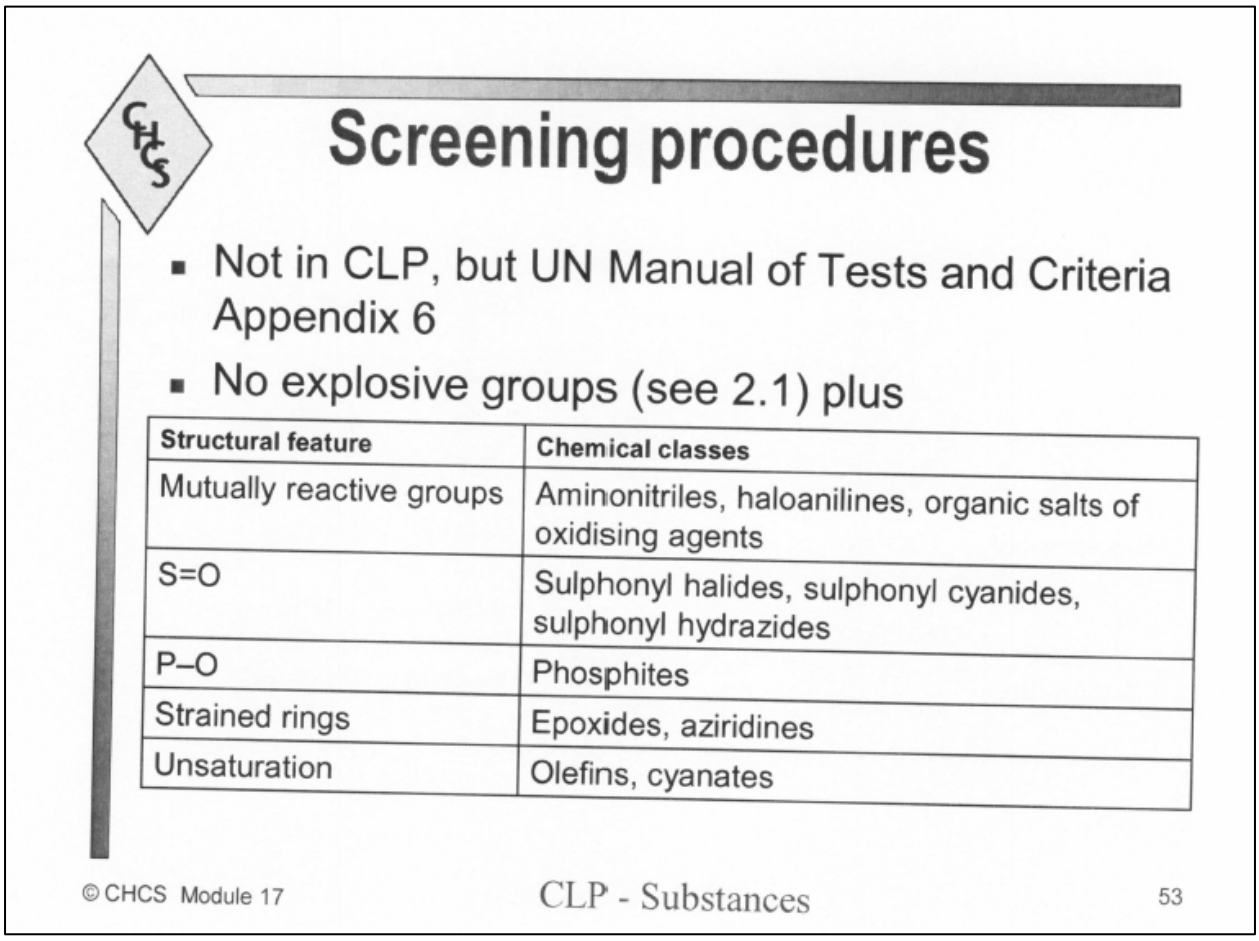
**Table R.7.1-28 Chemical groups associated with explosive properties**

| Chemical group | Chemical Class |
|---|---|
| -C≡C-<br>-C≡C-Metal<br>-C≡C-Halogen | Acetylenic Compounds<br>Metal Acetylides<br>Haloacetylene Derivatives |
|  | Diazo Compounds |
| -N=O -$NO_2$ | Nitroso and Nitro Compounds, |
| R-O-N=O<br>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',<br>[2]  | Peroxy Compounds:<br>[1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic);<br>[2] Peroxo acids (R'=H), Peroxyesters (R'=organic) |
| [1] ROOMetal,<br>[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-<br>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 H: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for ethylparaben. This is a new GreenScreen® assessment.

**Table 5: Change in GreenScreen® Benchmark™ for Methylparaben**

| Date | GreenScreen® Benchmark™ | GreenScreen® Version | Comment |
|---|---|---|---|
| April 12, 2023 | BM-2 | v. 1.4 | Original GreenScreen® assessment. |
| 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**

**Ethylparaben GreenScreen® Evaluation Prepared by:**

SIGNATURE BLOCK

Nancy Linde, M.S.
Senior Toxicologist
ToxServices LLC

**Ethylparaben GreenScreen® Evaluation QC'd by:**

SIGNATURE BLOCK

Bingxuan Wang, Ph.D., D.A.B.T.
Senior Toxicologist
ToxServices LLC