Amended Safety Assessment of Parabens as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (August 18, 2019) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.

The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Jinqiu Zhu, Ph.D., Toxicologist, and Priya Cherian, Scientific Analyst.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088
cirinfo@cir-safety.org

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 21 parabens as preservatives in cosmetic products. These ingredients are all reported to function in cosmetics as preservatives; however, five are reported to also function as fragrance ingredients. The Panel reviewed relevant data relating to the safety of these ingredients under the reported conditions of use in cosmetic formulations. The Panel concluded that 20 of the 21 parabens included in this report are safe in cosmetics in the present practices of use and concentration described in this safety assessment when the sum of the total parabens in any given formulation does not exceed 0.8%. However, the available data are insufficient to make a determination of safety for Benzylparaben.

INTRODUCTION

This is a re-review of the safety of parabens as used in cosmetics; included are the available scientific literature and unpublished data relevant to re-assessing safety of the previously reviewed ingredients and assessing other ingredients for the first time. According to the web-based *Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the ingredients in this group are primarily reported to function in cosmetics as preservatives, and five are reported to also function as fragrance ingredients (Table 1).[1]

In 2017, the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) agreed to re-open the parabens report that was published in 2008,[2] and to include the paraben salts and 4-Hydroxybenzoic Acid. The conclusions of previous CIR safety assessments of parabens are summarized in Table 2. The 21 ingredients in this current assessment thus comprise:

Benzylparaben*	Potassium Butylparaben	Sodium Ethylparaben
Butylparaben*	Potassium Ethylparaben	Sodium Isobutylparaben
Calcium Paraben	Potassium Methylparaben	Sodium Isopropylparaben
Ethylparaben*	Potassium Paraben	Sodium Methylparaben
Isobutylparaben*	Potassium Propylparaben	Sodium Paraben
Isopropylparaben*	Propylparaben*	Sodium Propylparaben
Methylparaben*	Sodium Butylparaben	4-Hydroxybenzoic Acid

* These ingredients were included in the 2008 safety assessment; at that time, the Panel concluded that these ingredients are safe in the present practices of use and concentration.[2]

This re-review was initiated because some of the ingredients being reviewed for the first time had high frequencies of use (e.g., Sodium Methylparaben was reported to be used in 436 cosmetic formulations at the time of prioritization). In addition, the Panel was concerned that new data from a developmental and reproductive toxicity (DART) study indicated reduced sperm counts and reduced expression of a specific enzyme, and a reduction in a specific cell marker in the testes of offspring of female rats orally dosed with 10 mg/kg/day Butylparaben during gestation and lactation periods. Reductions in anogenital distance and other effects were reported at 100 mg/kg/day in this study. In comparison, the previous CIR safety assessment of parabens included the calculation of margin of safety (MOS) values for adults and infants, assuming a no-observed–adverse-effect-level (NOAEL) of 1000 mg/kg/day from an older DART study. After careful consideration of all the new data regarding endocrine activity and DART studies, the Panel determined an adequate NOAEL value of 160 mg/kg/day for Butylparaben. An MOS was re-calculated accordingly, considering the different use concentrations and exposures of Butylparaben in various cosmetic product categories.

An exhaustive search of the world's literature was conducted for new data on the safety of parabens, as well as 4-Hydroxybenzoic Acid, in preparation of this report. A few short-term, but no new acute, subchronic or chronic toxicity studies, were discovered. This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Pertinent data were discovered in the European Chemicals Agency (ECHA) database.[3-11] Data were also discovered in reports by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[12] and the European Union's (EU) Scientific Committee on Consumer Safety (SCCS).[13-19]

Dermal penetration, toxicokinetic, short-term toxicity, DART, endocrine-activity, genotoxicity, biomonitoring, and epidemiology studies are briefly summarized in the body of the report, and in most cases, details are provided in tables. Toxicity studies conducted in animals exposed to individual parabens by subcutaneous injection are also briefly tabulated in the report; however, these studies lack relevance in assessing human exposure to parabens in cosmetics when dermal metabolism is by-passed. In addition, toxicity tests in animals exposed to mixtures of parabens with other compounds (e.g., phthalates), were not included due to their lack of relevance.

CHEMISTRY

Definition and Structure

The ingredients in this safety assessment are paraben phenolic acids, phenolic salts, the free carboxylic acid (4-Hydroxybenzoic Acid, a known metabolite of all of the other ingredients in this report), and its salts. The basic paraben structure is provided in Figure 1, and an example of a specific paraben (Butylparaben) is provided in Figure 2.

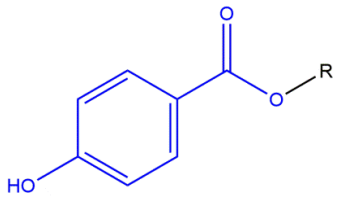


Figure 1. Paraben phenolic acids: a generic structure wherein R is an alkyl group from 1 to 4 carbons long, or is benzyl.

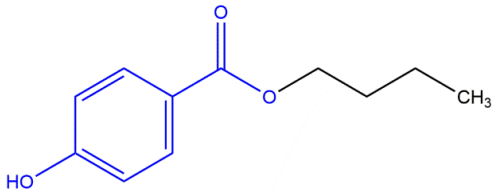


Figure 2. Paraben phenolic acids: an example, Butylparaben (wherein R from the generic structure in Figure 1, is an alkyl group 4 carbons long).

The salts of these phenolic acids have been included in this review of parabens. The phenolic proton is the most acidic in those parabens with an ester functional group, and the salt forms of these parabens share this same core structure (Figure 3). An example of a specific paraben salt (Potassium Butylparaben) is provided in Figure 4.

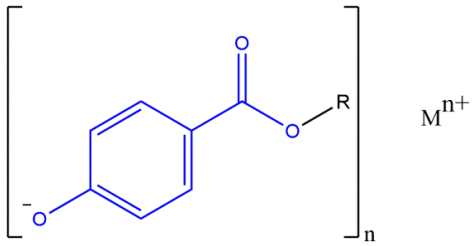


Figure 3. Paraben phenolic salts: generic structure wherein R is an alkyl group from 1 to 4 carbons long and M is sodium or potassium.

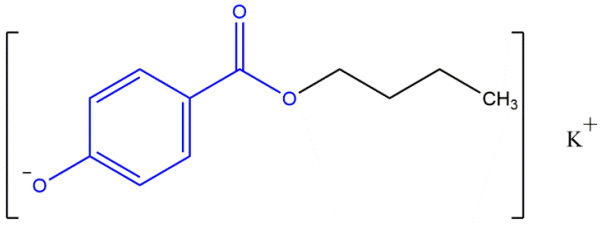


Figure 4. Paraben phenolic salts: an example, Potassium Butylparaben (wherein R, from the generic structure in Figure 3, is an alkyl group 4 carbons long and M is potassium).

Also included in this re-review are the free paraben carboxylic acid and its salts (i.e., not esters). The carboxylic proton (of 4-Hydoxybenzoic Acid) is the most acidic in those parabens without an ester functional group, and the salt forms of these parabens share this same core structure (Figure 5). An example of a specific paraben carboxylic salt (Calcium Paraben) is provided in Figure 6.

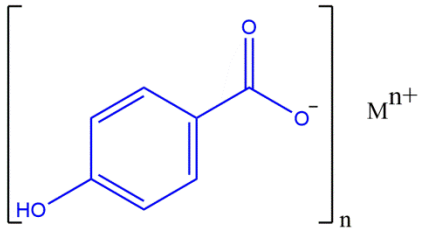


Figure 5. Paraben carboxylic salts: a generic structure wherein M is sodium, potassium, or calcium.

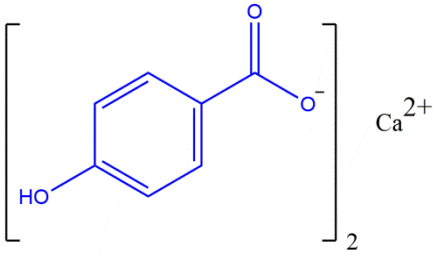


Figure 6. Paraben carboxylic salts: an example, Calcium Paraben (wherein M, from the generic structure in Figure 5, is calcium and n is 2).

Physical and Chemical Properties

Physical and chemical properties of parabens are presented in Table 3.

Parabens form small colorless crystals or white crystalline powders with practically no odor or taste.[2] Parabens are soluble in alcohol, ether, glycerin, and propylene glycol and slightly soluble or almost insoluble in water. As the alkyl chain length increases, water solubility decreases. Parabens are hygroscopic and have a high oil/water partition coefficient. Parabens are relatively stable against hydrolysis during autoclaving and resist saponification.[20]

The particle size distribution of some of the parabens included in the safety assessment is provided in Table 4.[3-6,8,9,11,21-23]

Method of Manufacture

Paraben phenolic acids (and salts) are prepared by esterifying 4-Hydroxybenzoic Acid with the corresponding alcohol (e.g., butanol to synthesize Butylparaben) in the presence of an acid catalyst, such as sulfuric acid, and an excess of the specific alcohol.[2] The acid is then neutralized with caustic soda, and the product is crystallized by cooling, isolated by centrifugation, washed, dried under vacuum, milled, and blended. Benzylparaben can also be prepared by reacting benzyl chloride with sodium 4-Hydroxybenzoic Acid. Paraben carboxylate salts may be prepared by deprotonating 4-Hydroxybenzoic Acid with an appropriate alkaline salt (e.g., sodium hydroxide could be used to prepare Sodium Paraben).[24]

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetic industry on the expected use of these ingredients in cosmetics. Use frequencies

of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentration by product category.

According to VCRP survey data received in 2019, Methylparaben was reported to be used in 11,739 formulations (9347 of which are leave-on formulations); this is an increase from the 8786 uses reported in 2006.[2,25,26] Propylparaben had the next highest number of reported uses at 9034 (7520 of which are leave-on formulations); this was an increase from 7118 uses reported in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to none.

The results of the concentration of use survey conducted by the Council in 2016 indicate Methylparaben had the highest reported maximum concentration of use; it is used at up to 0.9% in shampoos.[2,25] The highest maximum concentration of use reported for products resulting in leave-on exposure is 0.8% Methylparaben in a mascara, and for leave-on dermal exposure is 0.65% Ethylparaben in eye shadows. In 2006, Methylparaben had the highest reported maximum concentration of use at 1% in lipsticks. The maximum concentrations of use of the previously reviewed parabens have remained under 1% and the patterns of use are similar to those reported in the previous safety assessment.

Frequency and concentration of use data for all ingredients reported to be in use are provided in Table 5 and Table 6. The ingredients not in use, according to the VCRP and industry survey, are listed in Table 7.

Several of the parabens are reported to be used in products that can be incidentally ingested, used near the eye, come in contact with mucous membranes, or in baby products.[25,26] For example, Methylparaben is used at concentrations up to 0.35% in lipstick, 0.8% in mascara, 0.5% in bath oils, tablets and salts, and 0.4% in baby lotions, oils and creams.

Some of the parabens were reported to be used in cosmetic sprays (including hair sprays, hair color sprays, skin care products, moisturizing products, suntan products, deodorants, and other propellant and pump spray products)[25,26] and could possibly be inhaled. These ingredients are reportedly used at concentrations up to 0.9 % in spray products (e.g., Methylparaben in the category of other fragrance products). Although there are reported mean diameters as small as 37.8 μm (Sodium Propylparaben) for some of these materials, as pure, raw substances, those diameters are not indicative of particle sizes in final formulations.[6] Accordingly, those raw material mean particle diameters are not relevant to cosmetic safety. In practice, 95% - 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm with propellant sprays yielding a greater fraction of droplets/particles below 10 μm compared with pump sprays.[27-29] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[27,29] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[27] The maximum concentration of use recorded for deodorant sprays was 0.00012% (Methylparaben). However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Some of the parabens were reported to be used in dusting powders and face powders (e.g., Ethylparaben in face powders at up to 0.5%), and could possibly be inhaled. Conservative estimates of inhalation exposures to respirable particles during the use of loose-powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[30-32]

The Scientific Committee on Consumer Safety (SCCS) of the EU has published several opinions on parabens over the last few years (Table 8).[13-19] The current SCCS opinion (updated on May 2013) is:

> The use of butylparaben and propylparaben as preservatives in finished cosmetic products are safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19%... With regard to methylparaben and ethylparaben, the previous opinion, stating that the use at the maximum authorized concentrations can be considered safe, remains unchanged… Limited to no information was submitted for the safety evaluation of isopropyl-, isobutyl-, phenyl-, benzyl- and -pentylparaben. Therefore, for these compounds, the human risk cannot be evaluated. The same is true for benzylparaben….[17,19]

Based on SCCS opinions, the use of the different parabens is regulated by the EU Cosmetic Regulation, which has banned the use of Isopropylparaben, Isobutylparaben, Phenylparaben, Benzylparaben and pentylparaben as preservatives in cosmetic products,[33] and has established maximum concentration limits of 0.4% for Methylparaben or Ethylparaben (single esters and their salts), 0.19% for Propylparaben or Butylparaben (single esters and their salts), and 0.8% for mixtures of the these four parabens, wherein the sum of the individual concentration of Butylparaben and Propylparaben and their salts does not exceed 0.19 %.[33,34] In addition, "…Butylparaben and Propylparaben should be prohibited in leave-on cosmetic products designed for application on the nappy area of children under three years of age…."

Non-Cosmetic

2008

The European Food Safety Authority opinion cited reduction in daily sperm production in juvenile male rats fed Propylparaben at 10 mg/kg/day as the lowest-observable-adverse-effect-dose and contrasted these findings with the absence of effect for Methylparaben and Ethylparaben at doses up to 1000 mg/kg/day.[2] The opinion restated the acceptable daily intake (ADI) of 0 to 10 mg/kg/day for the sum of Methylparaben and Ethylparaben. The opinion stated that Propylparaben should not be included in the ADI, and failed to recommend an alternative ADI because of the lack of a clear NOAEL.

The US FDA considers Methylparaben and Propylparaben to be generally recognized as safe (GRAS) as antimicrobial agents in food. [21CFR184.1490; 21CFR184.1670] Butylparaben, Ethylparaben, and Propylparaben are approved for direct addition to food for human consumption as synthetic flavoring substances and adjuvants. [21CFR172.515] Ethylparaben may be used as an indirect food additive as a component of adhesives and coatings. [21CFR175.105] Methylparaben and Propylparaben are prior sanctioned food ingredients when used as antimycotics. [21CFR181.23] Methylparaben and Propylparaben have been used in diaper rash products, but there are inadequate data to establish general recognition of the safety and effectiveness. [21CFR310.545] Methylparaben is GRAS as a chemical preservative in animal drugs, feeds, and related products at levels not to exceed 0.1%. [21CFR582.3490] Residual Methylparaben and Propylparaben are not to exceed 0.1% when used as preservatives in pesticides for food. [40CFR180.930]

In pharmaceuticals, parabens are used as excipients (inactive ingredients). In the US FDA database of inactive ingredients, Methylparaben has been approved at a maximum potency of 1.8 mg in a tablet formulation and 2.6 mg/mL in an oral solution. Ethylparaben has been approved at a maximum potency of 0.6 mg in a granule formulation and 0.6 mg/mL in an oral solution. Propylparaben has been approved for use at a maximum potency of 0.2 mg in a tablet formulation and 0.2 mg/mL in an oral solution. Butylparaben has been approved for use at a maximum potency of 0.04 mg in a sustained action tablet formulation and 0.08 mg/mL in an oral solution.[35]

An evaluation by the JECFA determined that the acceptable daily intake (ADI) of the sum of the Ethylparaben and Methylparaben is up to 0 - 10 mg/kg.[36] In view of the adverse effects in male rats, Propylparaben was excluded from the ADI for use in food.[18]

In Australia's National Industrial Chemicals Notification and Assessment Scheme's (NICNAS) Human Health Tier II Assessment for parabens, it was found that no critical health effects associated with these chemicals have been established, although they do have very weak estrogenic activity.[36] There are no established adverse outcome pathways for this weak estrogenic activity.

NICNAS published the following conclusion in 2016:

> Current risk management measures are considered adequate to protect public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory.
>
> The main route of public exposure is expected to be through the skin, inhaled from products applied as aerosols, and potential oral exposure from lip and oral hygiene products.
>
> The available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak estrogenic activity, but there are no established adverse outcome pathways for this effect. Should further information on adverse outcome pathways in mammals associated with weak estrogenic activity become available, further assessment of these chemicals at Tier III could be required. [36]

TOXICOKINETIC STUDIES

Dermal Penetration

2008

Parabens in cosmetic formulations applied to skin penetrate the stratum corneum in inverse relation to the ester chain length.[2] Carboxylesterases present in keratinocytes hydrolyze parabens in the skin. The extent of the breakdown to 4-Hydroxybenzoic Acid is different between rodent and human skin. In vitro studies also indicate a difference in the extent of hydrolysis to 4-Hydroxybenzoic Acid, depending on whether viable whole skin or dermatomed human skin is used, with the former having a larger extent of hydrolysis. Chemicals that disrupt the stratum corneum may increase the skin penetration of Methylparaben and possibly Ethylparaben, but do not affect the penetration of parabens with longer ester chains.

In Vitro

In vitro dermal penetration studies are presented in Table 9.

In Franz-type diffusion cells, 2.3% - 3.3% of the applied dose of Methylparaben (0.1% in nine different vehicles) penetrated porcine skin (intact stored frozen) in 4 h.[37] The receptor fluid consisted of phosphate-buffered saline (pH 7.4) and 0.01% of Gentamicin-sulfate. In 24 h, 2.0% - 5.8% and 2.9% - 7.6% of un-metabolized Methylparaben penetrated previously frozen intact and tape-stripped skin, respectively. In full-thickness porcine skin stored frozen, permeability coefficients ranged from 31.3 ± 1.6 to 214.8 ± 40 cm/h x 10^{-4}, decreasing (Methylparaben > Ethylparaben > Propylparaben > Butylparaben) with increasing chain length.[38] Increasing the ethanol concentration in the vehicle or the exposure duration, increased the retention of the parabens in the dermis, relative to the epidermis. Binary combinations of the parabens reduced their permeation rates, which was attributed by the authors to high retention in the epidermis and dermis.

In a different study, the penetration of parabens from 3 commercial facial cream formulations through rabbit ear skin ranged from 20% - 60%, after 8 h in Franz-type diffusion cells, increasing with the water solubility of the paraben (Propylparaben < Ethylparaben < Methylparaben), regardless of the formulation tested.[39] Retention varied widely in the epidermis and dermis depending on the formulation.

Permeability coefficients estimated for Methylparaben, Propylparaben and Butylparaben in human cadaver skin (0.37 to 0.91 cm/h x 10^{-4}) and mouse skin (1.17 to 1.76 cm/h x 10^{-4}) were similar regardless of concentration tested (0.1% - 2%).[40] Residual quantities of parabens remaining in the skin increased as the test concentration increased, with greater amounts in the human epidermis than in mouse skin.

Abdominal skin samples were used to determine the dermal penetration of 0.1% Methylparaben, 0.08% Ethylparaben, 0.2% Propylparaben and 0.15% Butylparaben.[41] Previously frozen skin samples were thawed and mounted on Franz diffusion cells. A dose of 100 µL of lotion containing the test substance was applied to the skin once at t = 0 or multiple times at t = 0, t = 12 and t = 24. Thirty-six hours after a single application, penetration ranged from 0.007% ± 0.003 (Butylparaben) to 0.057% ± 0.03 (Methylparaben). Penetration 12 hours after the t = 24 dosing ranged from 0.04% ± 0.01% (Butylparaben) to 0.6% ± 0.1 (Methylparaben).

Human

Butylparaben

Dermal penetration was studied in 26 healthy Caucasian male volunteers aged 21 to 36 years old, after application of 2% (w/w) Butylparaben in a basic cream formulation which also contained 2% diethyl phthalate and 2% dibutyl phthalate.[42] Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week (control week) was followed by daily application of the cream with the test substances for 1 week. Butylparaben serum concentrations in the blood were undetectable in most samples during the control week, with maximum concentrations not exceeding 1.0 µg/L. Butylparaben concentrations increased rapidly (mean peak concentration = 135 ± 11 µg/L in 3 h) after the first application of cream containing the 3 test compounds. Twenty-four hours after the first application, but before the following application, the mean serum concentration was 18 ± 3 µg/L. Butylparaben could be detected in most serum samples collected throughout the second week of this study.

Penetration Enhancement

In Vitro

Methylparaben

Skin samples were collected within 24 h postmortem from the back of a 77-year-old woman and leg of a 73-year-old man and stored frozen.[43] Split thickness (~350 µm) samples were thawed and mounted in vertical-flow Neoflon™ diffusion cells, and exposed to a saturated aqueous solution of Methylparaben, with (saturated) and without 4-cyanophenol (CP). Receptor fluid (phosphate buffered saline [PBS]) and skin samples (diffusion area 0.64 cm^2) were maintained at 32°C. Solutions containing one or both compounds were added to the donor chamber at t = 0, and the receptor fluid was sampled hourly for 18 h for analysis by high-performance liquid chromatography (HPLC). Compared with the single-solute solutions, the steady-state flux was more than 5-fold larger for Methylparaben and 2.6-fold larger for CP in the binary solution (i.e., Methylparaben plus CP). The authors noted that the 5-fold increase in Methylparaben flux was consistent with a 6.4-fold increase in uptake of Methylparaben in the stratum corneum (SC), which occurred primarily in the nonlipid regions of the SC. However, the 1.6-fold increase in CP uptake was too small to explain the 2.6-fold increase in the CP flux. The authors concluded that the results above suggested CP enhanced skin permeation of Methylparaben primarily by increasing the solubility of Methylparaben in the SC (especially in the nonlipid regions), and Methylparaben increased skin permeation of CP by enhancing both the solubility and diffusivity of CP in the SC.

Absorption, Distribution, Metabolism, and Excretion (ADME)

1984

Parabens are quickly absorbed from the blood and gastrointestinal tract, hydrolyzed to 4-Hydoxybenzoic Acid, conjugated, and the conjugate excreted in the urine.[44] Data obtained from chronic administration studies indicate that parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Varying amounts of parabens are passed in the feces depending upon which paraben is administered and the size of the dose. Little or no unchanged paraben is excreted in the urine. Most of an administered dose can be recovered within 5 to 72 hours as 4-Hydoxybenzoic Acid or its conjugates. Parabens appear to be rapidly absorbed through intact skin.

1986

Metabolism of Benzylparaben is by sulfate conjugation of the parent compound.[45] Excretion is in the urine. Small amounts of the ester are excreted unmetabolized or hydrolyzed to the benzyl alcohol and 4-Hydoxybenzoic Acid.

1995

When male rabbits were administered either 800 mg/kg or 400 mg/kg of Isobutylparaben via a stomach tube, 77 - 85% of the ingredient was recovered as a form of 4-Hydoxybenzoic Acid; 20% was not recovered.[46]

2008

Ingested parabens are quickly absorbed from the gastrointestinal tract, hydrolyzed to 4-Hydoxybenzoic Acid, conjugated, and the conjugate excreted in the urine.[2] Data obtained from chronic administration studies indicate that parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Varying amounts of parabens are passed in the feces depending upon which paraben is administered and the size of the dose. Little or no unchanged paraben is excreted in the urine.

The ADME studies summarized below are presented in Table 10.

In Vitro

Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for α-fetoprotein (AFP).[47] On the other hand, the 50% inhibitory concentration (IC_{50}) of Benzylparaben was 0.012 µM. Butylparaben was de-esterified to 4-Hydoxybenzoic Acid in the S9 fraction of skin obtained from 5-week old male rats, with a maximum rate at saturating concentration (V_{max}) of 8.8 nmol/min/mg protein.[48]

Methylparaben and Ethylparaben were stable in human plasma, but Propylparaben, Butylparaben, and Benzylparaben concentrations decreased by 50% within 24 h.[49] All parabens tested were rapidly hydrolyzed when incubated with human liver microsomes (HLM), with rates depending on the alkyl chain length. Parabens, but not 4-Hydroxybenzoic Acid, were actively glucuronidated by liver microsomes and human recombinant uridine-5'-diphospho (UDP)-glucuronosyltransferases (UGTs).

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were hydrolyzed by rat liver microsomes (RLM) and HLM in in vitro tests.[50] Butylparaben was most effectively hydrolyzed by the RLM, which showed relatively low hydrolytic activity towards parabens with shorter and longer alkyl side chains. In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. Rat small-intestinal microsomes exhibited relatively higher activity toward longer-side-chain parabens. Human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes.

Metabolism rates of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben by HLM were inversely proportional to chain length (overall rate dominated by esterase-catalyzed hydrolysis, where the longer the alcohol moiety, the slower the hydrolysis).[51] This trend was also observed for human skin microsomes (HSM), but at much lower rates. Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the paraben. In contrast to human tissue fractions, the rat tissue fractions tested, including skin and liver fractions, hydrolyzed the parabens at rates that increased as the ester chain length increased. Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin.

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference. [52] Butylparaben was extensively hydrolyzed to 4-Hydroxybenzoic Acid as the major metabolite for both sexes and species. The other metabolite observed in the human hepatocytes was 4-hydroxyhippuric acid, which is the glycine conjugate (i.e., a Phase II metabolite) of 4-Hydroxybenzoic Acid.

Animal

Dermal

Nine rats were given a single dermal dose of 100 mg/kg bw 4-hydroxy [ring-U-^{14}C]-labeled Methylparaben, Propylparaben, or Butylparaben in 60% aqueous ethanol vehicle. C_{max} (≥ 693 and ≥ 614 ng eq/g in males and females, respectively) occurred within 8 h post-application, and blood concentrations decreased until the last quantifiable concentration within 24 h.[53] Most of the dosage (≥ 46.4%) was not absorbed, and less than 25.8% was found in the urine. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of [^{14}C]-Butylparaben was absorbed 72 h following application to the skin in rats.[52] Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in the liver.

Oral

In rats exposed to a single oral dosage of 100 mg/kg bw [ring-U-^{14}C]-labeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥ 11,432 and ≥ 21,040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h.[53] Radioactivity was eliminated rapidly, with averages ≥ 69.6% recovered in the urine during the first 24 h. Radioactivity was excreted predominantly in urine in rats orally exposed to a single 10, 100, or 1000 mg/kg bw/day dosage of [^{14}C]-Butylparaben.[52] The rate of urinary excretion was similar across all dosages, with ≥ 66% recovered in the first 24 h in males. Female rats excreted more Butylparaben in urine in the first 4 h after exposure, but there was no sex difference in the total dose excreted within 24 h.

Time-mated female SD rats were orally administered 0, 1500, 5000, or 15,000 ppm Butylparaben via NIH-07 feed, ad libitum, from gestation day (GD) 6 to postnatal day (PND) 28.[54] Dam plasma, amniotic fluid and fetuses were collected on GD 18 and plasma from both the pup and dam were collected on PNDs 4, 10, 14, 21, and 28 and analyzed for free (unconjugated) and total (unconjugated and conjugated) Butylparaben. Free Butylparaben was below the limit of quantitation in fetuses (LOQ 1.91 ng Butylparaben/g fetus) and amniotic fluid (LOQ 0.17 ng Butylparaben/mL amniotic fluid) at 1500 ppm. Analyte levels in amniotic fluid were less than 1% of maternal plasma, suggesting limited placental transfer. The total Butylparaben in PND 4 pup plasma was less than 5% of dam plasma in all exposure groups, suggesting low lactational transfer. However, at nearly all time points and exposure groups, there were higher levels of free Butylparaben in pup versus dam plasma, suggesting limited conjugation in pups. Pup conjugation of Butylparaben was age-dependent, not reaching the percent-conjugation in dams (> 99%) until PNDs 21 to 28. These data illustrate low placental and lactational transfer of dietary Butylparaben and that poor conjugation in pups during early lactation results in higher exposure to free Butylparaben in pups compared to dams.

Human

Dermal

All 26 male volunteers showed increased excretion of Butylparaben following daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben, 2% diethyl phthalate and 2% dibutyl phthalate.[55] Mean total Butylparaben excreted in urine during exposure was 2.6 ± 0.1 mg/24 h. The concentrations peaked in the urine 8 - 12 h after application.

Oral

Free and conjugated parabens and their major, non-specific metabolites (4-Hydroxybenzoic Acid and *p*-hydroxyhippuric acid) were detected in the urine samples of three subjects 24 h after an oral dose of deuterated Methylparaben, Butylparaben, and Isobutylparaben.[56] Minor metabolites discovered had hydroxy groups on the alkyl side chain or oxidative modifications on the aromatic ring.

Physiologically Based Pharmacokinetic (PBPK) Modelling

In one study, a PBPK model was developed and used to estimate the plasma free paraben concentration in adults consistent with 95th percentile urine concentration reported in US National Health and Nutrition Examination Survey (NHANES) program (2009 - 2010 collection period).[57] For the 2009 - 2010 sampling period, the predicted plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 70 kg male was 0.73, 0.21, and 0.052 µg/L, respectively; the predicted plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 60 kg female was 1.19, 0.54, and 0.58 µg/L, respectively. An in vitro based cumulative MOS was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the predicted free plasma paraben concentrations (Methylparaben + Ethylparaben + Butylparaben). The calculated cumulative MOS for adult females was 108, whereas the cumulative MOS for males was 444.

TOXICOLOGICAL STUDIES

Acute Dose Toxicity

No new published acute toxicity studies were discovered in the published literature, and no unpublished data were submitted. Acute subcutaneous studies are summarized in Table 11.

1984

Acute toxicity studies in animals indicate that parabens are practically nontoxic by various routes of administration.[44]

1986

Benzylparaben was not considered an acute toxic agent to mice or rats. Intravenous injections of Benzylparaben to dogs and cats caused no variation in blood sugar, circulation, and respiration.[45]

1995

Isobutylparaben had a subcutaneous LD_{50} of 2600 mg/kg in mice.[46]

Short-Term Toxicity Studies

1995

No significant histological changes were observed in mice dosed with 0.6% Isobutylparaben in the feed for 6 weeks. Mice dosed with 1.25% had atrophy of the spleen, thymus, and lymph nodes as well as multifocal degeneration and necrosis of the hepatic parenchyma. Mice dosed with 5% and 10% Isobutylparaben died within the first 2 weeks of the study.[46]

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.[2] *Fischer 344 male rats were treated by Methylparaben, Ethylparaben, Propylparaben, and Butylparaben at 4% for 9 - 27 days in the dry diet, and the magnitude of the proliferative effect in the prefundic area of the forestomach epithelium elevated as the alkyl chain length increases.*

The short-term toxicity studies that are summarized below are presented in Table 12.

Dermal

There were no significant changes in body and organ weights in any group when rats were dermally exposed to up to 600 mg/kg bw/day Isopropylparaben or Isobutylparaben for 28 days.[58] Macroscopic and microscopic examinations revealed mild-to-moderate skin damage in female rats treated by Isobutylparaben or Isopropylparaben at doses higher than 600 or 50 mg/kg bw/day, respectively. The weights of testes were significantly increased in male rats given a 1:1 mixture of Isobutylparaben and Isopropylparaben at doses of 600 or 1200 mg/kg bw/day. Follicle-stimulating hormone (FSH) concentration was dose-dependently decreased in males treated with mixture of Isobutylparaben and Isopropylparaben at a dose of 100 mg/kg bw/day or higher. The NOAELs for Isobutylparaben and Isopropylparaben for female skin damage were 600 mg/kg bw/day and 50 mg/kg bw/day, respectively.

Oral

At 100 and 300 mg/kg bw/day Propylparaben administered orally for four weeks, adult rats exhibited statistically-significant increases in relative liver weights, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, serum urea concentrations, lipid peroxidation and nitric oxide (NO) generation, and 17β-estradiol (E2) concentrations.[59] Statistically-significant decreases in total serum protein and albumin, glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities, serum testosterone concentrations, and T/E2 ratios, were also reported. Livers of affected rats exhibited dilated congested central and portal veins, highly proliferated bile ducts with fibrotic reactions, and multifocal areas of necrotic hepatocytes, and testes exhibited evidence of severe spermatogenic arrest, among other effects. Elevations of serum markers of lipid peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically significant in rats exposed to 250 mg/kg bw/day Methylparaben.[60] Malondialdehyde levels were elevated in the liver in a statistically significant, dose-dependent manner, among other effects, in mice orally exposed to 1.33 - 40 mg/kg bw/day Butylparaben for 30 days.[61]

Subchronic Toxicity Studies

No new published subchronic toxicity studies were discovered in the published literature, and no unpublished data were submitted, since the 1984 CIR report.

1984

Subchronic… oral studies indicate that parabens are practically nontoxic.[44] *A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses up to 2 g/kg/day.*

Chronic Toxicity Studies

No new published chronic toxicity studies were discovered in the published literature, and no unpublished data were submitted, since the 2008 CIR report. A chronic subcutaneous study has been summarized in Table 11.

1984

Chronic oral studies indicate that parabens are practically nontoxic.[44] A 60:40 mixture of the sodium salts of Propylparaben and Ethylparaben did not induce significant pathologic changes in rats treated at 1.4 g/kg bw/day for 18 months. At 2 percent of the diet, Methylparaben and Propylparaben exerted no toxic effect in rats after 96 weeks exposure. Weanling dogs treated by Methylparaben or Propylparaben at 1 g/kg bw/day for 378 to 422 days were in excellent condition throughout the experiment.

1995

Mice were orally dosed with 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks.[46] Upon necropsy, the only effect noted was amyloidosis in 58% of dosed males and 33% of dosed females surviving past 78 weeks, as compared with 25% of control males and 10% of control females.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

1984

Methylparaben was nonteratogenic in rabbits, rats, mice and hamsters, and Ethylparaben was nonteratogenic in rats.[44] Pregnant animals were given orally 5.0 to 550 mg/kg bw/day (rats, mice) or 3.0 to 300 mg/kg bw/day (hamsters) Methylparaben from Day 6 of gestation to Day 10 (hamsters) or 15 (rats, mice). Pregnant rabbits were orally administered 3.0 to 300 mg/kg bw/day Methylparaben daily from Day 6 of gestation to Day 18. Pregnant rats were dosed in diet of Ethylparaben at concentrations of 0.1, 1, or 10 % between gestation Days 8 and 15. On day 21 of pregnancy, rats were killed, and the number of fetal implantations, status of maternal visceral organs, fetal body weights, and numbers of skeletal, visceral, and external defects in fetuses were recorded. No apparent teratogenesis or toxicity was observed in 363 fetuses from rats fed up to10% Ethylparaben.

At the 10% level, cerebral hemorrhages, abnormal enlargement in the ventricles of the brain, and, in some, hydronephrosis and hypo-osteogenesis were observed in fetuses. Some fetuses at 1% Ethylparaben had no blood in the cardiac ventricle; some had intraperitoneal hemorrhages. Fetuses of rats of the 0.1% group had no significant visceral or skeletal defects.

2008

Methylparaben was nonteratogenic in rabbits, rats, mice, and hamsters, and Ethylparaben was nonteratogenic in rats.[2] Parabens, even at levels that produce maternal toxicity, do not produce terata in animal studies. One study examined the developmental toxicity of Butylparaben in rats and reported no effect on development up to an oral dose of 1000 mg/kg bw/day, even with some maternal toxicity at that dose. The maternal toxicity NOAEL dose was 1000 mg/kg bw/day.

Parabens have been extensively studied to evaluate male reproductive toxicity. In one in vitro study, sperm viability was eliminated by concentrations as low as 6 mg/ml Methylparaben, 8 mg/ml Ethylparaben, 3 mg/ml Propylparaben, or 1 mg/ml Butylparaben, but an in vivo study of 0.1% or 1.0% Methylparaben or Ethylparaben in the diet of mice for 8 weeks reported no spermatotoxic effects. Propylparaben did affect sperm counts at all levels from 0.01% to 1.0% (approximately 10 and 1000 mg/kg bw/day, respectively.). Epididymis and seminal vesicle weight decreases were reported in rats given a 1% oral Butylparaben dose, and decreased sperm number and motile activity in F1 offspring of rats maternally exposed to 100 mg/kg bw/day were reported. Decreased sperm numbers and activity were reported in F1 offspring of female rats exposed to Butylparaben subcutaneously at 100 or 200 mg/kg bw/day, but there were no abnormalities in the reproductive organs. The total treatment period was from gestation day 6 to postnatal day 20, with a 2-day interruption at parturition.

Methylparaben was studied using male rats at levels in the diet up to 10,000 ppm (estimated mean dose of 1141.1 mg/kg/day) with no adverse effects. Butylparaben was studied using rats at levels in the diet up to 10,000 ppm (estimated mean dose of 1087.6 mg/kg/day) in a repeat of the study noted above, but using a larger number of animals and a staging analysis of testicular effects. Rats received Butylparaben in the diet for a minimum of 56 days. No adverse reproductive effects were found.

Butylparaben, administered subcutaneously at 2mg/kg bw/day in male rats on postnatal days 2 to 18, produced only minor effects on epithelial cell height. No effect of Butylparaben on the expression of the water channel protein aquaphorin-1(APQ-1), efferent duct distension, or rete testis morphology was seen.

Dermal

No new published dermal DART studies were discovered and no unpublished data were submitted.

Oral

The oral DART studies summarized below are described in Table 13.

Time-mated rats were orally exposed to 10, 100, or 500 mg/kg bw/day of Butylparaben from GD 7 to PND 22.[62] The anogenital distance (AGD) of newborn male and female offspring was significantly reduced at 100 or 500 mg/kg bw/day. The reduced expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring was statistically significant at 10 mg/kg bw/day or above. In male offspring, epididymal sperm count decreased 76 - 78% compared to controls at all doses from 10 to 500 mg/kg bw/day. The reduction of epididymal sperm count showed the same effect at all doses (i.e. no dose-response effect was observed). Adult prostate weight reductions were statistically significant at 500 mg/kg bw/day. In prepubertal females, ovary weight reduction was statistically significant and mammary gland outgrowth was increased at 100 and 500 mg/kg bw/day. No clear effect was seen on mammary glands of adult female offspring.

Pregnant rats were orally exposed to 64, 160, 400, or 1000 mg/kg bw/day of Butylparaben from GD 7 to PND 21.[63] In the 400 and 1000 mg/kg bw/day groups of male offspring, reduced AGD and delayed preputial separation (PPS) were observed; the weights of the testes were significantly reduced and serum testosterone was reduced in a dose-response manner from PND 21 to PND 90. On PND 90, the number of the caudal epididymal sperm was significantly decreased by approximately 36% at 400 and 1000 mg/kg bw/day, and daily sperm production values were significantly decreased. In contrast, weights of the testes, epididymal cauda sperm counts, serum testosterone (T) and luteinizing hormone (LH) levels, and daily sperm production in male offspring did not change at doses of 64 and 160 mg/kg bw/day.

Estradiol (E_2) level was significantly elevated in weanling male rats orally exposed to Butylparaben at 50 mg/kg for 8 consecutive weeks, whereas serum levels of the hormones T, LH, and follicle-stimulating hormone (FSH), as well as ratios of T/E_2 and T/LH was decreased, compared to control groups.[64] Butylparaben treatment elevated markers of testicular DNA damage in a comet assay, such as the increase in the tail DNA%, tail length of DNA, and tail moment. In addition, the testicular malondialdehyde level was significantly elevated, along with a significant decrease in catalase enzyme activity. Histopathological examination showed a reduction in Leydig cells population along with pathological alternations of dilated congested subcapsular blood vessels and the dilation and congestion of interstitial vasculature.

The increase of CYP19 and estrogen receptor (ER)α expression; the reduction of steroidogenic acute regulatory protein (StAR), cytochrome cholesterol side-chain cleavage enzyme (P450scc), estrogen sulfotransferase (SULT1E1), and testes androgen receptor (AR) expression; and the reduced methylation rate of the ERα promoter, were statistically significant in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD 7 to GD 21.[65] Vimentin filaments showed shorter projections, concentration near the basal region, and disappearance of the apical extensions toward the lumen of the seminiferous tubules in 3-week old rats 6 h after a single 1000 mg/kg bw oral dosage of Butylparaben.[66] Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

Prepubertal female rats were exposed orally to Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, or Isobutylparaben in a dose-dependent manner (62.5, 250, and 1000 mg/kg bw/day) on PND 21 to PND 40. Rats treated with 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Isopropylparaben exhibited statistically-significant delays in vaginal opening.[67] In the 1000 mg/kg bw/day groups, there were statistically-significant decreases in the weights of the ovaries (Methylparaben or Isopropylparaben) and kidneys (Ethylparaben or Isopropylparaben), and increases in the weights of the adrenal glands (Methylparaben, Ethylparaben, or Propylparaben) and thyroid glands (Methylparaben). Liver weights increased at all dosage rates of Butylparaben. Morphological studies of the uterus revealed myometrial hypertrophy after exposure to 1000 mg/kg bw/day Propylparaben or Isopropylparaben and in animals of all dose groups of Butylparaben and Isobutylparaben. Among the statistically significant effects on serum hormone concentrations, estradiol concentrations were reduced (Ethylparaben or Isopropylparaben) and prolactin concentrations were increased (Methylparaben) in the 1000 mg/kg bw/day groups. Reduced plasma leptin concentrations were observed in male and female offspring of young adult female rats exposed orally to 100 mg/kg bw/day Butylparaben.[68]

F2 pups exhibited a statistically-significant greater mortality at PND 7 and thereafter, compared with controls, in a DART study in which F0 females and their F1 offspring were exposed to 0.105 mg/kg bw/day Methylparaben by gavage.[69] During lactation, treated "parous" F1 females exhibited mammary alveoli that were not always milk-filled, collapsed alveolar and duct structures with residual secretory content, and marked decrease in the size of the lobular structures.

There was no evidence of an effect on the weight of the male reproductive organs, epididymal sperm parameters, hormone concentrations, or histopathology in juvenile male rats exposed via lactation from maternal rats receiving up to 1000 mg/kg bw/day Propylparaben for 8 weeks.[70]

Methylparaben was associated with a statistically-significant higher incidence of abnormal sperm in rats exposed to 1000-ppm or 10,000-ppm in the diet for 8 weeks, mostly sperm with no head in 4% to 5% of sperm, compared with 2.3% in 100-ppm and control groups.[48] Measurements of hormone concentrations were generally not altered, except that testosterone (T) and follicle stimulating hormone (FSH) concentrations were higher in the 10,000-ppm Butylparaben-treated group, compared with the control group. The authors concluded that the no-observed-adverse-effect-concentration (NOAEC) was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of about 1140 and 1100 mg/kg/day for Methylparaben and Butylparaben, respectively.

Histopathologic examination revealed progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules and reduction and/or disappearance of tubular lumen 3 h after a single 1000 mg/kg oral dosage of Butylparaben in rats.[71] Terminal deoxynucleotidyl transferase (TdT)-mediated fluorescein- dUTP nick end labelling (TUNEL) assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h.

Subcutaneous

Subcutaneous DART studies have been summarized in Table 11.

Aquatic

Zebrafish embryos were exposed to sub-lethal concentrations of Methylparaben: 0.1, 1, 10, and 100 ppb. A significant inhibition in the acetylcholinesterase (AChE) activity, as well as an increase in cortisol levels, was observed in the exposed groups.[72] Alterations in developmental landmarks such as heart rate and hatching percentage were observed in embryos exposed to 10 ppb and 100 ppb of Methylparaben. Anxiety-like behavior was induced in larvae exposed to 0.1 ppb and 1 ppb of Methylparaben.

Exposure of zebrafish embryos to Methylparaben at 200, 400, 800, and 1000 µM for 96 h post fertilization (hpf), resulted in decreased heart rate and hatching rate and developmental abnormalities, including pericardial edema blood cell accumulation and bent spine.[73] The 96 hpf LC_{50} of Methylparaben was 428 µM (0.065 mg/L) and expression of vitellogenin was significantly upregulated compared to the control group in larval zebrafish exposed to 100µM (0.015mg/L) of Methylparaben till 96 hpf.

GENOTOXICITY STUDIES

1984

Numerous mutagenicity studies, including the Ames test, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the Methylparaben, Ethylparaben and Propylparaben are non-mutagenic.[44]

1995

Chinese hamster fibroblast cell lines treated with 0.03% Isobutylparaben had no chromosomal aberrations after 48 h.[46]

At a concentration of 1 mg/plate, Isobutylparaben and Isopropylparaben had negative Ames tests in Salmonella typhimurium. After 48 h, cells treated with 0.125 mg/ml Isopropylparaben or 0.6 mg/ml Isobutylparaben in ethanol had 2.0% and 3.0% polyploid cells, respectively. Both had a 1% incidence of structural chromosomal aberrations.

2008

A number of genotoxicity studies suggest the Methylparaben, Propylparaben, Isopropylparaben and Butylparaben are generally non-mutagenic.[2] Ethylparaben, Propylparaben, and Butylparaben induced 1% to 3% increases in polyploid cell production in an in vitro assay using Chinese hamster ovary (CHO) cells; Ethylparaben and Methylparaben were judged to induce significant chromosomal aberrations (11.0% and 15.0% increases, respectively) in the same study.

In Vitro

Methylparaben

Human spermatozoa were exposed to 13 mM Methylparaben for 2 or 5 h.[74] Methylparaben had no significant effect on DNA fragmentation as measured by the TUNEL and the sperm chromatin dispersion (SCD) assays in human spermatozoa. A statistically significant decrease in spermatozoa motility was observed after 2 and 5 h. After 5 h of exposure, a significant increase of the following parameters was observed in a time-dependent manner: annexin V and fluorescently labelled inhibitor of caspase assay (FLICA) signals, mitochondrial and total superoxide generation, as well as 8-hydroxy-2'-deoxyguanosine (8OHdG) production. In contrast, Methylparaben at a concentration of 2.5 mM did not induce any significant changes to the motility, vitality, mitochondrial reactive oxygen species (ROS) production, and 8OHdG formation over the 5-h time exposure period.

Propylparaben

Vero cells (derived from African green monkey kidney) were grown and incubated for 24 h with 0, 50, 200, 300, 400, or 500 µM Propylparaben at 37 °C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 5% fetal calf serum (FCS), 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine.[75] A statistically-significant, dose-dependent decrease in percentage of mitotic cells was observed across the concentrations tested (4-fold decrease at 500 µM, compared with control). Flow-cytometric analysis of DNA content revealed that the decline was attributable mainly to cell-cycle arrest at the G0/G1 phase. Immuno-detection techniques revealed statistically-significant induction of DNA DSBs (2-fold compared to control) verified by 8-OHdG staining at all concentrations tested (maximum intensity at 500 µM).

CHO cells were grown, and incubated for 1 or 3 h with 0, 0.5, 1, 1.5, 2, or 2.5 µM Propylparaben.[76] Sister chromatid exchange (SCE), chromosome aberration (CA), and DNA strand break (comet) assays were performed. Statistically-

significantly elevated SCEs/cell and CAs/cell were observed in cells incubated with Propylparaben (≥ 1.5 μM) and Propylparaben (≥ 1.0 μM) for 3 h, respectively.

Human spermatozoa were exposed to 2.5 mM Propylparaben for 2 or 5 h.[74] A statistically significant reduction in sperm motility as well as a stimulation of mitochondrial ROS was observed at both time points. After 2 h, Propylparaben exposure resulted in a significant loss of mitochondrial membrane potential (MMP).

Butylparaben

CHO cells were grown, and incubated for 1 or 3 h with 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mM or 0, 0.1, 0.25, 0.5, or 0.75 μM, respectively Butylparaben.[76] SCE, CA, and DNA strand break (comet) assays were performed. Statistically-significantly elevated indices of DNA fragmentation were observed in cells incubated for 1 h with ≥ 0.4 μM Butylparaben. Comparatively high incidences of fragmentation were observed. Statistically-significant, elevated SCEs/cell and CAs/cell were observed in cells incubated with 0.75 μM Butylparaben for 3 h.

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben

Human spermatozoa were exposed to a paraben mixture containing equal concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben for 24 h.[74] Significantly reduced motility was observed immediately after the treatment and was further exacerbated after 24 h at concentrations of 1, 2, and 4 mM (i.e., a mixture containing 250, 500, and 1000 μM of each paraben). After 24 h, spermatozoa that had been treated with 0.2 and 1 mM of the parabens mixture exhibited a significant increase in the generation of mitochondrial ROS, which then declined in concert with the loss of cell viability. An acute total superoxide response was also observed with dihydroethidium (DHE) shortly after parabens exposure, which became statistically significant at 2 and 4 mM. Caspase activation was observed following exposure to parabens concentrations above 1 mM and increased still further after 24 h.

In Vivo

No published in vivo genotoxicity studies were discovered in the published literature, and no unpublished data were submitted.

CARCINOGENICITY STUDIES

No new published dermal, oral, or inhalation carcinogenicity studies were discovered in the published literature, and no unpublished data were submitted, since the 1995 CIR report.

1984

Methylparaben was non-carcinogenic when administered intravaginally in rats and was not co-carcinogenic when injected with dibenzo[a,i]pyrene (DBP) subcutaneously in mice.[44] Propylparaben was noncarcinogenic in a study of transplacental carcinogenesis.

1995

No changes in either neoplasm incidence or time to neoplasm development were observed in mice dosed with 0.15, 0.3, or 0.6% Isobutylparaben in the feed for 102 weeks as compared with controls.[46]

2008

Isobutylparaben and Butylparaben were noncarcinogenic when given to mice in diet at levels of 0.15%, 0.3%, and 0.6% for 102 weeks ,respectively [2]

OTHER RELEVANT STUDIES

Endocrine Activity

2008

Butylparaben binds to estrogen receptors in isolated rat uteri, with an affinity orders of magnitude less than natural estradiol.[2] The estrogenic effect of parabens has been estimated by their competitive binding to the human estrogen receptors α and β. With diethylstilbestrol binding affinity set at 100, the relative binding affinity of the parabens increased as a function of chain length from not detectable for Methylparaben to 0.267 ± 0.027 for human estrogen receptor a and 0.340 ± 0.031 for human estrogen receptor β for Isobutylparaben. In a study of androgen receptor binding, Propylparaben exhibited weak competitive binding, but Methylparaben had no binding effect at all.

Methylparaben failed to produce any effect in uterotrophic assays in two laboratories, but did produce an effect in other studies from another laboratory. The potency of Methylparaben was 1000 to 20,000 less when compared to natural estradiol. The same pattern was reported for Ethylparaben, Propylparaben, and Butylparaben when potency was compared to natural estradiol; in positive studies the potency of Ethylparaben was 346 to 25,000 less; the potency of Propylparaben was 1612 to 20,000 less; and the potency of Butylparaben was 436 to 16,666 less. In two studies, Isobutylparaben did

produce an estrogenic response in the uterotrophic assay, but the potency was 240,000 to 4,000,000 less than estradiol. In one study, Benzylparaben produced an estrogenic response in the uterotrophic assay, but the potency was 330,000 to 3,300,000 less than estradiol.

Estrogenic activity of parabens and 4-Hydroxybenzoic Acid was increased in human breast cancer cells in vitro, but the increases were around 4 orders of magnitude less than that of estradiol. Several overviews of the endocrine disruption (estrogenic and androgenic effects) generally note that any effect of parabens is weak.

Another assessment of the endocrine disrupting/estrogenic potential of parabens noted that parabens do not have genotoxic, carcinogenic, or teratogenic potential and are rapidly hydrolyzed to 4-Hydroxybenzoic Acid and excreted. This assessment noted that parabens are able to bind estrogen and androgen receptors, activate estrogen-responsive genes, stimulate cellular proliferation, and increase levels of estrogen receptor protein. To place the in vitro data in context, the assessment cited the comparisons of parabens activity with 17β-estradiol and diethylstilbestrol (2 to 5 orders of magnitude lower) and phytoestrogens, including isoflavones (comparable or less). This assessment acknowledged increases or decreases in testes, epididymides, or prostate weights in male animals exposed to Butylparaben and Propylparaben and lower sperm counts in rats and mice exposed to Butylparaben and in rats exposed to Propylparaben, but discounted these effects as without pattern or dose-response.

The endocrine activity studies summarized below are described in Table 14.

In Vitro

Weak activation of murine peroxisome proliferator-activated receptor (mPPAR)α was seen in murine NIH-3T3-L1 cells at the highest concentrations of Butylparaben tested (100 μM).[77] Butylparaben activated mPPARγ with a lowest observed effect concentration (LOEC) of 30 μM and a maximal (4-fold) induction at 100 μM. The human data for Butylparaben (hPPARα and hPPARγ) were comparable to those obtained with mPPARα and mPPARγ, indicating a similar responsiveness.

Isobutylparaben antagonized the androgen receptor (AR) in CHO cells. The effect was statistically significant at ≥ 25 μM.[78] Butylparaben increased the number of BT-474 cells entering S-phase (concentration for half maximal stimulation of proliferation [EC_{50}] = 0.551 μM); the effect was enhanced in the presence of ligand heregulin (HRG; EC_{50} = 0.024 μM).[79] The EC_{50} for glucocorticoid-like activity in MDA-kb2 cells was 1.75 mM for Butylparaben and 13.01 mM for Propylparaben.[80] Butylparaben at 25 μM statistically-significantly enhanced the hydrocortisone-induced glucocorticoid receptor (GR) signal by 85%; Methylparaben, Ethylparaben, and Propylparaben did not have this effect.[81]

Butylparaben exhibited estrogen agonism at all concentrations tested in T47D-KBluc cells.[82] The maximum effect was observed at 10 μM.

The EC_{50}s for stimulating proliferation of MCF-7 cells ranged from 0.4-40 μM, LOECs from 0.1-20 μM, and no observed effects levels (NOECs) from 0.05 - 8 μM for the parabens tested.[83] The parabens tested, in descending order of these values, were Isobutylparaben > Butylparaben > Propylparaben > Ethylparaben > Methylparaben. In comparison, corresponding values for E2 were $EC_{50} = 2 \times 10^{-6}$ μM, LOEC = 10^{-6} μM, and 1×10^{-7} μM. Propylparaben at 10 μM resulted in deformed acini and filling of the acinar lumen in non-transformed MCF-12A and MCF-10A cells.[84] MCF-7 and HCI-7-Luc2 mammospheres treated with Methylparaben exhibited increased expression of ALDH1 (marker of human mammary stem cells) and were larger than control and E2-treated mammospheres.[85] Neither tamoxifen nor fulvestrant inhibited effects of Methylparaben on MCF-7 mammospheres.

Parabens enhanced differentiation of murine 3T3-L1 cells with potencies that increased with the length of the linear alkyl chain (Methylparaben < Ethylparaben < Propylparaben < Butylparaben), and the extension of the linear alkyl chain with an aromatic ring in Benzylparaben further augmented adipogenicity.[86] In the presence of differentiation media, 50 μM Butylparaben or Benzylparaben promoted lipid accumulation in human adipose-derived stem cells (hADSCs) as early as day 3 and throughout the differentiation process. Butylparaben had the strongest adipogenic effects of the parabens tested, whereas other parabens had no effect at 1 or 10 μM.

The US Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program (EDSP) program conducted a series of in vitro assays to examine the estrogenic properties of parabens.[87] There are 15, 14, 11, 5, and 2 positive results out of total 18 arrays for Butylparaben, Propylparaben, Ethylparaben, Methylparaben, and 4-Hydroxybenzoic Acid, respectively; while in vitro anti-androgen studies showed negative results.

Metabolites of Butylparaben and Isobutylparaben, 3-hydroxy n-butyl 4-hydroxybenzoate (3OH) and 2-hydroxy iso-butyl 4-hydroxybenzoate (2OH), exhibited estrogenic properties in MCF-7 and T47D human breast cancer cells.[88] The expression of estrogen-inducible gene (GREB1) was induced by Butylparaben, Isobutylparaben, 3OH, and 2OH at 10 μM, and blocked by co-administration of an ER antagonist (ICI 182, 780). The expression of a proproliferative, estrogen-inducible gene (GREB1) was significantly induced in MCF-7 cells treated by 10 μM Butylparaben, Isobutylparaben, 3OH, and 2OH for 2, 4, and 6h. Computational docking studies showed that the paraben compounds exhibited the potential for favorable ligand-binding domain interactions with human ERα in a manner similar to known x-ray crystal structures of E2 in complex with ERα.

In isolated mouse preantral follicle and human granulosa cell (hGC) cultures, Butylparaben adversely affected steroidogenesis at concentrations relevant to human exposure (100 nM), but no effects on follicular development or survival were noted in the culture systems.[89] Butylparaben attenuated di-(2-ethylhexyl) phthalate (DEHP) induced-reduction of progesterone concentrations in the spent media of hGC cultures. When present together, Butylparaben and DEHP decrease estradiol production.

Animal

Longer diestrus phases and a shortened interval of the estrous cycle were observed in 8-week old rats exposed to Propylparaben or Butylparaben at a dose of 100 mg/kg/day orally for 5 weeks.[90] No effect on the number of primary follicles was observed, while secondary follicles showed a decrease in the total number in all groups treated with Methylparaben, Propylparaben or Butylparaben. Propylparaben and Butylparaben decreased mRNA level of folliculogenesis-related genes (*Foxl2, Kitl,* and *Amh*). An increase in FSH levels in serum was observed, indicating an impairment of ovarian function.

Perinatal Methylparaben exposure in rats at doses mimicking human exposure (0.105 mg/kg/day) decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad.[91] Perinatal Methylparaben treatment was associated with a significant reduction in adipose tissue and more abundant glandular tissue. Long-term Methylparaben treatment from birth to lactation did not result in significant histological changes. In the pubertal window, expression alterations in 993 genes enriched in pathways including cholesterol synthesis and adipogenesis were observed.

Oral exposure to Methylparaben at 500 mg/kg/day caused morphological changes in gerbil prostates.[92] After 3, 7, and 21 days of treatment, male and female gerbils displayed similar alterations such as prostate/Skene's paraurethral gland epithelial hyperplasia, increased cell proliferation, and a higher frequency of androgen receptor binding activity.

Relative uterine weights were elevated in immature Sprague-Dawley rats after treatment with ≥ 0.16 mg/kg bw/day Benzylparaben on PND 21-PND 23.[93] Lowest-observed-effect-levels (LOELs) for increased relative uterine weight after treatment of immature female rats with Methylparaben or Ethylparaben on PND 21-PND 23 were 20 and 4 mg/kg bw/day, respectively.[94] No-observed-effect-levels (NOELs) for Methylparaben and Ethylparaben were 4 and 0.8 mg/kg bw/day, respectively. Ethylparaben and Propylparaben were negative for estrogen agonism and antagonism in ovariectomized female mice exposed to 1000 mg/kg bw/day by gavage for 7 days.[95]

Histopathologic examination revealed progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules and reduction and/or disappearance of tubular lumen 3 h after a single 1000 mg/kg oral dosage of Butylparaben in rats.[71] TUNEL assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h.

Human

In 26 healthy Caucasian males, minor differences in inhibin B, LH, estradiol, total thyroxine (T4), free thyroxine (FT4), and TSH concentrations were observed after daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben as well as 2% diethyl phthalate and 2% dibutyl phthalate, compared to the concentrations measured before the treatment.[42] The differences could not be attributed to the treatment.

Effects on Human Breast Cells

Methylparaben, Propylparaben, Butylparaben

MCF-10A non-transformed, immortalized human breast epithelial cells were exposed to 500 μM Methylparaben, 10 μM Propylparaben or Butylparaben in semi-solid 2% methylcellulose suspension culture, or 1 μM Methylparaben or 0.1 μM Propylparaben or Butylparaben in monolayer culture.[96] Ethanol served as the vehicle. The cells were grown in suspension culture (non-adherent conditions) to assess colony growth after a 17-day incubation period. Cells were grown in monolayer culture (adherent conditions) to assess cellular proliferation after a 7-day incubation period. In suspension culture, MCF-10A cells produced very few colonies and only of a small size. The presence of 500 μM Methylparaben or 10 μM Propylparaben or Butylparaben resulted in greater numbers of colonies per dish ($p < 0.05$) and greater average colony sizes ($p < 0.001$) compared with controls. Average colony sizes of cells grown with a paraben were comparable to those of cells grown with 17β-estradiol (70 nM). Concentration-response experiments showed that maximal numbers of colonies were formed at 100 μM Methylparaben or 1 μM Propylparaben or Butylparaben. Control experiments showed that the parabens did not influence the growth of MCF-10A cells under adherent conditions (i.e., monolayer cultures).

Human high-risk donor breast epithelial cells (HRBECs) were collected from the unaffected contralateral breasts of women undergoing breast surgery with a personal or family history of breast cancer, atypical neoplastic histopathology, and/or high mammographic density.[97] The cells were incubated for 7 days with 10 nM to 1 μM (vehicle not specified) Methylparaben in phenol red-free medium supplemented with 0.2% charcoal-stripped fetal bovine serums.[97] Some cells were exposed to 10 μM 4-hydroxy tamoxifen (OHT) or 1, 10, or 100 nM rapamycin for 24 h before functional analysis. Methylparaben substantially reduced the fraction of OHT-induced apoptotic cells in a concentration-dependent manner ($p = 0.001$) at all three concentrations: 57.82% ± 6.77% at 1 μM, 55.93% ± 10.54% at 100 nM, and 28.14% ± 11.3% at 10 nM. Methylparaben induced a detectable decline in endogenously accumulated reactive oxygen species (ROS) in all cell cultures.

In early passage HRBECs, average reduction in ROS by Methylparaben treatment was 38% ($p < 0.02$), without an evident concentration-response relationship. Prior exposure to Methylparaben resulted in a concentration-dependent, complete-to-partial evasion from the G1-phase arrest induced by OHT, and concurrent increase in the S-phase fraction. In contrast, the growth inhibitory effects of OHT were not reversed by a combination of luteal-phase serum concentrations of E2 and progesterone. The maintenance of S-phase in OHT-treated cells, like apoptosis evasion, was correlated with increasing concentrations of Methylparaben ($p < 0.001$).

Effects on Human Trophoblast Cells

Butylparaben

Human trophoblast cells, HTR8/SVneo, were exposed to Butylparaben at 50, 100, 200, and 400 µM.[98] Butylparaben inhibited cell proliferation and induced both apoptosis and endoplasmic reticulum stress at all doses. Butylparaben promoted the production of intracellular reactive oxygen species, increased Ca^{2+} concentration, and induced mitochondrial membrane depolarization. Butylparaben also inhibited the activation of PI3K/AKT pathways including AKT, ribosomal protein S6, P70 S6 kinase, and glycogen synthase kinase 3b. In addition, ERK1/2 activity was involved in Butylparaben-mediated signal transduction in HTR8/SVneo cells. The study author claimed that exposing human trophoblast cells to Butylparaben diminished normal physiological activity, leading to apoptosis and problems with early placental development.

Biomonitoring

The biomonitoring studies summarized below are described in Table 15.

Biomonitoring is the direct measurement of human exposure by measuring the parabens or their metabolites in human biological fluids (e.g., urine, blood), which account for both dietary intake (e.g., from foods and medicinal products with paraben preservatives) and dermal application of products with parabens. However, the presence of a substance in the blood or urine does not mean that it will cause effects or disease.[99] Chemical toxicity is related to its dose or concentration, in addition to a person's individual susceptibility. Small amounts may be of no health consequence, whereas larger amounts may cause adverse health effects.

The US NHANES program (the Fourth National Report) provides a large dataset for human spot urine levels of parabens, collected from 2005 to 2014, with 2013 - 2014 being the most recent collection period.[99] A total of 2686 urine specimens from a representative sample of persons ≥ 6 years of age in the US general population, was analyzed for the exposure level to Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. For the 2013 - 2014 sampling period, the median concentration of Methylparaben in urine was 48.1 µg/L (95th percentile: 819 µg/L), and Propylparaben in urine was 5.74 µg/L (95th percentile: 224 µg/L). For Butylparaben, the median concentration in urine was below the limit of detection (LOD, 0.1 µg/L) for all groups (age, gender, and race/ethnicity) in the 2011 - 2014 reporting period. In females, the median concentration of Ethylparaben in the 2013 - 2014 reporting period was 1.6 µg/L (95th percentile: 145 µg/L) while concentrations in males were below the LOD (1 µg/L).

Data from the US NHANES program were also used to analyze the exposure to parabens through oral hygiene products and sunscreen use.[100] Compared to individuals who reported "never" using mouthwash, individuals who reported daily use had significantly elevated urinary concentrations of Methylparaben and Propylparaben (30 and 39% higher, respectively). Individuals who reported "always" using sunscreen had significantly higher urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben (92, 102, and 151% higher, respectively) compared to "never" users of sunscreen. Associations between exposure biomarkers and sunscreen use were stronger in women compared to men, and associations with mouthwash use were generally stronger in men compared to women.

A community-based intervention study indicated that using personal care products (PCPs) that are labeled to be free of parabens, for 3 days, lowered urinary concentrations of Methylparaben and Propylparaben in 100 girls: Methylparaben and Propylparaben concentrations decreased by 43.9% (95% CI: –61.3, –18.8) and 45.4% (95% CI: –63.7, –17.9), respectively.[101] The GM concentration of Methylparaben decreased from 77.4 to 43.2 µg/L, and Propylparaben decreased from 22.6 to 12.3 µg/L. In contrast, the GM concentration of Ethylparaben increased from 2.9 to 4.2 µg/mL, and Butylparaben increased from 0.8 to 1.7 µg/mL. Concentrations of both Ethylparaben and Butylparaben were low overall and not detected in almost half the samples. In the same study population of 100 adolescent girls, participants who reported using "makeup" every day vs. rarely/never, had higher urinary concentrations of Methylparaben (120.5 ng/ mL vs. 13.4 ng/mL, $p < 0.01$) and Propylparaben (60.4 ng/mL vs. 2.9 ng/mL, $p < 0.01$).[102] However, ingredients (including Methylparaben and Propylparaben) in "makeup" products used by the girls were not disclosed. Other sources of parabens (food, pharmaceuticals, endogenous, etc.) were not considered.

A statistically significant difference was observed between serum parabens in 18 women who used lipstick containing Methylparaben and Propylparaben for 5 days compared with those not using this cosmetic ($p = 0.0005$ and 0.0016, respectively), and a strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202).[103]

One study reported the free and total paraben concentrations in 16 human serum samples in the US.[104] The mean total paraben concentrations in serum are 42.6 µg/L and 7.4 µg/L for Methylparaben and Propylparaben, respectively; whereas the

free concentration of Methylparaben and Propylparaben in the serum is 2.2 µg/L and 0.5 µg/L, respectively, indicating that parabens that are not hydrolyzed to 4-Hydroxybenzoic Acid are rapidly conjugated.

One or more of 5 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isobutylparaben) was detected in 99% of breast tissue samples collected from women with breast cancer, and all 5 were detected in 60% of the samples.[105] Median concentrations were highest for Propylparaben (16.8 ng/g tissue) and Methylparaben (16.6 ng/g tissue). Propylparaben concentrations were statistically significantly higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts.

Ethylparaben, Butylparaben, and Benzylparaben were detected in all placenta samples collected from healthy mothers.[106] The highest measured concentration was 11.77 ng Methylparaben/g tissue. The amount of Butylparaben, Ethylparaben, Methylparaben and Propylparaben was studied in human ovarian tumor samples.[107] The tissue mass fractions of the four parabens in the malignant tissues were at least twice as much as those present in the benign tissues. The tissue mass fractions of Methylparaben and Ethylparaben were higher than Propylparaben and Butylparaben.

Thirty-one pregnant women who provided multiple spot urine samples (n = 542) collected over two 24-h periods had their samples analyzed for Methylparaben, Propylparaben, Ethylparaben, Butylparaben, Isobutylparaben, and Benzylparaben.[108] These parabens were also measured in breast milk samples collected at approximately 3 months postpartum (n = 56 women). Women who used body and face lotions in the past 24 h had significantly higher geometric mean (GM) paraben concentrations (80 - 110%) in their urine than women who reported no use in the past 24 h. There was 100%, 72%, 96%, and 90% detection of Methylparaben, Butylparaben, Propylparaben, and Ethylparaben in urine, respectively. Lower detection rates were seen for Isobutylparaben (39%) and Benzylparaben (41%). Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben, respectively.

The conjugated or free species of six parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben), or their metabolite, 4-Hydroxybenzoic Acid, were measured in human adipose fat samples collected from 20 donors who underwent liposuction surgery.[109] Ethylparaben and Propylparaben were more frequently detected than the other parabens, at a detection frequency of 60% and 50%, and a GM concentration of 0.90 and 0.49 ng/g, respectively. GM concentrations of other parabens were not calculated due to their detection of lower than 50%. The GM concentration of the sum of six parabens and 4-Hydroxybenzoic Acid ($C_{\Sigma parabens}$) in adipose fat was 3420 ng/g. While a positive correlation between donor's age and $C_{\Sigma parabens}$ (75^{th} percentile of adipose concentrations; n = 15) was observed, no significant difference in concentrations of $C_{\Sigma parabens}$ between the two age groups were found (18 - 33 yr and 34 - 58 yr). However, the authors noted that total paraben measurements may have been compromised by alkaline hydrolysis in the tissue due to the use of alkali in the liposuction procedure.

The conjugated or free species of six parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben (not a cosmetic ingredient)), or their metabolite, 4-Hydroxybenzoic Acid, were measured in urine samples collected from 40 US children, 70 Chinese children, and 26 Chinese adults.[110] Parabens were present predominantly (> 90%) as conjugated species in urine. Among the six parabens analyzed, Methylparaben and Propylparaben were the predominant compounds, which accounted for 57 - 98% and 1.4 - 12%, respectively, of the total concentrations. The median concentrations of Methylparaben and Propylparaben in US adults were 43.9 and 9.1 ng/mL, respectively. The median concentration of the sum of six parabens in urine from US children was 54.6 ng/mL. The GM concentrations of 4-Hydroxybenzoic Acid in urine from US children were 752 ng/mL for girls and 628 ng/mL for boys, which were 2 - 3 times lower than the concentrations determined for Chinese children.

One or more of 7 parabens (Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben and Benzylparaben) were measured in 144 human adipose tissue samples collected from patients > 16 years old, who were undergoing non-cancer-related surgery, and presented no evidence of diagnosed hormone-related disease or cancer.[111] Detection frequencies and median concentrations were: Methylparaben (100.0%, 0.40 ng/g tissue), Ethylparaben (20.1%, < LOD), Propylparaben (54.2%, 0.06 ng/g tissue), Butylparaben (5.6%, < LOD), and Isobutylparaben (2.1%, < LOD). Isopropylparaben and Benzylparaben were not detected in any of the samples, while Butylparaben and Isobutylparaben concentrations above LOD were only recorded in 8 and 3 of the 144 samples. Methylparaben, Ethylparaben, and Propylparaben levels were significantly and positively correlated. No statistically significant relationship between age and paraben concentrations in human adipose tissue was identified. Of the seven parabens measured, only a positive association between age and Methylparaben concentrations was found (close to, but not statistically significant, p = 0.06).

The Environment and Reproductive Health (EARTH) study examined the association between the use of 14 PCPs and the urinary concentrations of parabens in 400 men (18 - 55 year of age).[112] The largest percent increase for parabens was associated with the use of suntan/sunblock lotion (66 - 156%) and hand/body lotion (79 - 147%). A subset of 10 PCPs that were used within 6 h of urine collection contributed to at least 70% of the weighted score and predicted elevated urinary concentrations of Methylparaben, Propylparaben, and Butylparaben (788%, 1333%, and 254% higher, respectively). GM concentrations of Methylparaben, Propylparaben, and Butylparaben in urine were 28, 2.86, and 0.26 µg/L, respectively.

The EARTH study also showed that, among 346 infants, none of the maternal preconception parabens concentrations were associated with birth weight.[113] Maternal preconception Methylparaben concentration was associated with a decreased head

circumference of 0.27 cm (95% CI: −0.54, 0), while no associations were observed between Ethylparaben, Propylparaben, and Butylparaben concentrations and head circumference.

Six parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben) and 4-Hydroxybenzoic Acid were measured in 143 urine samples from healthy, premenopausal women.[114] 4-Hydroxybenzoic Acid was associated with increased FSH 0.07 (95% CI: 0.01, 0.13) and paraben concentrations were associated with increased E2 0.21 (95% CI: (0.15, 0.28) and increased progesterone 0.32 (95% CI: 0.23, 0.41).

Among 1003 pregnant women, median concentrations of Butylparaben were 2-fold greater than US women from the NHANES program, while concentrations of Methylparaben, Ethylparaben and Propylparaben were lower.[115] There was correlation between the four parabens, particularly between Methylparaben and Propylparaben (Spearman r =0.78). In addition, the study authors observed that increasing concentrations of parabens were present as the age of the subjects increased.

The associations between maternal urinary parabens (Methylparaben, Ethylparaben, Propylparaben, and Butylparaben) and plasma inflammatory markers across pregnancy were examined in 130 preterm birth cases and 352 controls.[116] An interquartile range increase in Methylparaben (359 ng/mL) was positively associated with a 6.69% increase in interleukin-6 (IL-6) (95% CI: 0.02, 13.8), while an increase in Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in IL-1β (95% CI: −14.1, −0.86). However, the authors stated that it is difficult to make conclusions about the magnitude by which parabens contribute towards inflammatory processes during pregnancy due to the complexity of receptor signaling in immune cells.

Urinary paraben concentration and reproductive and thyroid hormones were measured in 602 pregnant women in Puerto Rico.[117] Butylparaben, Methylparaben, and Propylparaben were associated with decreases in the sex hormone-binding globulin (SHBG) by 5.27% (95% CI: -9.4, − 1.14), 3.53% (95% CI: -7.37, 0.31) and 3.74% (95% CI: -7.76, 0.27), respectively. Methylparaben was associated with decreases in reproductive hormones, including an 8% decrease (95% CI: -15.4, 0.61) in estriol, a suggestive 3% increase (95% CI: -2.95, 9.61) in the progesterone/estriol ratio, and a suggestive 6.7% decrease (95% CI: -13.13, 0.29) in testosterone at 16 - 20 weeks.

Effects on Adhesin Genes in *Candida glabrata*

Culture of *Candida glabrata* (a yeast pathogen) in Synthetic Complete (SC) medium containing 1.5 mM Methylparaben and 165 µM Propylparaben induced expression of EPA6 adhesin gene, leading to increased adherence to cultured human Lec2 epithelial cells as well as primary human vaginal epithelial cells.[112] Culture of *Candida glabrata* in a variety of over-the-counter (OTC) vaginal products (concentrations ranged from 15% to 25%) also induced expression of EPA6.[118-120]

DERMAL IRRITATION AND SENSITIZATION STUDIES

1984

Methylparaben (10% and 100%), Propylparaben (10%), and Ethylparaben (10% and 100%) were, at most, mildly irritating when applied to rabbit skin.[44]

Parabens are practically nonirritating in the [human] population with normal skin… Skin irritation and sensitization tests on product formulations containing from 0.1 to 0.8 percent of one or two of the parabens, including Methylparaben, Ethylparaben, Propylparaben and Butylparaben, showed no evidence of significant irritation or sensitization potential for these ingredients.

Parabens are practically nonsensitizing in the [human] population with normal skin. Practically all animal sensitization tests indicate that the parabens are nonsensitizing.

1986

Benzylparaben was not a skin irritant when tested in rabbits.[45]

Sensitization to Benzylparaben has been observed in eczematous patients. A 3% mixture of Benzylparaben, Methylparaben, Ethylparaben, Propylparaben, and Butylparaben produced positive reactions ranging from 1 to 3.7%. The cross-sensitization potential of paraben esters was demonstrated in patients previously sensitized to a paraben mixture. Two thirds of the patients sensitive to one paraben ester also reacted to one or more of the other esters.

2008

Benzylparaben applied directly (0.5 g) to rabbit skin produced no significant irritation.[2]

Parabens are practically non-irritating in the population with normal skin. Skin irritation tests on product formulations containing from 0.1% to 0.8 % of one or two of the parabens showed no evidence of significant irritation for these ingredients.

In Vitro

The parabens were tested individually for irritancy and sensitization potential in co-cultured human keratinocyte and peripheral blood mononuclear cells (PBMCs).[120] The keratinocytes were isolated from skin received as residual material from plastic surgery; PBMCs were enriched from buffy coats by density centrifugation. The cells were co-cultured in serum-free keratinocyte growth medium (KGM-2) on 12-well cell culture plates. The co-culture was incubated for 48 h with or without a paraben. The concentrations tested were not specified, but likely ranged around 1 - 1000 µM, in dimethyl sulfoxide (DMSO; vehicle). Fluorescence-activated cells sorting (FACS) was used to identify and characterize dendritic cell-related cells (DC-rcs). Categorization of compounds as potential irritants and sensitizers was based on EC_{50}s calculated from concentration-response data for cell death (irritancy) and CD86-expression (sensitization), compared with vehicle controls. Substances with EC_{50}s for cell death of ≤ 50 µM were considered to be irritating, with EC_{50}s ranging from 50 - 1000 µM were considered weakly irritating, and substances that did not reach the 50% threshold for cytotoxicity, or for which EC_{50} > 1000 µM, were considered non-irritating. Substances with an EC_{50} for CD86-expression of ≤ 12.5 µM were categorized as extreme sensitizers, > 12.5 µM < 50 µM as strong sensitizers, > 50 µM < 100 µM as moderate sensitizers, and > 100 EC_{50} as non-sensitizers. Methylparaben and Ethylparaben showed no potential for irritation in this test. Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben appeared to be weak irritants. The sensitization potential of the parabens tested was correlated with side-chain length: Methylparaben, Ethylparaben, Propylparaben, and Isopropylparaben were classified as weak sensitizers; and Butylparaben, Isobutylparaben, and Benzylparaben were strong sensitizers in this study.

Photosensitization/Phototoxicity

1984

Photo-contact sensitization and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methylparaben, Propylparaben, and/or Butylparaben gave no evidence for significant photoreactivity.[44]

In Vitro

Methylparaben

Normal human keratinocytes (HaCaT cells) were exposed to 0, 0.003%, 0.03%, and 0.3% (0, 0.197, 1.97, and 19.7 mM, respectively) Methylparaben in an ethanol vehicle.[118] The cells were grown and incubated, with or without Methylparaben, for 6 or 24 h in DMEM supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine, and 100 U/mL penicillin/streptomycin at 37°C. Methylparaben-treated and -untreated cells were exposed to medium-wavelength ultraviolet light (UVB; 15 or 30 mJ/cm^2) after replacing the culture medium with PBS. The UVB source was a bank of six fluorescent sunlamps with an emission spectrum of 275 - 375 nm, mainly in the UVB range, peaking at 305 nm, and including a small amount of long-wavelength ultraviolet light (UVA) and short-wavelength ultraviolet light (UVC). After irradiation, the cells were incubated in culture medium without Methylparaben for various durations. Methylparaben reduced cell viability in a statistically significant manner within 6 h at 0.3% and within 24 h at 0.03%. Fluorescent microscopy using a fluorescent micro-plate reader revealed little evidence of reactive oxygen species (ROS) or nitric oxide (NO) production after Methylparaben exposure. UVB irradiation at 30 mJ/cm^2 (but not at 15 mJ/cm^2) induced small amounts of late apoptosis and necrosis. Methylparaben induced statistically significant elevation of ($p < 0.5$) UVB-induced cell death, as evaluated by immunocytochemistry and flow cytometry; the propidium iodide (PI) index increased 3- and 7-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 15 mJ/cm^2, and 2- and 3-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 30 mJ/cm^2. Methylparaben at both concentrations elevated ($p < 0.05$) measurements of ROS and NO production and lipid peroxidation, and activated NFκB and AP-1 in UVB-irradiated cells.

OCULAR IRRITATION STUDIES

1984

Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits.[44] A primary eye irritation study in humans showed Methylparaben to be nonirritating at concentrations up to 0.3%.

1986

Benzylparaben was neither an eye nor skin irritant when tested in rabbits.[45]

2008

A number of rabbit eye irritation studies have been conducted on products containing Methylparaben, Ethylparaben, Propylparaben, and/or Butylparaben at concentrations of 0.1% to 0.8%. Most products produced no signs of eye irritation. Other products produced slight or minimal eye irritation, with scores of 1.0 to 3.3/110.[2]

In Vitro

Methylparaben

Wong-Kilbourne-derived human conjunctival epithelial cells (WCCs) and immortalized human corneal epithelial cells (HCEs) were exposed to 0, 0.001%, 0.0025%, 0.005%, 0.0075%, 0.01%, 0.025%, 0.05%, 0.075%, and 0.1% Methylparaben.[121] The cells were cultured under standard conditions in Hank's balanced salt solution supplemented with 10% FCS, 1% L-glutamine, and 1% penicillin-streptomycin. HCEs were cultured under standard conditions in keratinocyte serum-free medium supplemented with 0.05 mg/mL bovine pituitary extract, 5 ng/mL epidermal growth factor, 0.005 mg/mL human insulin, and 500 ng/mL hydrocortisone. When the cells reached 75% - 80% of confluency, the medium was replaced with testing solutions and incubation continued for 1 h; after which the solutions were replaced with an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazonium bromide) solution, incubation continued for 4 h, and the MTT solution was replaced with MTT-solubilization solution (10% Triton X-10) that was spectrophotometrically analyzed. Metabolic activity/number of viable cells, measured via the MTT assay, was reduced in both cell lines in a concentration-dependent manner after exposure to Methylparaben; 0.001% Methylparaben (the lowest concentration tested) reduced activity/viability by 36.41% ± 33.95% in HCEs and by 24.48% ± 23.24% in WCCs. The highest concentration tested (0.1%) reduced activity/viability by 77.3% ± 33.8% in HCEs and by 73.92% ± 26.25% in WCCs.

CLINICAL STUDIES

Adverse Event Reports

1984

Industry complaint experience data showed low to moderate numbers of safety-related complaints with the incidence depending on the product.[44]

Paraben sensitization has occurred, especially when paraben-containing medicaments have been applied to damaged or broken skin. Even when applied to patients with chronic dermatitis, parabens generally induce sensitization in less than 3 percent of such individuals. Of 27,230 patients with chronic skin problems, 2.2 percent were sensitized by preparations of parabens at concentrations of 1 to 30 percent. Many patients sensitized to paraben-containing medications can wear cosmetics containing these ingredients with no adverse effects.

Parabens were designated "non-allergen" of the year (2019) by the American Contact Dermatitis Society.[122,123] Monitoring for paraben allergy followed with studies reporting paraben testing in standard screening fashion since 1940. The frequency of allergic contact sensitization to parabens has remained low and remarkably stable for many decades despite wide use. Parabens have been considered relatively nonirritating at levels used in current formulations, as verified in extensive experience with the mix at current applied patch test concentrations.

Retrospective and Multicenter Studies

In one retrospective analysis, 1363 cumulative irritation test studies in more than 45,000 subjects, who use-tested 151 different paraben-containing formulations (along with other ingredients), did not demonstrate parabens to be irritating in typical in-use conditions and irritation scores did not correlate with preservative concentrations.[124]

Allergic contact dermatitis caused by paraben mixture was analyzed on the basis of data collected by the European Surveillance System on Contact Allergies (ESSCA) network between 2009 and 2012 from 12 European countries (Table 15).[125] Of the 52,586 tests during the study period, parabens yielded less than 1% positive reactions. Of the results obtained from 2362 TRUE-test®, the paraben mixture yielded only 0.4% positive reactions. The allergic contact dermatitis data are summarized in Table 16.

EPIDEMIOLOGICAL STUDIES

The epidemiological studies summarized below are described in Table 17.

Prospective Studies

In vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility.[126] No significant associations were observed between current exposure levels of Methylparaben, Ethylparaben, and Propylparaben in Chinese pregnant women and size of infants at birth.[127]

Urinary Methylparaben and Propylparaben concentrations were associated with an increase in gestational age in northern Puerto Rico.[128] Methylparaben, Butylparaben, and Propylparaben were associated with a 34 - 50% decrease in the odds of Small for Gestational Age (SGA).

Among 501 male partners of couples planning to become pregnant, urinary concentrations of Methylparaben, Ethylparaben, and Butylparaben were associated with diminished sperm count and several sperm motility parameters.[129] In contrast, hydroxylated paraben metabolites (methyl-protocatechuic acid and ethyl-protocatechuic acid) were positively associated with select semen quality parameters. The median urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben among 419 participants who provided both urine and semen samples are 6.51, 0.36, 1.39, 0.03, and 0.02 ng/mL, respectively. In the same study population, no associations were observed between paraben concentration in seminal plasma and 35 semen quality parameters among 339 male partners after false discovery rate (FDR) adjustment.[130] In addition, seminal plasma concentrations of Ethylparaben and Benzylparaben were associated with an increased percentage of sperm motility.

Among 936 men of couples seeking infertility treatment, urinary concentrations of Methylparaben and Propylparaben remained stable over the study period between 2000 and 2017.[131] The downward trends in sperm concentration and normal morphology were not affected when including urinary paraben concentrations in linear regression models; i.e., parabens did not substantially change the downward trends in semen parameters (volume, sperm concentration, count, motility, and morphology).

Among 482 pregnant women, an interquartile range increase of urinary Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in pro-inflammatory marker interleukin-1β (95% CI: −14.1, −0.86).[116] However, the association between Ethylparaben and interleukin-1β differed across pregnancy, becoming positive at the end of the study.

In Latino girls, at age 9, earlier thelarche, pubarche, and menarche were associated with urinary Methylparaben concentrations, and earlier pubarche was associated with urinary Propylparaben concentrations.[132] In boys, no prenatal parabens were associated with pubertal timing, while one association of earlier gonadarche with urinary Propylparaben concentrations was observed. However, associations of peripubertal measurements with parabens may reflect reverse causality: children going through puberty early may be more likely to use products that expose them to parabens.

Urinary paraben concentrations (Methylparaben, Propylparaben, and Butylparaben) and pregnancy blood glucose levels during the 1st and/or 2nd trimester were measured in 241 women.[133] Investigating parabens individually did not provide any significant results. However, when investigating these parabens as a mixture, positive associations of Butylparaben (e.g., comparing the 4th and 1st quartiles) with glucose levels were observed for both the 1st trimester (adjusted difference = 12.5 mg/dL; 95% CI: 0.9, 24.2) and 2nd trimester (adjusted difference = 11.2 mg/dL; 95% CI: 0.2, 22.3), and a negative association between 1st trimester Propylparaben and glucose (adjusted difference = −22.3 mg/dL; 95% CI: −43.2, −1.4).

Maternal urinary paraben levels of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben were measured in 850 mother-infant pairs.[134] In all infants, each doubling increase in average Ethylparaben was associated with −2.82% (95% CI: −5.11%, −0.53%) decrease in weight z-score (standard deviation scores) at birth. In addition, age-specific association of Ethylparaben with −3.96% (95% CI: −7.03%, −0.89%) and −3.38% (95% CI: 6.72%, −0.03%) reduction in weight z-scores were observed at 1 and 2 years in males, respectively. Third-trimester Ethylparaben was negatively associated with weight z-scores at birth, 1, and 2 years in males.

Among 473 pregnant women in France, 4 parabens (Methylparaben, Ethylparaben, Propylparaben, and Butylparaben) were measured in spot urine samples collected between weeks 23 and 29 of gestation.[135] A positive association between the sum of parabens and placental weight was identified ($\beta = 7.12$, $p = 0.04$).

Among 1087 pregnant women in China, five parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben) concentrations were measured in spot urine samples collected between 8 and 16 gestational weeks.[136] A total of 103 (9.5%) women were diagnosed with gestational diabetes mellitus (GDM). Urinary Ethylparaben was associated with GDM. The relative risks (RRs) = 1.12 (95% CI: 0.63, 2.01) for the second quartile, RRs = 1.11 (95% CI: 0.64, 1.93) for the third quartile, and RRs = 1.70 (95% CI: 1.02, 2.82) for the highest quartile, compared with the lowest quartile. In contrast, there was no evidence of associations between urinary Methylparaben or Propylparaben and GDM.

Five parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben) were measured in three spot urine samples (in the first, second, and third trimesters) of 478 pregnant women in China.[137] Each 2-fold increase in average prenatal paraben concentration was associated with lower mental development index (MDI) scores among girls ($\beta = -1.08$, 95% CI: −2.10, −0.06) and ($\beta = -1.51$, 95% CI: −2.69, −0.32) for Methylparaben and sum of combined parabens (Σparabens), respectively; but, the association was not statistically significant among boys.

Among 392 women, Methylparaben, Propylparaben, and Butylparaben were measured in two spot urine samples collected during pregnancy.[102] T-helper 1 (Th1) and T-helper 2 (Th2) cells were measured in offspring blood samples at ages two, five, and seven; probable asthma and aeroallergies were assessed at age 7. Methylparaben was associated with lower Th1% (RR: −3.35, 95% CI: −6.58, −0.02) and Th2% at borderline significance (RR: −4.45, 95% CI: −8.77, 0.08). Propylparaben was associated with decreased odds of probable asthma (OR: 0.86, 95% CI: 0.74, 0.99).

Among 480 pregnant women, 130 cases of preterm birth were identified, including 75 cases of spontaneous preterm birth and 37 cases of placental preterm birth.[116] Regression analyses indicated Ethylparaben was associated with increased risk for placental preterm birth OR = 1.47 (95% CI: 1.14 – 1.91).

One study examined 420 women undergoing in vitro fertilization (IVF) treatment. [126] Urinary concentrations of parabens (Methylparaben and Propylparaben) were not associated with any IVF outcome, such as endometriosis, diminished ovarian reserve, tubal or ovulatory disorders.

Of 252 adolescents participating in a New Bedford Cohort (NBC) study, urine concentrations of parabens were not associated with any maladaptive behavior, e.g., internalizing and externalizing behavior, Behavioral Symptoms Index (BSI), adaptive skills, and Developmental Social Disorders (DSD).[138]

Among 152 pregnant women, a significant decrease in diastolic blood pressure was associated with exposure to parabens, including Methylparaben, Ethylparaben, and Butylparaben, in the second trimester ($\beta = -0.62$ mmHg; 95% CI: −1.16, −0.08 per doubling of Methylparaben concentrations).[139]

Retrospective Studies

Preterm birth (PTB) was associated with umbilical cord blood concentrations of Butylparaben (OR = 60.77; 95% CI = 2.60 - 1419.93) and Benzylparaben (OR = 0.03, 95% CI = 0.01 - 0.44).[140] The authors stated that the OR of 0.03 for Benzylparaben indicated a "protective effect" of Benzylparaben for preterm birth. Linear regression analysis indicated an association between maternal urinary concentrations and decreased gestational age and body length in newborns. No statistically-significant associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated (i.e., body length, gestational age at birth, birth weight, head circumference). No statistically-significant associations were found between prenatal or postnatal growth of male newborns and maternal urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben.[141]

The incidence of cryptorchidism and/or hypospadias, combined, was associated with placental concentrations of Methylparaben ≥ 1.96 ng/g (OR = 3.18; 95% CI = 0.88 - 11.48) and Propylparaben concentrations ≥ 1.16 ng/g (OR = 4.72; 95% CI = 1.08 - 20.65).[142] Of 436 children at 3 years of age, the median values of estimated daily intake of Methylparaben, Ethylparaben, Propylparaben, Butylparaben and Benzylparaben were 12.10, 5.68, 4.50, 0.06 and 0.17 μg/kg bw/day, respectively.[143] Urinary Ethylparaben concentrations of boys were positively associated with weight z scores ($\beta = 0.16$, 95% CI: 0.04, 0.29, $p = 0.01$) and height z scores ($\beta = 0.15$, 95% CI: 0.03, 0.27; $p = 0.01$). Positive associations were found between the sum of molar concentrations of all five parabens and height z scores among all children ($\beta = 0.24$, 95% CI: 0.04, 0.45; $p = 0.02$). All regression coefficients calculated for girls and all other coefficients for boys were not statistically significant.

Mean percent change (MPC) and the results of statistical tests for trends were not statistically significant in a study of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben in women undergoing infertility evaluation and ovarian volume (OV) or antral follicle count (AFC) measurements.[144]

No statistically-significant associations were found between the urinary concentrations of Methylparaben, Propylparaben, or Butylparaben and serum hormone concentrations, semen quality parameters and motion characteristics or all but one indicator of sperm damage in a comet assay.[145] The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

Cross-Sectional Studies

Among 315 men under 45 years of age who attended an infertility clinic for diagnostic purposes in Poland, urinary concentrations of Ethylparaben and Butylparaben were associated with an increase in the percentage of sperm with abnormal morphology.[146] Urinary Isobutylparaben concentrations were significantly associated with an increase in the percentage of sperm with high DNA stainability. Urinary concentrations of parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben and Isobutylparaben) were not associated with the level of reproductive hormones, including FSH, T and E_2. In addition, urinary concentrations of Methylparaben and Propylparaben were not related to any of the examined semen quality parameters, sperm DNA damage, or the level of reproductive hormones. The unadjusted GM urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Isobutylparaben were 14.7, 1, 4.3, 0.3, and 0.4 μg/L, respectively.

In cord plasma of 27 healthy pregnant women (37th week of pregnancy), Methylparaben, Propylparaben and the sum of all measured parabens (Methylparaben + Ethylparaben + Propylparaben + Butylparaben) were inversely associated with T levels.[147]

Urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were measured in 215 young healthy men (18 - 23 years old), 94% of whom had detectable urinary concentrations of parabens.[148] Urinary concentrations of parabens were not significantly associated with any semen parameters or any of the reproductive hormone levels, including FSH, LH, T, inhibin B and E_2. The unadjusted GM urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben were 11.2, 1.1, and 0.64 ng/mL, respectively.

Among 42 partners (36.8 ± 5.4 years old) of couples who visited a gynecology clinic in Tokyo for infertility consultation, no significant association was found between semen parameters (sperm volume, concentration and motility) and urinary paraben

concentrations in regression analyses.[149] The GM urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were 48.2, 1.88, 1.13, and 0.184 ng/mL, respectively.

Linear regression analyses of data from the US NHANES program indicated an association between reduced serum thyroxine (T4) concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben.[150]

Analysis of data from the NHANES program indicated an association between aeroallergen and food sensitization, combined, and urinary concentrations of Methylparaben (OR = 1.74; CI = 1.02 - 3.22), Propylparaben (OR = 2.04; CI = 1.12 - 3.74), and Butylparaben (OR = 1.55; CI = 1.02 - 2.33).[151] The results also indicated an association between urinary concentrations of Methylparaben and nonatopic asthma (OR = 0.025; CI = 0.07 - 0.90) and nonatopic wheeze (OR = 0.23; CI = 0.05 - 0.99).

Urine samples were collected from 696 pregnant women in China.[152] The detection rates for the five parabens in the urine samples were 97.70% (Methylparaben), 71.26% (Ethylparaben), 96.55% (Propylparaben), 15.80% (Butylparaben), and 2.73% (Benzylparaben). No significant association was found between parabens and GDM among the overall population. However, significant non-linear associations of Propylparaben and the summed estrogenic activity of parabens with GDM were found in the stratified analysis by pre-pregnancy body mass index (BMI) in the overweight/obese population, with adjusted ORs of 3.47 (95% CI: 1.28, 9.42) and 2.87 (95% CI: 1.07, 7.73) for GDM in the second tertile of urinary Propylparaben and the summed estrogen activity, respectively, when compared to the first tertile.

One study examined the association between parabens and asthma morbidity among 450 children with asthma and with asthma prevalence among 4023 children participating the US NHANES program (2005-2014).[153] An increased prevalence of reporting emergency department visits were observed for every 10-fold increase in Methylparaben and Propylparaben concentrations among boys with asthma [(prevalence OR = 2.61, 95% CI:1.40-4.85) and (OR = 2.18, 95% CI: 1.22-3.89, respectively)]. Among children in the general population, no overall associations with current asthma were observed, although there was a positive trend with Propylparaben and a current asthma diagnosis.

Among 1693 black women aged 23 – 34 years, morbid obesity (BMI $\geq$ 35 kg/m^2) was inversely associated with Butylparaben and Methylparaben concentrations.[154] Methylparaben concentrations were 30.7% lower for BMI $\geq$ 35 vs. < 25 kg/m^2 (95% CI: −48.0%, −7.7%), and Butylparaben concentrations were 30.6% lower for BMI $\geq$ 35 vs. < 25 kg/m^2 (95% CI: −49.6%, −4.6%).

Among 156 men under 45 years of age who attended the infertility clinic for diagnostic purposes with normal semen concentration, a positive association was found between urinary level of Butylparaben and XY18 disomy ($p = 0.045$) and Propylparaben and disomy of chromosome 13 ($p = 0.007$).[146]

RISK ASSESSMENT

Margin of Safety

For the purpose of this risk assessment, the Panel determined an adequate NOAEL value of 160 mg/kg/day for Butylparaben in consideration of the new data in the category of endocrine activity and from DART studies.[62,63,69,70,155-157] Specifically, the NOAEL has been derived from a study where pregnant rats were orally exposed to Butylparaben by gavage from gestation day 7 through postnatal day 21.[63] Above a dose of 160 mg/kg/day, Butylparaben exerted adverse effects on the reproductive system in male offspring, including delayed preputial separation, reduced reproductive organ weights at several ages, reduced luteinizing hormone level, and elevated estradiol and progesterone levels in serum from prepubertal male rats. Importantly, Butylparaben exposure in utero and during lactation significantly reduced epididymal cauda sperm counts, daily sperm production, and serum testosterone in a dose-dependent manner.

In comparison, the SCCS chose an NOEL of 2 mg/kg bw/day for the calculation of the MOS of Butylparaben. The NOEL was derived from a study in which three neonatal male rats were exposed subcutaneously to 2 mg/kg bw/day Butylparaben from PND 2 to PND 18 (Table 13).[155] No effects on any of the measured reproductive parameters were documented, compared with the control group. DART parameters examined in this study included testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts, and immunoexpression of the APQ-1. However, the Panel considered that such study suffers from several critical limitations: it involves a route of subcutaneous exposure; it is not an OECD TG study; only one postpartum dose was tested; did not examine the generation toxicity and typical DART endpoints, such as AGD, weight of the epididymis / seminal vesicle, sperm counts, reproductive hormone levels, etc.

For the purposes of an MOS calculation, the Panel considered a scenario wherein a consumer would use a set of cosmetic products containing Butylparaben; aggregate exposure to seventeen cosmetic products is calculated to be 17.4 g/day based on addition of deterministic values for a range of products (Table 18).[158] These seventeen cosmetic products are divided into four main categories:[159] (1) oral products, (2) eye products, (3) non rinse-off products and (4) rinse-off product; the global daily exposure of products for each category was estimated using the data summarized in Table 18.

The Panel also considered the different use concentrations and exposures of Butylparaben in each main cosmetic product category. For purposes of worst-case assumption, the maximum use concentration of Butylparaben was set to represent the concentrations of use across the products in that category. The Council's concentration of use survey indicates that the

maximum use concentration of Butylparaben in the category of (1) oral products, (2) eye products, (3) leave-on products, and (4) rinse-off product is 0.2%, 0.5%, 0.24%, and 0.33%, respectively.[25] (Table 18)

The Panel noted that the measured extent of dermal penetration of parabens is variable ranging from 1% to 75%, probably due to differences in animal species used, matrix effects, and other experimental conditions.[159,160] For purposes of calculating an MOS, the systemic availability of un-metabolized Butylparaben after topical application to human skin is of the primary concern. A human toxicokinetic study has been conducted in 26 young adult males with dermal repeated exposure to Butylparaben at a daily dose of 10 mg/kg bw/day for five days.[42,55] No effects of Butylparaben on serum hormonal levels were observed during the exposure time of 5 days; and, about 2.1% un-metabolized Butylparaben was detected in the urine of the participants. Note that Butylparaben was applied to the whole-body in this human study (10 mg/kg bw/day), while a conservative estimation indicates that daily exposure of consumers to Butylparaben is much lower (0.66 mg/kg bw/day, as shown in Table 18). In addition, the available in vitro percutaneous absorption studies using human split- or full thickness skin suggest a conservative assumption of human dermal penetration of un-metabolized Butylparaben at 3.7% (which was used by SCCS to calculate the MOS of Butylparaben and then to derive the recommended maximum use concentration of Butylparaben in the EU).[15,17] Taking into account that dermal absorption of lower molecular weight parabens is likely to be higher, the Panel selected an estimate of a 50% dermal absorption of un-metabolized parabens in the calculation of the MOS, which thereof represents a very conservative assumption.

For adults (60 kg body weight), the relevant calculations are:

> Global daily exposure (GDE, Butylparaben) = (2.36 g/day of oral products x 0.2 % maximum use concentration) + (0.05 g/day of eye products x 0.5 % maximum use concentration) + (13.93 g/day of non-rinse-off products x 0.24 % maximum use concentration) + (1.04 g/day of rinse-off products x 0.33 % maximum use concentration) = 0.042 g/day
>
> Systemic exposure dose (SED, Butylparaben) = GDE ÷ 60 kg body weight x 50 % dermal absorption x 1000 mg/g conversion factor = 0.35 mg/kg/day
>
> MOS (adult, Butylparaben) = NOAEL/SED = 160 mg/kg/day / 0.35 mg/kg/day = 457

Since alkyl parabens undergo the similar enzymatic hydrolysis to form 4-Hydroxybenzoic Acid, such a conservative MOS of Butylparaben for adults could then be inferred to other single alkyl parabens.

The Panel considered exposures to cosmetic products containing multiple parabens. A protective MOS level of 100 was used to calculate a maximum safe use concentration for combined paraben use in a single formulation.

> NOAEL/MOS (adult, multiple paraben) = SED = 160 mg/kg/day / 100 = 1.6 mg/kg/day
>
> (SED x body weight) / (dermal absorption x conversion factor x GDE) =
>
> (1.6 mg/kg/day x 60 kg) / (50% x 1000 mg/g x 17.4 g/day) = maximum use concentration = 1.1 %

Accordingly, the Panel determined that the commonly used limitation of 0.8% for Σparabens is ultra-conservative and is safe for human health when parabens are used in combination in cosmetic products.

Estimate and Refinement of Aggregate Exposure

Estimate of Aggregate Exposure

In addition to cosmetic and personal care products, parabens are also widely used in drugs and foods. According to one study, considering aggregate exposure to parabens from various sources, the total combined exposure was 76 mg/ day: with cosmetics and personal care products accounting for 50 mg/day; drugs, 25 mg/day; and foods, 1 mg/day.[161]

The Dutch National Institute for Public Health and the Environment (RIVM) conducted an exposure assessment in consideration of the aggregated exposure to parabens via three major sources: PCPs, foods, and medicinal products.[160] For Methylparaben, adding exposures results in an aggregate exposure estimate of 3.0 mg/kg/day for both adults and children. The estimate for medicinal products contributes 70 - 74% of this value, while the contribution of food is less than 1%. For Propylparaben, adding the exposures results in an aggregate exposure estimate of 1.2 mg/kg/day for both children and adults; 64 - 72% of the exposure is from medicinal products, and less than 1% from food. For Ethylparaben, due to the lack of use information on medicinal products, the summation of exposure via PCPs and exposure via foods will result in an aggregate exposure of 0.2 mg/kg/day for adults and children and, as with Methylparaben and Propylparaben, the contribution of foods is less than 1%. However, the authors noted that such an aggregation estimate was based on a series of studies with varying levels of information and uncertainties.

Refinement of Aggregate Exposure

In current risk assessments, aggregate exposure of parabens is commonly estimated by using a simplistic approach of summing the exposures from all the individual product types in which parabens are used. However, this summation will result in an unrealistic estimation because 1) the use frequency of products and the amount of product applied are over-estimated, 2) parabens may not be used in all products of a given type (e.g., all make-up products), 3) the extent of use factors for parabens in products is not considered, 4) individuals in the population vary in their patterns of product use including co-use and non-use, and 5) the extent to which parabens are absorbed from the skin into the internal system warrants further studies. Use of multiple exposure models help provide realistic estimates in comparison with observational biomonitoring data.[162] A recent study indicated that approximately 60 - 90% of the model predictions from five implemented models were within a factor of 10 of the observed paraben exposures, while 30 - 40% of the predictions were within a factor of 3 (i.e., a factor of 3 or 10, above or below the minimum observed absorbed doses). These models included three of the screening models (i.e., RIVM ConsExpo, SCCS notes of guidance algorithms, and the Risk Assessment Identification and Ranking-Indoor and Consumer Exposure (RAIDAR-ICE)) and two higher tier probabilistic models (US EPA's Stochastic Human Exposure and Dose Simulation – High Throughput (SHEDS-HT), and Creme Care & Cosmetics). A number of uncertainties affect interpretation of the modeled vs. measured exposures, such as parabens in preservative product concentrations, dermal absorption parameters, and degree of metabolism following dermal absorption.

An approach has been developed to refine the aggregate exposure estimates using four of the more commonly used parabens (i.e., Methylparaben, Ethylparaben, Propylparaben, and Butylparaben).[159] The relative refinement allowed co-use and non-use data, as well as the extent of parabens use data, to be developed for nine cosmetic and skin care products, including body lotion, body cream, facial mask, hand lotion, foundation/liquid make-up, facial moisturizer, lip color, night cream and facial cleanser. Simple summed aggregate exposure from these nine cosmetic and skin care products was 1.61, 0.80, 1.70, and 0.016 mg/kg/day for Methylparaben, Propylparaben, Ethylparaben, and Butylparaben, respectively. When the refining factors were applied, and a conservative dermal penetration rate of 80% was chosen, the aggregate exposure compared to the simple addition approach was reduced by 51%, 58%, 90%, and 92% for Methylparaben, Propylparaben, Butylparaben, and Ethylparaben, respectively. In comparison, estimated internal exposure based on the 95th percentile values of parabens concentration in human urine was 19.9, 8.2, 1.39, and 0.86 µg/kg/day for Methylparaben, Propylparaben, Ethylparaben, and Butylparaben, respectively. This means that in all cases the aggregate exposure estimates are significantly greater than the exposures derived from the biomonitoring data.[159] If exposure via food was included, the aggregate exposure for Methylparaben and Propylparaben, which are used extensively in foods, would only increase by 1% and 4%, respectively. That is, estimates for exposure to Methylparaben and Propylparaben via food are at least 25-fold lower than the estimates for aggregate exposure resulting from dermal exposure to cosmetic products.[159,161]

Another study takes population variability of individual characteristics and behavior within the female US population into account.[163] Daily parabens intake was estimated based on skin permeation coefficient models, product use characteristics, and multi-pathway exposure model, i.e., aqueous dermal uptake, gaseous dermal uptake, inhalation intake, and environmentally mediated intake due to disposal after parabens use. The mean (2.5th - 97.5th percentiles) modeled population intakes were 0.2 (0.003 - 0.8), 0.03 (0 - 0.2), 0.06 (0 - 0.3), and 0.02 (0 - 0.1) mg/kg/day for Methylparaben, Ethylparaben, Propylparaben, and Butylparaben, respectively. This intake estimate represents a consumer who uses the following eleven PCPs which all contain parabens: shampoo, conditioner, body lotion, facial cream, night cream, facial cleanser, deodorant, body wash, foundation, eye shadow, and lipstick. The environmentally mediated parabens intake from disposal stage was three to four orders of magnitude lower than use stage.[163]

SUMMARY

This is a safety assessment of the available scientific literature and concentration of use data relevant to assessing the safety of 20 parabens and 4-Hydroxybenzoic Acid as used in cosmetics. According to the *Dictionary*, parabens primarily function in cosmetics as preservatives, although five of the ingredients also are reported to function as fragrance ingredients.

According to VCRP survey data received in 2019, Methylparaben was reported to be used in 11,739 formulations; this is an increase from 8786 uses reported in 2006. Propylparaben had the next highest number of reported uses at 9034; this was an increase from 7118 uses reported in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to zero.

The results of the concentration of use survey conducted by the Council in 2016 indicate Methylparaben had the highest reported maximum concentration of use, up to 0.9% in shampoos. The highest maximum concentration of use reported for products resulting in leave-on dermal exposure is Ethylparaben in eye shadows at 0.65%. In 2006, Methylparaben had the highest reported maximum concentration of use at 1% in lipsticks. The maximum concentrations of use of the previously reviewed parabens have remained under 1%, and the patterns of use are similar to those reported in the previous safety assessment.

The US FDA considers Methylparaben and Propylparaben to be GRAS as antimicrobial agents in food.

Parabens may be classified as moderate penetrants. Penetration was inversely proportional to the lipophilicity of the parabens tested (Methylparaben > Ethylparaben > Propylparaben > Butylparaben). Residual quantities of parabens remaining in the skin increased as the test concentration increased, with greater amounts in the human epidermis than in mouse skin.

After application of 2% (w/w) Butylparaben in cream (also contains 2% diethyl phthalate and 2% dibutyl phthalate) in 26 healthy Caucasian men, Butylparaben was detected in the serum, with maximum concentrations not exceeding 1.0 µg/L. Butylparaben concentrations increased rapidly within 3 h after the first application of cream containing the three test compounds, and could be detected in most serum samples collected throughout the second week of this study.

In in vitro tests, Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for AFP. Conversely, the IC_{50} of Benzylparaben was 0.012 µM. Butylparaben was metabolized to 4-Hydoxybenzoic Acid with maximum rate at saturating concentration (V_{max}) of 8.8 nmol/min/mg protein. CP enhances skin permeation of Methylparaben primarily by increasing the solubility of Methylparaben in the SC (especially in the nonlipid regions).

Methylparaben and Ethylparaben were stable in human plasma, but Propylparaben, Butylparaben and Benzylparaben concentrations decreased by 50% within 24 h. All parabens tested were rapidly hydrolyzed when incubated with HLM, depending on the alkyl chain length. Parabens, but not 4-Hydroxybenzoic Acid, were actively glucuronidated by liver microsomes and human recombinant UGTs.

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference. Butylparaben was extensively hydrolyzed to 4-Hydroxybenzoic Acid as the major metabolite for both sexes and species. Methylparaben, Ethylparaben, Propylparaben and Butylparaben were hydrolyzed by RLM and HLM in in vitro tests. In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. Rat small-intestinal microsomes exhibited relatively higher activity toward longer-side-chain parabens. Human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes.

Metabolism rates of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben by HLM and HSM were inversely proportional to chain length. Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the paraben. In contrast to human tissue fractions, all rat tissue fractions tested hydrolyzed the parabens at rates that increased as the ester chain length increased. Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin.

Nine rats were given a single dermal dose of 100 mg/kg bw [ring-U-^{14}C]-labeled Methylparaben, Propylparaben, or Butylparaben. C_{max} (≥ 693 and ≥ 614 ng eq/g in males and females, respectively) occurred within 8 h post-application, and blood concentrations decreased until the last quantifiable concentration within 24 h. Most of the dosage (≥ 46.4%) was not absorbed, and less than 25.8% was found in the urine. Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in liver.

In rats exposed to a single oral dosage of 100 mg/kg bw [ring-U-^{14}C]-labeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥ 11,432 and ≥ 21,040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h. Radioactivity was eliminated rapidly, with averages ≥ 69.6% recovered in the urine during the first 24 h. The rate of urinary excretion was similar across all dosages, with ≥ 66% recovered in the first 24 h in males.

Time-mated female SD rats were orally administered 0, 1500, 5000, or 15,000 ppm Butylparaben via NIH-07 feed, ad libitum, from GD 6 to PND 28. Low placental and lactational transfer of dietary Butylparaben were observed. Poor conjugation in pups during early lactation results in higher exposure to free Butylparaben in pups compared to dams.

All 26 male volunteers showed increased excretion of Butylparaben following daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben. Mean total Butylparaben excreted in urine during exposure was 2.6 ± 0.1 mg/24 h. The concentrations peaked in the urine 8 to 12 h after application. Free and conjugated parabens and their major, non-specific metabolites (4-Hydroxybenzoic Acid and *p*-hydroxyhippuric acid) were detected in the urine samples of 3 subjects 24 h after an oral dose of deuterated Methylparaben, Butylparaben, and Isobutylparaben.

There were no significant changes in body and organ weights in any group when rats were dermally exposed to up to 600 mg/kg bw/day Isopropylparaben or Isobutylparaben for 28 days. Macroscopic and microscopic examinations revealed mild-to-moderate skin damage in female rats. NOAELs for Isobutylparaben and Isopropylparaben were 600 mg/kg bw/day, and 50 mg/kg bw/day, respectively.

At 100 and 300 mg/kg bw/day Propylparaben administered orally, rats exhibited statistically-significant increases in relative liver weights, serum ALT, AST, ALP, and LDH activities. Significant decreases in total serum protein and albumin, GSH, CAT and SOD activities, serum testosterone concentrations, and T/E2 ratios, were also reported. Livers of affected rats exhibited dilated congested central and portal veins, highly proliferated bile ducts with fibrotic reactions, and multifocal areas of necrotic hepatocytes, and testes exhibited evidence of severe spermatogenic arrest.

Elevations of serum markers of lipid-peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically significant in rats exposed to 250 mg/kg bw/day Methylparaben. Malondialdehyde levels were elevated in the liver in a

statistically-significant, dose-dependent manner, among other effects, in mice orally exposed to 1.33 - 40 mg/kg bw/day Butylparaben for 30 days.

Time-mated rats were orally exposed to 10, 100, or 500 mg/kg bw/day of Butylparaben from GD7 to PND22. The AGD of newborn male and female offspring was significantly reduced at 100 or 500 mg/kg bw/day. The expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring was statistically-significantly reduced at 10 mg/kg bw/day or above. In male offspring, epididymal sperm count decreased 76 - 78% compared to controls at all doses from 10 to 500 mg/kg bw/day. The reduction of epididymal sperm count showed the same effect at all doses. In prepubertal females, ovary weight reduction was statistically significant and mammary gland outgrowth was increased at 100 and 500 mg/kg bw/day.

Statistically-significant, dose-dependent reductions in anogenital distance and ovary weights were observed in offspring of female rats exposed orally to 100 or 500 mg/kg bw/day Butylparaben from GD7 to GD21.

E_2 level was elevated in male rats orally exposed to Butylparaben at 50 mg/kg for 8 weeks, whereas serum levels of the hormones T, LH, and FSH was decreased. Testicular DNA damage and a reduction in Leydig cells population were recorded in Butylparaben treated groups.

CYP19 and ERα expression were significantly increased, and the expression of StAR, P450scc, SULT1E1, and AR in the testes and methylation rate of the ERα promoter were significantly reduced, in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD7 to GD21.

Weights of the testes, epididymal cauda sperm counts, and daily sperm production in male offspring were significantly reduced in the 400 and 1000 mg/kg bw/day groups of rats orally exposed to Butylparaben on GD7 to PND21. Vimentin filaments showed shorter projections, concentration near the basal region, and disappearance of the apical extensions toward the lumen of the seminiferous tubules in 3-week old rats 6 h after a single 1000 mg/kg bw oral dosage of Butylparaben.

Prepubertal female rats exposed orally to 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Isopropylparaben on PND21 to PND40 exhibited statistically-significant delays in vaginal opening. Decreases in the weights of the ovaries, increases in the weights of the adrenal glands, thyroid glands, and liver, as well as myometrial hypertrophy were observed in the 1000 mg/kg bw/day groups. Reduced plasma leptin concentrations were observed in male and female offspring of young adult female rats exposed orally to 100 mg/kg bw/day Butylparaben.

F2 pups exhibited statistically-significantly greater mortality at PND7 when F0 females and their F1 offspring were exposed to 0.105 mg/kg bw/day Methylparaben by gavage. During lactation, treated “parous” F1 females exhibited mammary alveoli that were not always milk-filled, collapsed alveolar and duct structures with residual secretory content, and marked decrease in the size of the lobular structures. There was no evidence of an effect on the weight of the male reproductive organs, epididymal sperm parameters, hormone concentrations, or histopathology in juvenile male rats exposed via lactation from maternal rats receiving up to 1000 mg/kg bw/day Propylparaben for 8 weeks.

Methylparaben was associated with a statistically-significantly higher incidence of abnormal sperm in rats exposed to 1000-ppm or 10,000-ppm in the diet for 8 weeks, mostly sperm with no head in 4% to 5% of sperm, compared with 2.3% in 100-ppm and control groups. Measurements of hormone concentrations were generally not altered, except that T and FSH concentrations were higher in the 10,000-ppm Butylparaben-treated group, compared with the control group.

Zebrafish embryos exposure to Methylparaben at 10 ppb and 100 ppb caused alterations in developmental landmarks such as heart rate and hatching percentage. Anxiety-like behavior was induced in larvae exposed to 0.1 ppb and 1 ppb of Methylparaben.

Exposure of zebrafish embryos to Methylparaben at 200 μM, 400 μM, 800 μM, and 1000 μM for 96 hpf resulted in decreased heart rate and hatching rate, and developmental abnormalities. Expression of vitellogenin I was significantly upregulated in larval zebrafish exposed to 100μM of Methylparaben for 96 hpf.

Three neonatal male rats were exposed subcutaneously to 2 mg/kg bw/day Butylparaben on PND 2 to PND 18. No effects on any of the measured reproductive parameters were detected.

Human spermatozoa were exposed to 13 mM Methylparaben for 2 or 5 h. Methylparaben had no significant effect on DNA fragmentation, while a statistically significant decrease in spermatozoa motility was observed. Methylparaben at a concentration of 2.5 mM did not induce any significant changes to the motility, vitality, mitochondrial ROS production, or 8OHdG formation.

A dose-dependent decrease in the percentage of mitotic cells was observed in Vero cells exposed to Propylparaben. Induction of DNA DSBs was also observed. Statistically significant elevations of SCEs/cell and CAs/cell were observed in cells incubated with Propylparaben ($\geq$ 1.5 μM) and Propylparaben ($\geq$ 1.0 μM) for 3 h, respectively.

Statistically significant, elevated indices of DNA fragmentation were observed in CHO cells incubated for 1 h with $\geq$ 0.4 μM Butylparaben. Elevated SCEs/cell and CAs/cell were observed in CHO cells incubated with 0.75 μM Butylparaben for 3 h.

Human spermatozoa were exposed to a paraben mixture containing equal concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben. Significantly reduced motility was observed immediately after the treatment and was

further exacerbated after 24 h at doses of 1, 2 and 4 mM. Caspase activation was observed following exposure to parabens concentrations above 1 mM and increased still further after 24 h.

Weak activation of PPARα and PPARγ was observed in NIH-3T3-L1 cells exposed to Butylparaben. Isobutylparaben antagonized the AR in CHO cells. Butylparaben increased the number of BT-474 cells entering S-phase; the effect was enhanced in the presence of ligand heregulin. Butylparaben significantly enhanced the GR signal, while Methylparaben, Ethylparaben, and Propylparaben did not have this effect.

Butylparaben exhibited estrogen agonism in T47D-KBluc cells. MCF-7 and HCI-7-Luc2 mammospheres treated with Methylparaben exhibited increased expression of ALDH1. Parabens enhanced differentiation of murine 3T3-L1 cells with potencies that increased with the length chain. Butylparaben and Benzylparaben promoted lipid accumulation in hADSCs.

EPA's EDSP program conducted a series of in vitro assays to examine the estrogenic properties of parabens compounds. There were 15, 14, 11, 5, and 2 positive results out of total 18 arrays for Butylparaben, Propylparaben, Ethylparaben, Methylparaben, and 4-Hydroxybenzoic Acid, respectively; while in vitro anti-androgen studies showed negative results.

Metabolites of Butylparaben and Isobutylparaben, 3OH and 2OH, exhibited estrogenic properties in MCF-7 and T47D human breast cancer cells. The expression of GREB1 was induced by 3OH and 2OH metabolites, and blocked by co-administration of an ER. The estrogenic activity of the 3OH and 2OH metabolites is mediated by classical ER mediated signaling. 3OH and 2OH metabolites showed the potential for favorable ligand-binding domain interactions with human ERα.

Longer diestrus phases and shortened the intervals of the estrous cycle were observed in rats orally exposed to Propylparaben or Butylparaben at a concentration of 100 mg/kg/day for 5 weeks. Propylparaben and Butylparaben decreased mRNA level of folliculogenesis-related genes (*Foxl2, Kitl* and *Amh*). An increase in FSH levels in serum was observed, indicating an impairment of ovarian function.

Perinatal Methylparaben exposure in rats at doses mimicking human exposure (0.105 mg/kg/day) decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad. Prepubertal Methylparaben treatment was associated with a significant reduction in adipose tissue and more abundant glandular tissue. Long-term Methylparaben treatment from birth to lactation did not result in significant histological changes.

Oral exposure to Methylparaben at 500 mg/kg/day caused morphological changes in gerbil prostates. Male and female gerbils displayed similar alterations such as prostate epithelial hyperplasia, increased cell proliferation, and a higher frequency of androgen receptor binding activity.

In isolated mouse preantral follicle and human granulosa cell (hGC) cultures, Butylparaben adversely affected steroidogenesis at concentrations relevant to human exposure (100 nM), but no effects on follicular development or survival were noted in the culture systems. Butylparaben attenuated di-(2-ethylhexyl) phthalate (DEHP) induced-reduction of progesterone concentrations in the spent media of hGC cultures.

The presence of 500 μM Methylparaben or 10 μM Propylparaben or Butylparaben in MCF-10A non-transformed cells resulted in significant increase of colony numbers and sizes compared with control. Concentration-response experiments showed that maximal numbers of colonies were formed at 100 μM Methylparaben or 1 μM Propylparaben or Butylparaben.

Methylparaben induced a detectable decline in endogenously accumulated ROS in HRBECs cells. Methylparaben substantially reduced the fraction of OHT-induced apoptotic cells in a concentration-dependent manner. The maintenance of S-phase in OHT-treated cells, like apoptosis evasion, was correlated with increasing concentrations of Methylparaben.

Butylparaben inhibited human HTR8/SVneo cell proliferation and induced both apoptosis and endoplasmic reticulum stress at 50, 100, 200, and 400 μM.

Data from the NHANES program showed that, for the 2013 - 2014 sampling period of a representative sample of the US general population, the median concentration of Methylparaben in urine was 48.1 μg/L (95^{th} percentile: 819 μg/L), and Propylparaben in urine was 5.74 μg/L (95^{th} percentile: 224 μg/L). For Butylparaben, the median concentration in urine was below the LOD (0.1 μg/L). In females, the median concentration of Ethylparaben was 1.6 μg/L (95^{th} percentile: 145 μg/L) while males were below the LOD (1 μg/L).

Analysis of data from the NHANES program showed that compared to individuals who reported "never" using mouthwash, individuals who reported daily use had significantly elevated urinary concentrations of Methylparaben and Propylparaben (30 and 39% higher, respectively). Individuals who reported "always" using sunscreen had significantly higher urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben (92, 102, and 151% higher, respectively) compared to "never" users of sunscreen.

Women who used body and face lotions in the past 24 h significantly higher paraben concentrations (80 - 110%) in their urine than women who reported no use. There was 100%, 72%, 96%, and 90% detection of Methylparaben, Butylparaben, Propylparaben, and Ethylparaben in urine, respectively. Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben, respectively.

A community-based intervention study indicated that using PCPs that are labeled to be free of parabens for 3 days lowered some parabens urinary concentrations in 100 adolescent girls: Methylparaben and Propylparaben concentrations decreased by 43.9% and 45.4%, respectively. Girls who reported using specific makeup (e.g., foundation, blush, and mascara) every day vs. rarely/never had higher urinary concentrations of Methylparaben (120.5 ng/ mL vs. 13.4 ng/mL, $p < 0.01$) and Propylparaben (60.4 ng/mL vs. 2.9 ng/mL, $p < 0.01$).

A statistically significant difference was observed between serum parabens in 18 women who used lipstick containing Methylparaben and Propylparaben for 5 days compared with those not using this cosmetic ($p = 0.0005$ and 0.0016, respectively), and a strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202).

The mean concentrations of Methylparaben and Propylparaben measured in serum of 16 human are 42.6 μg/L and 7.4 μg/L, respectively; whereas the free concentrations of Methylparaben and Propylparaben in the serum are 2.2 μg/L and 0.5 μg/L, respectively.

One or more of 5 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isobutylparaben) was detected in 99% of breast tissue samples collected from women with breast cancer, and all 5 were detected in 60% of the samples. Median concentrations were highest for Propylparaben (16.8 ng/g tissue) and Methylparaben (16.6 ng/g tissue). Propylparaben concentrations were higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts.

Methylparaben, Butylparaben, and Benzylparaben were detected in all placenta samples collected from healthy mothers. The highest measured concentration was 11.77 ng Methylparaben/g tissue.

The amounts of Butylparaben, Ethylparaben, Methylparaben and Propylparaben were studied in human ovarian tumor samples. The tissue mass fractions of the four parabens in malignant tissues were at least twice as much as those present in the benign tissues. The tissue mass fractions of Methylparaben and Ethylparaben were higher than Propylparaben and Butylparaben.

One or more of 6 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, heptylparaben (not a cosmetic ingredient)) as well as 4-Hydroxybenzoic Acid were detected in 20 human adipose fat samples. Ethylparaben and Propylparaben were more frequently detected than the other parabens, at a detection frequency of 60% and 50%, and a GM concentration of 0.90 and 0.49 ng/g, respectively. Paraben concentrations in adipose fat samples of Caucasian volunteers were higher than those of African Americans.

One or more of 6 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben (not a cosmetic ingredient)) as well as 4-Hydroxybenzoic Acid, were measured in urine samples collected from 40 US children, 70 Chinese children, and 26 Chinese adults. Parabens were present predominantly (> 90%) as conjugated species in urine. The median concentrations of Methylparaben and Propylparaben in US adults were 43.9 and 9.1 ng/mL, respectively. The GM concentrations of 4-Hydroxybenzoic Acid in urine from US children were 752 ng/mL for girls and 628 ng/mL for boys, which were 2 - 3 times lower than the concentrations determined for Chinese children.

One or more of 7 parabens (Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben and Benzylparaben) were detected in 144 human adipose tissue samples. Detection frequencies and median concentrations were: Methylparaben (100.0%, 0.40 ng/g tissue), Ethylparaben (20.1%, < LOD), Propylparaben (54.2%, 0.06 ng/g tissue), Butylparaben (5.6%, <LOD), and Isobutylparaben (2.1%, <LOD). Isopropylparaben and Benzylparaben were not detected in any of the samples.

EARTH study indicated the largest percent increase for parabens was associated with the use of suntan/sunblock lotion (66 - 156%) and hand/body lotion (79 - 147%). GM concentrations of Methylparaben, Propylparaben, and Butylparaben in urine were 28, 2.86, and 0.26 μg/L, respectively. Among 346 infants, none of the maternal preconception parabens concentrations were associated with birth weight. Maternal preconception Methylparaben concentration was associated with a decreased head circumference of 0.27 cm (95% CI: −0.54, 0).

Six parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben) and 4-Hydroxybenzoic Acid were measured in 143 urine samples from healthy, premenopausal women. 4-Hydroxybenzoic Acid was associated with increased FSH 0.07 (95% CI: 0.01, 0.13) and paraben concentrations were associated with increased E2 0.21 (95% CI: (0.15, 0.28) and increased progesterone 0.32 (95% CI: 0.23, 0.41).

Among 1003 Puerto Rican pregnant women, median concentrations of Butylparaben were 2-fold greater than US women from the NHANES program, while concentrations of Methylparaben, Ethylparaben, and Propylparaben were lower. Positive correlation was identified between Methylparaben and Propylparaben (Spearman $r = 0.78$). And trends were observed for increasing concentration of four parabens with increasing age categories.

The associations between maternal urinary parabens (Methylparaben, Ethylparaben, Propylparaben, and Butylparaben) and plasma inflammatory markers across pregnancy were examined in 130 preterm birth cases and 352 controls. An interquartile

range increase in Methylparaben (359 ng/mL) was positively associated with a 6.69% increase in IL-6 (95% CI: 0.02, 13.8), while increase in Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in IL-1β (95% CI: −14.1, −0.86).

Among 602 pregnant women in Puerto Rico, urinary Butylparaben, Methylparaben, and Propylparaben were associated with decreases in SHBG by 5.27% (95% CI: -9.4, − 1.14), 3.53% (95% CI: -7.37, 0.31) and 3.74% (95% CI: -7.76, 0.27), respectively. Methylparaben was associated with decreases in reproductive hormones, including an 8% decrease (95% CI: -15.4, 0.61) in estriol, a suggestive 3% increase (95% CI: -2.95, 9.61) in the progesterone/estriol ratio, and a suggestive 6.7% decrease (95% CI: -13.13, 0.29) in testosterone at 16 - 20 weeks.

Among 420 women undergoing IVF treatment, urinary concentrations of Methylparaben and Propylparaben were not associated with IVF outcomes. Of 252 adolescents participating in NBC Cohort study, urine concentrations of parabens were not associated with any maladaptive behavior.

Among 152 pregnant women, a significant decrease in diastolic blood pressure was associated with exposure to parabens including Methylparaben, Ethylparaben, and Butylparaben in the second trimester (β = −0.62 mmHg; 95%CI: −1.16, −0.08 per doubling of Methylparaben concentrations).

Culture of *Candida glabrata* in SC medium containing 1.5 mM Methylparaben and 165 μM Propylparaben induced expression of EPA6 adhesin gene, leading to increased adherence to cultured human Lec2 epithelial cells as well as primary human vaginal epithelial cells.

In in vitro assay, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben appeared to be weak irritants. The sensitization potential of the parabens tested was correlated with side-chain length: Methylparaben, Ethylparaben, Propylparaben, and Isopropylparaben were classified as weak sensitizers; and Butylparaben, Isobutylparaben, and Benzylparaben were strong sensitizers in this study.

Methylparaben elevated UVB-induced cell death in a statistically significant manner. Methylparaben elevated measurements of ROS and NO production and lipid peroxidation, and activated NFκB and AP-1 in UVB-irradiated cells. Metabolic activity/number of viable cells was reduced in WCCs and HCEs in a concentration-dependent manner after exposure to Methylparaben.

Data collected by the ESSCA network between 2009 and 2012 indicated that parabens yielded less than 1% positive actions of allergic contact dermatitis in the 52,586 tests.

In prospective studies, in vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility. No significant associations were observed of the current exposure levels of Methylparaben, Ethylparaben, and Propylparaben in Chinese pregnant women with size of infants at birth. Urinary Methylparaben and Propylparaben concentrations were associated with an increase in gestational age, and Methylparaben, Butylparaben, and Propylparaben were all associated with a 34–50% decrease in the odds of SGA.

Among 501 male partners of couples planning to become pregnant, urinary concentrations of Methylparaben, Ethylparaben, and Butylparaben were associated with diminished sperm count and several sperm motility parameters. However, seminal plasma concentrations of Ethylparaben and Benzylparaben in 339 males were associated with an increased percentage of sperm motility.

A urinary concentration increase of parabens was associated with the use of suntan/sunblock lotion (66 - 156%) and hand/body lotion (79 - 147%) in 400 men who reported the use of 14 PCPs. GM concentrations of Methylparaben, Propylparaben, and Butylparaben in urine were 28, 2.86, and 0.26 μg/L, respectively.

Among 346 infants, none of the maternal preconception paraben concentrations were associated with birth weight.[113] Maternal preconception Methylparaben concentration was associated with a decreased head circumference of 0.27 cm (95% CI: −0.54, 0).

The downward trends in sperm concentration and normal morphology among 936 men who sought infertility treatment were not affected when including urinary paraben concentrations in linear regression models, indicating that parabens exposure was not associated with the downward trends in semen parameters.

An interquartile range increase of urinary Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in pro-inflammatory marker interleukin-1β (95% CI: −14.1, −0.86). In Latino children, peripubertal urinary Methylparaben or Propylparaben concentrations were associated with altered pubertal timing; however, the causality could not be determined.

In retrospective studies, the incidence of cryptorchidism and/or hypospadias, combined, was associated with placental concentrations of Methylparaben ≥ 1.96 ng/g (OR = 3.18; CI = 0.88 - 11.48) and Propylparaben concentrations ≥ 1.16 ng/g (OR = 4.72; CI = 1.08 - 20.65). Linear regression analyses indicated an association between urinary Ethylparaben concentrations in 3-year old boys and their body weights and heights.

Among 241 pregnant women, urinary concentrations of Butylparaben were positively associated with blood glucose levels for both the 1st trimester (adjusted difference = 12.5 mg/dL; 95% CI: 0.9, 24.2) and 2nd trimester (adjusted difference = 11.2 mg/dL; 95% CI: 0.2, 22.3), when assessed as a mixture with two other parabens, Methylparaben and Propylparaben. In

contrast, a negative association between 1st trimester propylparaben and glucose (adjusted difference = −22.3 mg/dL; 95% CI: −43.2, −1.4).

Maternal urinary paraben levels of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben were measured in 850 mother-infant pairs. In all infants, each doubling increase in average Ethylparaben was associated with −2.82% (95% CI: −5.11%, −0.53%) decrease in weight z-score (standard deviation scores) at birth. In addition, age-specific association of Ethylparaben with −3.96% (95% CI: −7.03%, −0.89%) and −3.38% (95% CI: 6.72%, −0.03%) reduction in weight z-scores were observed at 1 and 2 years in males, respectively. Third-trimester Ethylparaben was negatively associated with weight z-scores at birth, 1 and 2 years in males.

Among 473 pregnant women, four parabens (Methylparaben, Ethylparaben, Propylparaben, and Butylparaben) were measured in spot urine samples collected between weeks 23 and 29 of gestation. A positive association between the sum of parabens and placental weight has been identified ($\beta = 7.12$, $p = 0.04$).

Among 1087 pregnant women in China, a total of 103 (9.5%) women were diagnosed with gestational diabetes mellitus (GDM). Urinary Ethylparaben was associated with GDM. The RRs = 1.12 (95% CI: 0.63, 2.01) for the second quartile, RRs = 1.11 (95% CI: 0.64, 1.93) for the third quartile, and RRs = 1.70 (95% CI: 1.02, 2.82) for the highest quartile, compared with the lowest quartile.

Five parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben) were measured in three spot urine samples of 478 pregnant women in China. Each 2-fold increase in average prenatal paraben concentration was associated with lower MDI scores among girls $\beta = -1.08$ (95% CI: −2.10, −0.06) and $\beta = -1.51$(95% CI: −2.69, −0.32) for Methylparaben and Σparabens, respectively.

Methylparaben was associated with lower Th1% (RR: −3.35, 95% CI: −6.58, −0.02) and Th2% at borderline significance (RR: −4.45, 95% CI: −8.77, 0.08) in their children. Propylparaben was associated with decreased odds of probable asthma (OR: 0.86, 95% CI: 0.74, 0.99).

Among 480 pregnant women, 130 cases of preterm birth were identified. Regression analyses indicated Ethylparaben was associated with increased risk for placental preterm birth OR = 1.47 (95% CI: 1.14 – 1.91). Urinary concentrations of Methylparaben and Propylparaben were not associated with any IVF outcomes in 420 women undergoing IVF. In a different study, urine concentrations of parabens were not associated with any maladaptive behaviors. A significant decrease in diastolic blood pressure was associated with exposure to parabens in 152 pregnant women in their second trimester.

Preterm birth was associated with umbilical cord blood concentrations of Butylparaben (OR = 60.77; CI = 2.60 - 1419.93) and Benzylparaben (OR = 0.03, CI = 0.01 - 0.44). The authors stated that the OR of 0.03 for Benzylparaben indicated a "protective effect" of Benzylparaben for preterm birth. Linear regression analysis indicated an association between maternal urinary concentrations and decreased gestational age and body length in newborns.

No statistically significant associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated (i.e., body length, gestational age at birth, birth weight, head circumference). No statistically significant associations were found between prenatal or postnatal growth of male newborns and maternal urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben.

Linear regression analyses of data from the US NHANES program indicated an association between reduced serum T4 concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben. MPC and the results of statistical tests for trends were not statistically significant in a study of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben in women undergoing infertility evaluation and OV or AFC measurements.

Analysis of data from the US NHANES program indicated an association between aeroallergen and food sensitization, combined, and urinary concentrations of Methylparaben (OR = 1.74; CI = 1.02 - 3.22), Propylparaben (OR = 2.04; CI = 1.12 -3.74), and Butylparaben (OR = 1.55; CI = 1.02 - 2.33). The results also indicated associations between urinary concentrations of Methylparaben and nonatopic asthma (OR = 0.025; CI = 0.07 - 0.90), and nonatopic wheeze (OR = 0.23; CI = 0.05 - 0.99).

Urine samples were collected from 696 pregnant women in China. No significant association was found between parabens and GDM among the overall population. However, significant non-linear associations of Propylparaben and the summed estrogenic activity of parabens with GDM were found in the stratified analysis by pre-pregnancy body mass index (BMI) in the overweight/obese population, with adjusted ORs of 3.47 (95% CI: 1.28, 9.42) and 2.87 (95% CI: 1.07, 7.73) for GDM in the second tertile of urinary Propylparaben and the summed estrogen activity, respectively, when compared to the first tertile.

One study examined the association between parabens and asthma morbidity among 450 children with asthma and with asthma prevalence among 4023 children participating in the US NHANES program (2005 - 2014). An increased prevalence odds of reporting emergency department visits were observed for every 10-fold increase in Methylparaben and Propylparaben concentrations among boys with asthma [(prevalence OR = 2.61, 95% CI: 1.40-4.85) and (OR = 2.18, 95% CI: 1.22-3.89, respectively)]. Among children in the general population, no overall associations with current asthma were observed, although there was a positive trend with Propylparaben and a current asthma diagnosis.

Among 1693 black women aged 23 - 34 years, Methylparaben and Butylparaben concentrations were 30 % lower for BMI ≥ 35 vs. < 25 kg/m^2 [(95% CI: −48.0%, −7.7%) for Methylparaben and (95% CI: −49.6%, −4.6%) for Butylparaben, respectively].

Of 156 men under 45 years of age who attended the infertility clinic for diagnostic purposes with normal semen concentration, a positive association was found between urinary level of Butylparaben and XY18 disomy ($p = 0.045$) and Propylparaben and disomy of chromosome 13 ($p = 0.007$).

No statistically significant associations were found between the urinary concentrations of Methylparaben, Propylparaben, or Butylparaben and serum hormone concentrations, semen quality parameters, and motion characteristics (for all but one indicator). The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

Urinary levels of Ethylparaben and Butylparaben were associated with an increase in the percentage of sperm with abnormal morphology. Urinary Isobutylparaben concentrations were significantly associated with an increase in the percentage of sperm with level of Isobutylparaben increased high DNA stainability. Neither categories of urinary concentrations of parabens nor continuous concentrations of parabens were associated with the level of reproductive hormones. Urinary concentrations of Methylparaben and Propylparaben were not related to any of the examined semen quality parameters, sperm DNA damage, or the level of reproductive hormones.

Urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were measured in 215 young healthy men, 94% of whom had detectable urinary concentrations of parabens. Urinary concentrations of parabens were not significantly associated with any semen parameters or any of the reproductive hormone levels.

Among 42 partners of couples who visited a gynecology clinic for infertility consultation, no significant association was found between semen parameters (sperm volume, concentration, and motility) and urinary paraben concentrations, in regression analyses.

In cord plasma of 27 healthy pregnant women, Methylparaben, Propylparaben and the sum of all measured parabens (Methylparaben + Ethylparaben + Propylparaben + Butylparaben) were inversely associated with T levels.

A conservative risk assessment was performed. Therein, an NOAEL value of 160 mg/kg/day for Butylparaben was determined to be adequate in consideration of the new data in the category of endocrine activity and from DART studies. For the purposes of an MOS calculation, the Panel considered a scenario wherein a consumer would use a set of cosmetic products containing Butylparaben. Therein, an aggregate exposure to four main categories of products was considered: (1) oral products, (2) eye products, (3) leave-on products and (4) rinse-off product; the global daily exposure of products for each category was estimated using the maximum use concentration of Butylparaben in each category, 0.2%, 0.5%, 0.24%, and 0.33%, respectively. The Panel noted that the available in vitro percutaneous absorption studies using human split- or full thickness skin suggest a conservative assumption of human dermal penetration of un-metabolized Butylparaben at 3.7%, though this could vary due to differences in animal species used, matrix effects, and other experimental conditions. Considering the variables, and taking into account that dermal absorption of lower molecular weight parabens is likely to be higher, the Panel selected a 50% dermal absorption rate of un-metabolized parabens as adequately conservative for the calculation of the MOS. The MOS for adults was 457 for Butylparaben. Since alkyl parabens undergo the similar enzymatic hydrolysis to form 4-Hydroxybenzoic Acid, such a conservative MOS of Butylparaben for adults could then be inferred to other single alkyl parabens.

Since multiple parabens are commonly combined for use in a single formulation, and no use data are available for such combinations, the Panel used the above parameters to calculate a maximum combined parabens (Σparabens) use concentration, starting from an MOS of 100. Utilizing this protective margin of safety for Σparabens, the maximum use concentration was calculated to be 1.1%.

A human paraben PBPK model developed to predict the plasma free paraben concentration based on 95th percentile parabens concentration in urine reported in US NHANES program (2009 - 2010 collection period). An in vitro based cumulative MOS was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the predicted free plasma paraben concentrations (Methylparaben + Ethylparaben + Butylparaben). The calculated cumulative MOS for adult females was 108, whereas the cumulative MOS for males was 444.

Considering aggregate exposure from various sources, e.g., cosmetics, food, and pharmaceutical use, the total combined exposure to parabens was estimated. Refinement techniques were applied in comparison with simply summed exposures from all multiple cosmetic product types. Approximately 60 - 90% of the model predictions from five implemented models were within a factor of 10 of the observed paraben exposures, while 30 - 40% of the predictions were within a factor of 3. More importantly though, in all cases, aggregate exposure estimates were significantly greater than the exposures derived from experimental biomonitoring data.

DISCUSSION

The Panel expressed concern about new data from DART studies that indicated lower NOAEL values than the one used in the previous CIR safety assessment of the parabens. One of these studies indicated reduced sperm counts and reduced expression of testicular CYP19a1, and a reduction of the Sertoli/Leydig cell marker Nr5a1 in the testes of offspring of female rats orally dosed with 10 mg/kg bw/day Butylparaben during the gestation and lactation periods. The Panel noted that the reduction of epididymal sperm count has shown the same effect across all doses from 10 to 500 mg/kg bw/day in this study, decreasing 76 - 78% compared to controls; while a dose -response relationship is expected between estrogen agonists exposure and sperm count decrease. The Panel also noted that wide variation exists in measuring epididymal sperm count between different laboratories and/or different experimental technicians, thus the decline in sperm counts in this study warrants further validation, by making comparisons to historical sperm count control databases. In addition, the Panel noted that the data, in terms of the DART endpoints of AGD, epididymal sperm count, and histological examinations, did not show consistency at doses ranging from 10 to 100 mg/kg bw/day when compared to other DART studies that followed similar Butylparaben exposure scenarios. In contrast, data are more consistent at doses ranging from 160 to 1000 mg/kg bw/day.

The Panel also discussed the conflicting data from other DART studies, and agreed that 1) many of these reports are irrelevant to the routes of exposure associated with intended cosmetic use, or otherwise did not account for the extensive metabolism of parabens (to metabolites with no known DART activity); 2) are the result of poorly designed studies; and 3) were not verified by other methods. Thus, after careful consideration of all the new data, the Panel determined a NOAEL of 160 mg/kg bw/day for Butylparaben. The Panel determined the different use concentrations and exposures of Butylparaben in various cosmetic products category should be considered when estimating the systemic exposure levels for the MOS calculation.

The Panel recognized that the study chosen by the SCCS for the calculation of the MOS of Butylparaben examined DART endpoints in male rats, involved subcutaneous instead of oral administration of Butylparaben during the lactation period. The SCCS acknowledged an NOEL of 2 mg/kg bw/day, instead of an NOAEL, for deriving the MOS of Butylparaben. In order to obtain an acceptable MOS $\geq$ 100, the SCCS recommended the maximum use concentration of Butylparaben in the finished cosmetic products set to be 0.19% (0.14% as acid). The calculation is based on the assumptions of the maximum exposure to preservatives of an adult (60 kg body weight) at 17.4 g/day and a human dermal penetration rate of un-metabolized Butylparaben at 3.7%.

However, the Panel considered that the study with an NOEL of 2 mg/kg bw/day suffers from several critical limitations: 1) this study involves route of subcutaneous exposure which may result in chemicals circumventing the physiological barriers and bypassing the portal of entry metabolism, and therefore not considered suitable for quantitative risk assessment in the context of cosmetic usage; 2) this study is not an OECD TG study (e.g., the Butylparaben treated group contained only 3 rats and the control group contained only 5 rats); 3) only one postpartum dose at 2 mg/kg bw/day was tested; 4) male rats were exposed to Butylparaben postnatally, which did not examine the generation toxicity (e.g., a more robust study design should involve gestational exposure of paraben to pregnant rats while examining toxicity in the male offspring); and 5) typical DART endpoints were not covered, such as AGD, PPS, weight of the epididymis and seminal vesicle, sperm counts, reproductive hormone levels, etc.

The Panel considered an NOAEL should be derived from studies that provide reliable reproduction toxicity data that are reproducible and with little variability. Because multiple parabens are commonly used in any given cosmetic product, a maximum safe use concentration for combined paraben (Σparabens) use in a single formulation was calculated using a protective MOS level of 100. The maximum use concentration was calculated to be 1.1%. Accordingly, the Panel determined that the commonly used (e.g., in the EU) limitation of 0.8% is ultra-conservative and is safe for human health when parabens are used in combination in a cosmetic product.

The Panel also recognized that these ingredients can enhance the penetration of other ingredients through the skin. The Panel cautioned that care should be taken in formulating cosmetic products that may contain these ingredients in combination with any ingredients whose safety was based on their lack of dermal absorption data, or when dermal absorption was otherwise a concern.

The Panel noted that the EU Cosmetic Regulation has banned the use of Isopropylparaben, Isobutylparaben, Phenylparaben, Benzylparaben, and pentylparaben as preservatives in cosmetic products. The scientific rationale for restricting these ingredients warrants further justification.

The Panel noted that both in vitro and in vivo studies indicate a rapid and effective metabolism of parabens by carboxyl-esterases after oral or dermal exposure. Parabens are further metabolized by conjugation with glucuronide, sulfate, or glycine prior to excretion. When applied to human skin, parabens are metabolized to 4-Hydroxybenzoic Acid. Whereas older studies suggested that un-metabolized parabens are not excreted, recent studies with more sensitive analytical methods have measured un-metabolized parabens and their metabolites following dermal exposures. Because each of these alkyl parabens (i.e., excluding Benzylparaben) undergo extensive metabolism in a similar way, the Panel felt that safety data for one of these alkyl parabens could be used to support the safety of the other alkyl parabens.

The Panel discussed concerns about the relevance of the oral animal studies to human risk assessment in that the rapid and effective metabolism of parabens in rodents does not occur in humans. Species differences in the esterase affinities and activities must be carefully taken account for deriving a safe level of exposure in humans. The Panel noted that uncertainties relate to data gaps on dermal absorption of un-metabolized parabens by human skin in vivo and in vitro. One human toxicokinetic study indicates after dermal repeated exposure to Butylparaben at a daily dose of 10 mg/kg bw/day for five days, about 2.1% un-metabolized Butylparaben was detected in the urine of the participants. However, the Panel noted that a conservative estimation shows that daily exposure of consumers to Butylparaben is much lower (0.66 mg/kg bw/day). While SCCS derived the value of 3.7%, based on in vitro studies using human split- or full thickness skin, as a worst-case assumption for the dermal absorption of un-metabolized Butylparaben, uncertainties need to be addressed considering that absorption may be variable due to differences in animal species used, matrix effects, other experimental conditions, dermal absorption of lower molecular weight parabens (which is likely to be higher). In light of these facts, the Panel selected an estimate of a 50% dermal absorption rate of un-metabolized parabens in the calculation of MOS, which represents a very conservative assumption.

The Panel discussed the bioaccumulation potential of parabens. The Panel noted that, as lipid-soluble chemicals, parabens may distribute to tissues despite metabolism. Recent studies have demonstrated the presence of parabens in various human tissues. However, the data are equivocal regarding cumulative storage in such tissues.

The Panel noted that recent epidemiology studies suggested paraben exposure association with different types of health outcomes, such as lower mental developmental index in girls, adverse impacts on fetal and childhood growth, decreased diastolic blood pressure during pregnancy, increased risk for placental preterm birth, disturbance of reproductive hormone levels, and disomy of chromosome; although, these were not confirmed by subsequent or previous epidemiologic investigations. Sources of parabens exposure in these studies are broadly from the environment and not specified. More importantly, parabens exposures of the study population are always coupled with other preservatives and active ingredients that are used in a wide variety of consumer products, including phthalates, BPA, TCS, etc. Therefore, the currently available scientific evidence lacks the clarity regarding any cause-and-effect relationship between parabens and human health outcomes. It remains to be determined whether the costimulatory effects require multiple such exposures. Further studies in larger populations and with more repeated measures across pregnancy would be useful to confirm these findings, and better understand if the hormone changes may affect downstream maternal and infant health outcomes. The Panel also noted that several studies suggested urinary paraben concentrations were associated with glucose levels in women at high risk of GDM, however, a causal relationship cannot be established. In one study, a positive association (Propylparaben) was identified among overweight/obese pregnant women, but not in the overall population; and importantly, evidence available in other studies indicates either no association or negative association between urinary Propylparaben concentration and GDM.

The Panel noted that measurements of total parabens in human adipose tissue warrant further investigation with larger sample sizes and unbiased analytical methods. In one study, total paraben measurements (the sum concentration of free and conjugated parabens and their metabolite 4-Hydroxybenzoic Acid) were compromised by alkaline hydrolysis in the tissue due to the use of alkali in the liposuction procedure, i.e., high concentrations of 4-Hydroxybenzoic Acid could be an artifact from the reaction of paraben esters with sodium bicarbonate solution used in liposuction procedures. In another study, while a positive, though not statistically significant, association between age and Methylparaben concentrations in human adipose tissue was observed, a positive association with age might also be a consequence of the commonly lower metabolic activity in older individuals (which may delay the metabolism and clearance of chemicals).

The Panel noted that paraben exposures are attributed to cosmetic products, foods, medicines, and other sources. Refined aggregate exposure models suggest that cosmetic product use is a major source of parabens dermal exposure. However, the vast quantity of biomonitoring data indicate that systemic exposure resulting from the cosmetic use of these ingredients is very low.

The Panel also reviewed data from a kinetic-based study which expands the use of human biomonitoring data in safety assessment. As biomonitoring data integrates all routes (inhalation, dermal, and oral) and sources of exposure (cosmetics, foods, drugs, etc.), it provides valuable perspective to help evaluate aggregate exposure to parabens. The human paraben PBPK model was used to estimate the plasma free paraben concentration in adults consistent with 95th percentile urine concentration reported in US NHANES program (2009 - 2010 collection period). Based on the model, the calculated cumulative MOS for adult females was 108, and for males was 444. Both cumulative MOS derived from human epidemiological survey are sufficient to ensure human safety.

The Panel also discussed the safety of parabens as used in vaginally-applied cosmetic products. One published reference was submitted to the Panel along with the assertion that these ingredients cause irreparable damage to sperm and may preclude fertilization in users. However, of the multiple endpoints asserted in the reference, each was either constructed around an improperly chosen/designed assay to make such assertions unequivocally, and/or resulted in no significant effects. Another published reference asserted these ingredients may increase the chances of developing a vaginal yeast infection. However, the cell culture studies performed therein were dosed with extremely high concentrations compared to cosmetic use (i.e. 15 - 25% preservative in these studies vs a maximum use concentration of parabens in cosmetics of 0.5%). The Panel classified

these studies as illustrations of potential, general hazards, which fail to demonstrate risks relevant to cosmetic safety in the context of concentration of use.

The Panel discussed the issue of incidental inhalation exposure to paraben. The Panel noted that some of the parabens were reported to be used in cosmetic powder and sprays, at very low concentrations, which may result in incidental inhalation exposure; e.g., Ethylparaben in face powders at up to 0.5%. The Panel noted that in aerosol products that are widely applied, e.g., hair sprays, 95% - 99% of droplets/particles would not be respirable to any appreciable amount. The Panel also noted that, while particle/droplet size is an important parameter, the physicochemical properties of ingredients in a spray formulation, as well as the realistic exposure factors under in-use conditions also play significant roles in evaluating inhalation safety of parabens as spray formulation. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel considered the potential for exposure to these ingredients to cause irritation or induce skin sensitization. The Panel noted that skin tests on product formulations containing from 0.1 to 0.8 percent of, one or a combination of two, of the parabens showed no evidence of significant irritation or sensitization potential for these ingredients. All animal sensitization tests indicated that the parabens are non-sensitizing.

However, the Panel concluded that the available data are insufficient to determine the safety of Benzylparaben. The data needed to determine the safety of this ingredient comprise an NOAEL derived from DART studies. The Panel noted that this ingredient is not reported to be in current use.

CONCLUSION

The CIR Expert Panel concluded that the following 20 parabens are safe in cosmetics in the present practices of use and concentration described in the safety assessment when the sum of the combined concentration of parabens in any given formulation does not exceed 0.8 %.

Butylparaben
Calcium Paraben*
Ethylparaben
Isobutylparaben
Isopropylparaben
Methylparaben
Potassium Butylparaben*
Potassium Ethylparaben*
Potassium Methylparaben*
Potassium Paraben*
Potassium Propylparaben*
Propylparaben
Sodium Butylparaben
Sodium Ethylparaben
Sodium Isobutylparaben
Sodium Isopropylparaben*
Sodium Paraben*
Sodium Methylparaben
Sodium Propylparaben
4-Hydroxybenzoic Acid*

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that each would be used in product categories and at concentrations comparable to others in this group.*

The CIR Expert Panel also concluded that the available data are insufficient to make a determination of safety for Benzylparaben. (*This ingredient is not reported to be in current use.*)

TABLES

Table 1. Definitions, structures, and functions of parabens in this safety assessment. [1; CIR Staff]

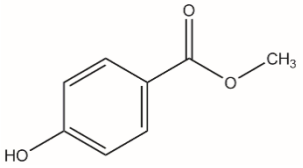
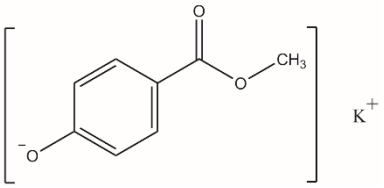
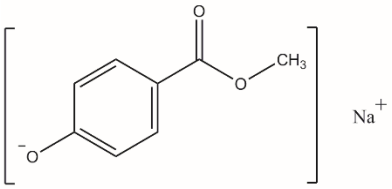
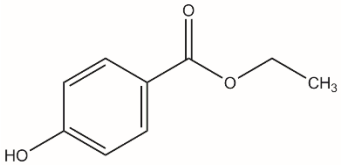
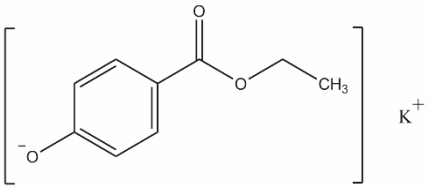
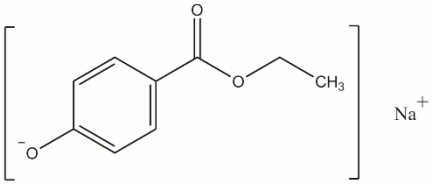
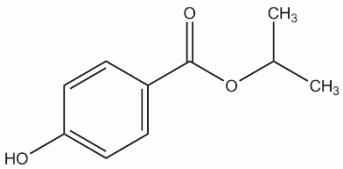
Ingredient CAS No.	Definition & Structure	Function
Parabens and Paraben Salts		
Methylparaben 99-76-3	Methylparaben is the ester of methyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Methylparaben 26112-07-2	Potassium Methylparaben is the potassium salt of Methylparaben that conforms to the formula: 	Preservative
Sodium Methylparaben 5026-62-0	Sodium Methylparaben is the sodium salt of Methylparaben that conforms to the formula: 	Preservative
Ethylparaben 120-47-8	Ethylparaben is the ester of ethyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Ethylparaben 36457-19-9	Potassium Ethylparaben is the potassium salt of Ethylparaben that conforms to the formula: 	Preservative
Sodium Ethylparaben 35285-68-8	Sodium Ethylparaben is the sodium salt of Ethylparaben that conforms to the formula: 	Preservative
Isopropylparaben 4191-73-5	Isopropylparaben is the ester of isopropyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Preservative

Table 1. Definitions, structures, and functions of parabens in this safety assessment. [1; CIR Staff]

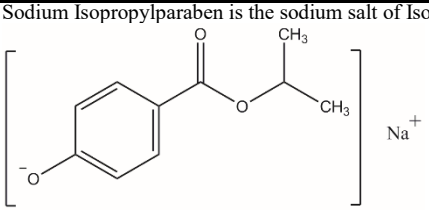
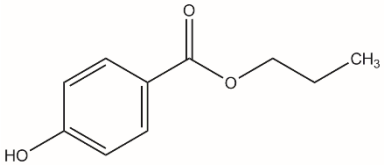
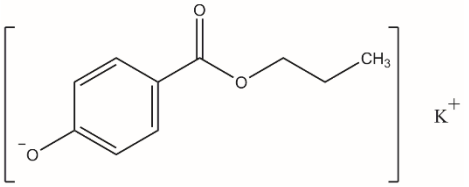
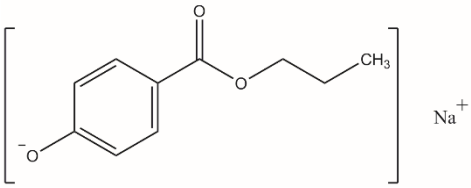
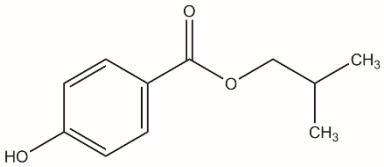
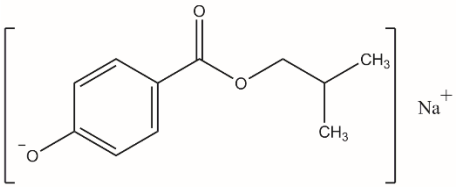
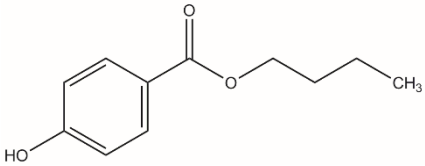
Ingredient CAS No.	Definition & Structure	Function
Sodium Isopropylparaben	Sodium Isopropylparaben is the sodium salt of Isopropylparaben: 	Preservative
Propylparaben 94-13-3	Propylparaben is the ester of n-propyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Propylparaben 84930-16-5	Potassium Propylparaben is the potassium salt of Propylparaben that conforms to the formula: 	Preservative
Sodium Propylparaben 35285-69-9	Sodium Propylparaben is the sodium salt of Propylparaben that conforms to the formula: 	Preservative
Isobutylparaben 4247-02-3	Isobutylparaben is the ester of isobutyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Preservative
Sodium Isobutylparaben 84930-15-4	Sodium Isobutylparaben is the sodium salt of Isobutylparaben: 	Preservative
Butylparaben 94-26-8	Butylparaben is the ester of butyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Fragrance ingredient, preservative

Table 1. Definitions, structures, and functions of parabens in this safety assessment. [1; CIR Staff]

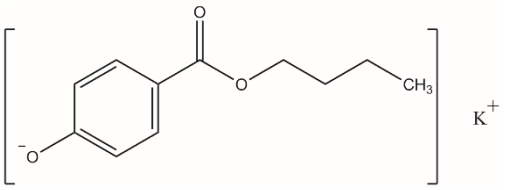
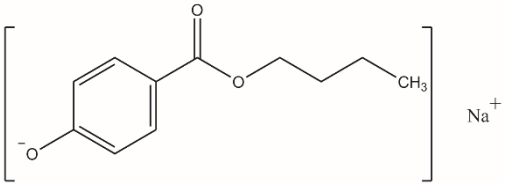
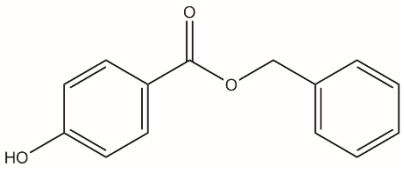
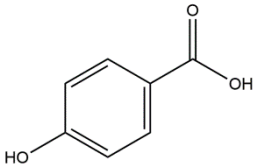
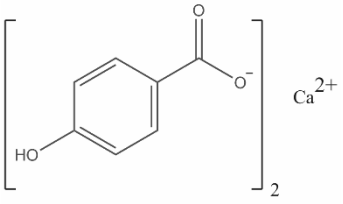
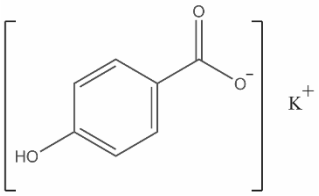
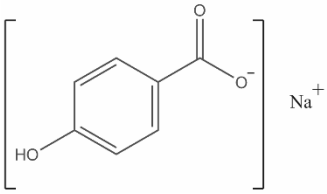
Ingredient CAS No.	Definition & Structure	Function
Potassium Butylparaben 38566-94-8	Potassium Butylparaben is the potassium salt of Butylparaben that conforms to the formula: 	Preservative
Sodium Butylparaben 36457-20-2	Sodium Butylparaben is the sodium salt of Butylparaben that conforms to the formula: 	Preservative
Benzylparaben 94-18-8	Benzylparaben is the ester of benzyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Preservative
Paraben Carboxylic Salts and Free Acid (non-esters)		
4-Hydroxybenzoic Acid 99-96-7	4-Hydroxybenzoic Acid is the aromatic acid that a conforms to the formula: 	Fragrance ingredient; preservative
Calcium Paraben 69959-44-0	Calcium Paraben is organic salt that conforms to the formula: 	Preservative
Potassium Paraben 16782-08-4	Potassium Paraben is the organic salt that conforms to the formula: 	Preservative
Sodium Paraben 114-63-6 85080-04-2	Sodium Paraben is the organic salt that conforms to the formula: 	Preservative

Table 2. Previous CIR safety assessments of parabens

Parabens	Conclusion	Reference
Methylparaben, Ethylparaben, Propylparaben, and Butylparaben	Safe as cosmetic ingredients in the present practices of use	1984[44]
Benzylparaben	Available data are insufficient to support the safety	1986[45]
Isobutylparaben and Isopropylparaben	Safe as cosmetic ingredients in the present practices of use	1995[46]
Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, Isopropylparaben, and Isobutylparaben	Safe in the present practices and concentrations	2008[2]

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Benzylparaben		
Physical Form	Solid, crystalline	7
Color	White	7
Odor	Odorless	7
Molecular Weight g/mol	228.25	2
Density g/cm^3 @ 20ºC	1.224±0.06 est.	164
Vapor Density mmHg	0 est.	7
Melting Point ºC	110-112	2
Boiling Point ºC	389.8±17.0 est.	164
Water Solubility g/L @ 25ºC	1.08	7
	10	2
Other Solubility g/L		
Propylene glycol	130	2
log P_{ow}	3.97	7
Disassociation constants (pKa, pKb)		
pK_a	8.18±0.15 est.	164
Butylparaben		
Physical Form	Crystals or powder	165
Color	White	165
Odor	Odorless	165
Molecular Weight g/mol	194.23	165
Vapor pressure mmHg @ 25ºC	$1.86x10^{-4}$	165
Melting Point ºC	68-69	2
	68-72	2
Boiling Point ºC	309.2±15.0	164
Water Solubility g/L @ 20ºC	$0.0027x10^2$	165
	Insoluble	2
Other Solubility g/L		
Alcohol	Soluble	2
Ether	Soluble	2
Glycerin	Slightly soluble	2
Disassociation constants (pKa, pKb)		
pK_a	8.37	2
	8.47	165
Ethylparaben		
Physical Form	Crystals or powder	166
Color	Colorless or white	166
Molecular Weight g/mol	166.18	2
Density @ 20ºC	1.291	4
Vapor pressure mmHg @ 25ºC	$9.29x10^{-5}$	166
Melting Point ºC	116-118	2
	115-118	2
Boiling Point ºC	297-298	2
Water Solubility g/L @ 25ºC	0.885	166
Other Solubility		
Alcohol	Very soluble	2
Ether	Very soluble	2
Glycerin	Slightly soluble	2
log K_{ow}	2.47	4,166
	2.27	39
Disassociation constants (pKa, pKb)		

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
pK_a	8.22	2
	8.34	166
Isobutylparaben		
Physical Form	Solid, powder	22
Color	White	22
Molecular Weight g/mol	194.25	2
Density g/cm^3 @ 20ºC	1.105±0.06	164
Vapor pressure mmHg @ 25ºC	0.000381	22
Melting Point ºC	72.95 est.	22
Boiling Point ºC	302.3±15.0	164
Water Solubility g/L @ 25ºC	2.24	22
log P_{ow}	3.04	22
Isopropylparaben		
Molecular Weight g/mol	180.22	2
Melting Point ºC	96-97	167
Boiling Point ºC	294	168
Methylparaben		
Physical Form	Powder	20
	Liquid	20
Color	White or colorless	20
Odor	Characteristic	20
Molecular Weight g/mol	152.16	2
Density g/cm^3 @ 137.2ºC	1.1208	169
@ 20ºC	1.209±0.06 est.	164
Vapor pressure mmHg @ 25ºC	$2.37x10^{-4}$	20
Melting Point ºC	131	2
	125-128	2
Boiling Point ºC	270-280	2
	265	168
	140-141	170
Water Solubility g/L @ 25ºC	$2.50x10^3$	20
	Slightly soluble	2
Other Solubility		
Alcohol	Very soluble	2
Benzene	Slightly soluble	2
Ether	Very soluble	2
Glycerin	Slightly soluble	2
log K_{ow}	1.93	39
Disassociation constants (pKa, pKb)		
pK_a	8.17	2
Propylparaben		
Physical Form	Crystal or powder	171
Color	Colorless or white	171
Odor	Odorless or faint	171
Molecular Weight g/mol	180.21	2
Density	1.0630	2
	1.28	171
Vapor pressure mmHg @ 25ºC	$5.55x10^{-4}$ est.	171
Melting Point ºC	96.2-98	2
	95-98	2
Boiling Point ºC	294	168
	271	171
Water Solubility g/L	0.0500	171
	Insoluble	2
Other Solubility		
Alcohol	Soluble	2
Ether	Soluble	2
log K_{ow}	2.34	5
	2.81	39
Disassociation constants (pKa, pKb)		
pK_a	8.35	2
Calcium Paraben		
Formula Weight g/mol	314.306	172
Potassium Butylparaben		
Formula Weight g/mol	232.32	173

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Potassium Ethylparaben		
Formula Weight g/mol	204.266	[174]
Potassium Methylparaben		
Formula Weight g/mol	190.239	[175]
Potassium Paraben		
Formula Weight g/mol	176.212	[176]
Potassium Propylparaben		
Formula Weight g/mol	218.293	[177]
Sodium Butylparaben		
Formula Weight g/mol	216.212	[178]
Sodium Ethylparaben		
Physical Form	Solid, powder	[23]
Color	White	[23]
Formula Weight g/mol	188.157	[36]
Density g/cm^3 @ 20ºC	1.34	[23]
Melting Point ºC	268	[23]
Water Solubility g/L @ 23ºC & pH 10.4	> 1000	[23]
log K_{ow}	-0.14	[23]
Sodium Isobutylparaben		
Formula Weight g/mol	216.212	[179]
Sodium Methylparaben		
Physical Form	Crystalline solid	[3]
Color	White	[3]
Formula Weight g/mol	174.131	[180]
Density g/ml @ 20ºC	1.42	[3]
Melting Point ºC	313	[3]
Water Solubility g/L @ 20ºC & pH 11.4	> 10.0	[3]
log P_{ow}	-0.63	[3]
Disassociation constants pKa @ 23ºC	8.4	[3]
Sodium Paraben		
Formula Weight g/mol	160.104	[181]
Sodium Propylparaben		
Physical Form	Solid, powder	[6]
Color	White	[6]
Formula Weight g/mol	202.185	[182]
Density @ 20ºC	1.24	[6]
@ 25ºC	1.24	[6]
Vapor pressure mmHg @ 20ºC	< 0.001	[6]
Melting Point ºC	302	[6]
Boiling Point ºC	310 (decomp)	[6]
Water Solubility g/L @ 23ºC	> 100	[6]
log P_{ow}	0.27	[6]
4-Hydroxybenzoic Acid		
Molecular Weight g/mol	138.12	[183]
Melting Point ºC	214.5	[184]
Boiling Point ºC	336.2 est.	[183]
log K_{ow}	1.39 est.	[185]
Disassociation constants (pKa, pKb) pK$_a$	4.57±0.10 est	[186]

Decomp=decomposes on melting

Table 4. Particle size distribution of parabens in this safety assessment.

Ingredient	D_{10} (µm)	D_{50} (µm)	D_{90}/D_{100} (µm)	Fraction <10 µm diameter (vol %)	Reference
Butylparaben	28.5 ± 0.9	114.8 ± 2.4	332.9 ± 16.4	2.1 ± 0.2	[9]
Isobutylparaben	3.1 ± 0.2	25.4 ± 1.5	80.5 ± 4.1	--	[22]
Isopropylparaben	--	150 (6.82%) 106 (35.38%) 75 (27.51 %) 53 (3.15 %)	--	--	[21]
Methylparaben	22.0 ± 0.9	141.7 ± 18.4	426.7 ± 82.6	3.7 ± 0.2	[8]
Sodium Methylparaben	7.9 ± 3	117.1 ± 17.5	693.5 ± 96.8	11.6 ± 2.2	[3]
Ethylparaben	50 ± 4.3	307.5 ± 21.9	770.6	3.0 ± 0.2	[4]
Propylparaben	2.6 ± 0.1	16.2 ± 0.7	113 ± 5	37.8 ± 1.0	[5]
Sodium Ethylparaben	6.5 ± 0.3	49.5 ± 6.4	147.1 ± 28.3	--	[23]
Sodium Propylparaben	6.7 ± 0.3	37.8 ± 4.9	164.5 ± 36.7	--	[6]
4-Hydroxtbenzoic acid	--	≥ 59.5 - < 85.5	--	No detection	[11]

Table 5. Current and historical frequency and concentration of use of parabens according to duration and exposure.

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Benzylparaben				**Butylparaben**			
	2019[26]	**2006[2]**	**2016[25]**	**2003[2]**	**2019[26]**	**2006[2]**	**2016[25]**	**2003[2]**
Totals*	**NR**	**1**	**NR**	**NR**	**3884**	**3001**	**0.00000006-0.5**	**0.00002-0.54**
Duration of Use								
Leave-On	*NR*	*1*	*NR*	*NR*	*3127*	*2409*	*0.00000006-0.5*	*0.00002-0.4*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*734*	*551*	*0.0000004-0.33*	*0.00004-0.54*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*23*	*41*	*0.00002-0.1*	*0.00004-0.07*
Exposure Type								
Eye Area	NR	NR	NR	NR	777	812	0.000002-0.5	0.00002-0.3
Incidental Ingestion	NR	NR	NR	NR	273	219	0.0000026-0.2	0.0008-0.1
Incidental Inhalation-Spray	NR	NR	NR	NR	13; 709[a]; 671[b]	27; 453[a]; 320[c]	0.00000011-0.1; 0.00059-0.22[a]	0.0004-0.2; 0.03-0.4[a]; 0.0004-0.4[c]
Incidental Inhalation-Powder	NR	NR	NR	NR	119; 671[b]; 6[c]	88; 21[b]; 320[c]	0.0057-0.3; 0.0001-0.24[c]	0.07-0.14; 0.05[b]; 0.0004-0.4[c]
Dermal Contact	NR	1	NR	NR	3156	2406	0.0000004-0.4	0.00004-0.54
Deodorant (underarm)	NR	1[a]	NR	NR	8[a]	10[a]	0.000025[d]	0.002[a]
Hair - Non-Coloring	NR	NR	NR	NR	283	246	0.00000011-0.22	0.0004-0.25
Hair-Coloring	NR	NR	NR	NR	33	28	0.0000005-0.05	0.03
Nail	NR	NR	NR	NR	43	21	0.00000006-0.07	0.003-0.2
Mucous Membrane	NR	NR	NR	NR	517	312	0.0000026-0.2	0.00004-0.11
Baby Products	NR	NR	NR	NR	11	28	NR	0.05
	Ethylparaben				**Isobutylparaben**			
	2019[26]	**2005**[2]	**2016[25]**	**2003[2]**	**2019[26]**	**2006[2]**	**2016[25]**	**2003[2]**
Totals*	**3802**	**2679**	**0.00000032-0.65**	**0.00002-0.98**	**1918**	**642**	**0.00000006-0.3**	**0.000007-0.5**
Duration of Use								
Leave-On	*2878*	*2066*	*0.00000032-0.65*	*0.00002-0.6*	*1447*	*435*	*0.00000006-0.3*	*0.000007-0.5*
Rinse-Off	*893*	*562*	*0.0000008-0.5*	*0.0001-0.98*	*446*	*178*	*0.0000004-0.23*	*0.0001-0.4*
Diluted for (Bath) Use	*31*	*51*	*0.005-0.1*	*0.00004-0.15*	*25*	*29*	*0.000012-0.005*	*0.00002-0.2*
Exposure Type								
Eye Area	545	543	0.000002-0.65	0.00002-0.49	213	59	0.00000006-0.14	0.000007-0.5
Incidental Ingestion	64	72	0.000008-0.3	0.0002-0.2	63	11	0.000004-0.09	0.0001-0.4
Incidental Inhalation-Spray	13; 786[a]; 370[b]	23; 431[a]; 330[c]	0.000031-0.22; 0.00059-0.2[a]; 0.06-0.15[b]	0.02-0.2; 0.0001-0.6[a]; 0.0004-0.4[c]	7; 383[a]; 444[b]	7; 109[a]; 129[c]	0.00004-0.023; 0.00002-0.18[a]	0.01-0.2; 0.0002-0.3[a]; 0.02-0.4[c]
Incidental Inhalation-Powder	64; 370[b]; 12[c]	122; 12[b]; 330[c]	0.0057-0.5; 0.06-0.15[b]; 0.0002-0.48[c]	0.04-0.5; 0.0004-0.4[c]	21; 444[b]; 2[c]	8; 5[b]; 129[c]	0.0029-0.0086; 0.0000007-0.24[b]	0.00001-0.04; 0.02-0.4[c]
Dermal Contact	2988	2147	0.000002-0.65	0.00004-0.98	1577	525	0.0000006-0.3	0.00001-0.5
Deodorant (underarm)	10[a]	10[a]	not spray: 0.5 spray: 0.00005[d]	0.002-0.1[a]	5[a]	3[a]	NR	0.002[a]
Hair - Non-Coloring	102	229	0.0000008-0.3	0.001-0.6	141	83	0.0000004-0.17	0.01-0.3
Hair-Coloring	115	92	0.000004-0.2	0.2	30	1	0.000036-0.00008	NR
Nail	15	10	0.00000032-0.2	0.01-0.2	37	3	NR	0.006
Mucous Membrane	310	170	0.000008-0.3	0.00004-0.2	265	63	0.000004-0.09	0.00002-0.4
Baby Products	15	15	0.032	NR	5	7	NR	NR

Table 5. Current and historical frequency and concentration of use of parabens according to duration and exposure.

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Isopropylparaben				**Methylparaben**			
	2019[26]	**2006[2]**	**2016[25]**	**2003[2]**	**2019[26]**	**2006[2]**	**2016[25]**	**2003[2]**
Totals*	**274**	**48**	**0.000005-0.32**	**0.00001-0.3**	**11,739**	**8786**	**0.000001-0.9**	**0.0003-1**
Duration of Use								
Leave-On	*231*	*39*	*0.00004-0.32*	*0.00001-0.3*	*9347*	*6468*	*0.0000043-0.8*	*0.0008-1*
Rinse-Off	*42*	*8*	*0.000005-0.22*	*0.03-0.2*	*2333*	*2105*	*0.000001-0.9*	*0.001-0.46*
Diluted for (Bath) Use	*1*	*1*	*NR*	*0.005*	*59*	*213*	*0.21-0.5*	*0.0003-0.5*
Exposure Type								
Eye Area	45	10	0.19	0.06-0.2	1797	1610	0.000002-0.8	0.07-0.6
Incidental Ingestion	31	1	0.12	0.2	305	301	0.000032-0.35	0.07-1
Incidental Inhalation-Spray	2; 88[a]; 21[b]	2; 6[a]; 6[c]	0.00004; 0.00004[a]	0.0005-0.3[a]; 0.1-0.2[c]	86; 3299[a]; 1851[b]	111; 1382[a]; 968[c]	0.0000043-0.41 0.0024-0.5[a]; 0.25-0.6[b]	0.1-0.35; 0.07-0.5[a]; 0.15-0.44[c]
Incidental Inhalation-Powder	6; 21[b]	5; 6[c]	NR	0.00001-0.00002; 0.1-0.2[c]	346; 1851[b]; 20[c]	376; 33[b]; 968[c]	0.004-0.4; 0.0024-0.6[b] 0.001-0.8[c]	0.1-0.5; 0.2-0.4[b]; 0.15-0.44[c]
Dermal Contact	197	39	0.031-0.32	0.00001-0.3	9310	6898	0.000001-0.6	0.0003-0.7
Deodorant (underarm)	NR	NR	NR	NR	20[a]	35[a]	not spray: 0.15-0.4 spray: 0.000075-0.00012	0.0008-0.3[a]
Hair - Non-Coloring	23	6	0.000005-0.22	0.001	1500	1137	0.0002-0.9	0.1-0.4
Hair-Coloring	NR	NR	NR	NR	237	197	0.0000016-0.4	0.05-0.35
Nail	6	NR	0.00012	0.1	68	37	0.0000012-0.41	0.002-0.4
Mucous Membrane	52	2	0.12	0.005-0.2	833	751	0.000001-0.5	0.0003-1
Baby Products	NR	NR	NR	NR	36	60	0.13-0.4	0.2-0.4

	Propylparaben			
	2019[26]	**2006[2]**	**2016[25]**	**2003[2]**
Totals*	**9034**	**7118**	**0.00000014-0.7**	**0.00002-0.7**
Duration of Use				
Leave-On	*7520*	*5585*	*0.00000014-0.7*	*0.00002-0.7*
Rinse-Off	*1465*	*1422*	*0.00000026-0.3*	*0.01-0.5*
Diluted for (Bath) Use	*49*	*140*	*0.0001-0.3*	*0.04-0.3*
Exposure Type				
Eye Area	1564	1477	0.00000014-0.7	0.02-0.5
Incidental Ingestion	586	527	0.000004-0.3	0.03-0.62
Incidental Inhalation-Spray	35; 2532[a]; 1349[b]	62; 996[a]; 706[c]	0.00000014-0.31; 0.0003-0.25[a]; 0.02-0.25b[c]	0.1-0.3; 0.001-0.5[a]; 0.03-0.4[c]
Incidental Inhalation-Powder	272; 1349[b]; 21[c]	308; 31[b]; 706[c]	0.0018-0.3; 0.02-0.25[b]; 0.0001-0.3[c]	0.1-0.7; 0.2[b]; 0.03-0.4[c]
Dermal Contact	7232	5598	0.00000014-0.4	0.00002-0.7
Deodorant (underarm)	13[a]	29	not spray: 0.025-0.15 spray: 0.000025-0.000058	0.002-0.2[a]
Hair - Non-Coloring	749	623	0.0000055-0.4	0.03-0.5
Hair-Coloring	168	150	0.00000026-0.25	0.04-0.5
Nail	58	27	0.0000003-0.2	0.002-0.4
Mucous Membrane	983	832	0.000004-0.3	0.02-0.62
Baby Products	35	56	0.15	0.05-0.2

Totals=Rinse-off + Leave-on + Diluted for Bath Product Uses.
*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
** Suspected to be a typo in the publication and may actually be 2006.
NR – no reported use
[a] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 6. Frequency (2019)[26] and concentration (2016)[25] of use according to duration and exposure of parabens.

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Sodium Butylparaben		Sodium Ethylparaben		Sodium Isobutylparaben	
Totals*	**2**	**NR**	**27**	**0.000012-0.062**	**2**	**NR**
Duration of Use						
Leave-On	*2*	*NR*	*25*	*0.000012-0.062*	*2*	*NR*
Rinse-Off	*NR*	*NR*	*2*	*0.0036*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	10	0.0036	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a]	NR	5[a]; 4[b]	NR	2[a]	NR
Incidental Inhalation-Powder	NR	NR	4[b]	0.0036[c]	NR	NR
Dermal Contact	2	NR	24	0.0036-0.062	2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	0.0036	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.000012	NR	NR
Mucous Membrane	NR	NR	2	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Sodium Methylparaben		Sodium Paraben		Sodium Propylparaben	
Totals*	**414**	**0.000005-0.4**	**NR**	**0.008**	**134**	**0.000015-0.28**
Duration of Use						
Leave-On	*216*	*0.00001-0.4*	*NR*	*0.008*	100	*0.000017-0.28*
Rinse Off	*189*	*0.000005-0.4*	*NR*	*NR*	30	*0.000015-0.1*
Diluted for (Bath) Use	*9*	*NR*	*NR*	*NR*	4	*NR*
Exposure Type						
Eye Area	46	0.000012-0.4	NR	NR	18	0.004-0.28
Incidental Ingestion	NR	NR	NR	NR	NR	0.1
Incidental Inhalation-Spray	2; 46[a]; 79[b]	0.00002; 0.00022-0.3[b]	NR	NR	15[a]; 16[b]	NR
Incidental Inhalation-Powder	79[b]	0.00013; 0.00016-0.3[c]	NR	NR	16[b]	0.0051[c]
Dermal Contact	257	0.000005-0.4	NR	0.008	124	0.0004-0.28
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	72	0.00002-0.4	NR	NR	3	0.000015
Hair-Coloring	75	0.3-0.4	NR	NR	1	0.0051
Nail	NR	0.000046	NR	NR	NR	0.000017
Mucous Membrane	23	0.25	NR	NR	10	0.1
Baby Products	NR	NR	NR	NR	1	NR

Totals=Rinse-off + Leave-on + Diluted for Bath Product Uses.

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

NR=Not Reported

[a] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

[c] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 7. Parabens with no current reported use according to VCRP data (2019) and the Council survey (2016).[2,25,26]

Benzylparaben	Potassium Paraben
Calcium Paraben	Potassium Propylparaben
Potassium Butylparaben	Sodium Isopropylparaben
Potassium Ethylparaben	4-Hydroxybenzoic Acid
Potassium Methylparaben	

Table 8. SCCS/SCCP (Scientific Committee on Consumer Products, predecessor of SCCS) opinions on parabens

Year	Conclusion	Reference
2005	It is the opinion of the SCCP that, viewing the current knowledge, there is no evidence of demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics containing parabens.	[13]
2005	Methylparaben and Ethylparaben can be safely used up to the maximum authorized concentration as actually established (0.4%). The available data do not enable a decisive response to the question of whether propyl, butyl and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%. More information is needed in order to formulate a final statement on the maximum concentration of propyl, isopropyl, butyl and isobutyl paraben allowed in cosmetic products.	[14]
2006	The conclusion of opinion SCCP/0873/05 (i.e., ref.16) remains unchanged.	[15]
2008	As already concluded in earlier opinions, Methyl Paraben and Ethyl Paraben are not subject of concern. The SCCP is of the opinion that, based upon the available data, the safety assessment of Propyl and Butyl Paraben cannot be finalized yet.	[15,16]
2011	The use of Butylparaben and Propylparaben as preservatives in finished cosmetic products as safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19%. With regard to Methylparaben and Ethylparaben, the previous opinion, stating that the use at the maximum authorized concentrations can be considered safe, remains unchanged. Limited to no information was submitted for the safety evaluation of isopropyl- and isobutyl-paraben. Therefore, for these compounds, the human risk cannot be evaluated. The same is true for Benzylparaben.	[17]
2011	For general cosmetic products containing parabens, excluding specific products for the nappy area, the SCCS considers that there is no safety concern in children (any age group) as the MOS was based on very conservative assumptions, both with regards to toxicity and exposure. In the case of children below the age of 6 months, and with respect to parabens present in leave-on cosmetic products designed for application on the nappy area, a risk cannot be excluded in the light of both the immature metabolism and the possibly damaged skin in this area. Based on a worst-case assumption of exposure, safety concerns might be raised. Given the presently available data, it is not possible to perform a realistic quantitative risk assessment for children in the pertinent age group as information on internal exposure in children is lacking. With regard to pregnant women, the unborn fetus will be better protected than the neonate/newborn or early infant exposed dermally to parabens by the more efficient systemic parabens inactivation by the mother.	[18]
2013	The concerns of the SCCP/SCCS expressed previously and reiterated in recent Opinions remain unchanged and reinforced after the evaluation of both the reproductive toxicity and the toxicokinetic studies on Propylparaben recently submitted to the SCCS. The same data were extrapolated for the evaluation of the risk by Butylparaben exposure. The additional submitted data does not remove the concern expressed in the previous opinions on the relevance of the rat model for the risk assessment of parabens. Although much toxicological data on parabens in rodents exists, adequate evidence has not been provided for the safe use of propyl- or Butylparaben in cosmetics. For these reasons, the 22 SCCS reiterates its previous conclusions and requests regarding an improvement of the data, in particular a) on the exposure of humans including children to Propyl- and Butylparaben in cosmetic products and b) the toxicokinetics of Propylparaben and Butylparaben in humans.	[18,19]

Table 9. In vitro dermal penetration studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
Methylparaben	Pig	Skin from the upper half of the ears of 6-month-old pigs	0.1% in aqueous, or hydrogel or emulsion oil-in-water formulations with and without a penetration enhancer (urea, Transcutol or propylene glycol), 0.1%, pH=5.5	Porcine skin used fresh or after storage at 4°C for 18 h or frozen, clamped between donor and receptor chambers of Franz-type diffusion cells	Receptor fluid (phosphate-buffered saline and 0.01% of Gentamicin-sulphate) and skin samples (~3.3 cm^2 discs, intact or tape-stripped 20 times; diffusion area 2 cm^2) maintained at 32°C; nine formulations, representing the most frequently types of MP-containing topical leave-on products, were prepared with a combination of difference concentrations of the following chemicals: aqua, urea, ethoxydiglycol, propylene glycol, olea europaea oil, glyceryl stearate, C12-14 Pareth-3, cetyl alcohol, carbomer, sodium hydroxide, and lactic acid; 20 µL aqueous solution was added to the donor chamber or ~20 mg of hydrogel or emulsion was applied to the skin sample at t=0; 50 µL samples removed from the receptor chamber at intervals for up to 4 h or 24 h (depending on the experiment) for analysis by HPLC and replaced by fresh receptor medium	For freshly excised intact skin and previously frozen intact skin, concentrations of unmetabolized Methylparaben in receptor fluid <LOD-2.3% and 2.3%-3.3% of applied dose, respectively, after 4-h exposure; for previously frozen intact and tape-stripped skin, concentrations of unmetabolized Methylparaben in receptor fluid were 2.0%-5.8% and 2.9%-7.6% respectively, after 24-h exposure; absorption rate was higher from emulsions vs. hydrogels, enhancer-containing formulations vs. enhancer-free formulations, and when skin was tape stripped	[37]
Methylparaben Ethylparaben Propylparaben Butylparaben	Pig	Ears (~1 mm thick) collected from young animals	0.1% in 20%(v/v) or 50% (v/v) ethanol/PBS	Full-thickness porcine skin, stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (20% or 50% ethanol/PBS) and skin samples (diffusion area 1.77 cm^2); system maintained at 37°C; 2 mL solution added to the donor chamber at t=0; 400 µL samples removed from the receptor chamber at intervals for up to 6 h or 7.5 h (depending on the experiment) for analysis by capillary electrophoresis (CE) and replaced by fresh receptor medium	Permeability coefficients (cm/h x 10^{-4}), in descending order: Methylparaben, 214.8 ± 40, Ethylparaben, 197.5 ± 10; Propylparaben, 101.9 ± 15; Butylparaben 31.3 ± 1.6; skin penetration was inversely proportional to lipophilicity; Increasing ethanol concentration in the vehicle and exposure duration increased parabens retention in dermis compared epidermis; Binary combinations of the parabens reduced their permeation rates, attributed by the authors to high retention in the epidermis and dermis	[38]
Methylparaben Ethylparaben Propylparaben	Rabbit (mixed breed)	Skin excised from ears of 6-month-old animals	3 commercial facial moisturizing creams containing 0.23%-0.32% (w/w) Methylparaben, 0%-0.1% Ethylparaben, and 0.04%-0.19% Propylparaben.	Full-thickness skin, stored frozen, thawed and mounted on Franz-type diffusion cells	Receptor fluid (saline) and skin samples (diffusion area 0.6 cm^2); Donor chamber filled with 2 mg/cm^2 cream at t=0; 300 µL samples removed from the receptor chamber at intervals for up to 8 h for analysis by HPLC and replaced by fresh receptor medium	Percentage of applied dose in receptor fluid after 8 h exposure, in descending order: Methylparaben, 60%; Ethylparaben, 40%; Propylparaben, 20% of PP – penetration decreased with decreasing water solubility, regardless of the formulation tested; Retention varied widely in the epidermis (14.0-253.0 µg/g) and dermis (0-19.3 µg/g), depending on the formulation	[39]
Methylparaben Propylparaben Butylparaben	Human Mouse (hairless)	Human cadaver epidermis (commercially available) Skin from 8-week-old male mice	0.1%, 0.4%, and 2% in a general oil-in-water cream formulation	Human epidermis (~0.03 mm thick) and mouse skin (~0.25 mm thick), stored frozen, thawed and	Receptor fluid (1:1 ethanol/water, v/v) and skin samples (diffusion area 0.785 cm^2) maintained at 32°C; 10 mg cream applied to the skin surface at t=0; 1 mL samples removed from the receptor chamber at intervals for up to 24 h for analysis by LC-	Permeability coefficients (K_ps; cm/h x 10^{-4}) were similar regardless of concentration tested; K_ps were directly related to paraben concentration K_ps for human skin ranged from 0.74 ± 0.19 to 0.91 ± 0.44 for Methylparaben, 0.54 ± 0.14 to	[40]

Table 9. In vitro dermal penetration studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
				mounted on Franz diffusion cells	MS/MS and replaced by fresh receptor medium	0.91 ± 0.22 for Propylparaben, and 0.37 ± 0.15 to 0.56 ± 0.32 for Butylparaben K_ps for mouse skin ranged from 1.41 ± 0.12 to 1.66 ± 0.21 for Methylparaben, 1.52 ± 0.13 to 1.76 ± 0.39 for Propylparaben, and 1.17 ± 0.15 to 1.27 ± 0.20 for Butylparaben Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin; Residual quantities in human epidermis (µg/ml x 10^{-4}): Methylparaben, 235 ± 132 to 7198 ± 4662; Propylparaben, 375 ± 212 to 4120 ± 2344; Butyl paraben, 436 ± 226 to 5480 ± 2593; Residual quantities in mouse skin: Methylparaben, 14 ± 5 to 286 ± 104; Propylparaben, 21 ± 9 to 410 ± 112; Butyl paraben, 15 ± 2 to 358 ± 118 Authors state results show that parabens may be classified as moderate penetrants	
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	Abdominal skin samples collected during surgery from 8 women	Commercial body lotion containing 0.1% (w/w) Methylparaben, 0.08% Ethylparaben, 0.2% Propylparaben, and 0.15% Butylparaben.	Human skin samples, stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (3% bovine serum albumin in isotonic saline solution) and skin samples (diffusion area 3.14 cm^2) maintained at 32°C; single 100 µL (45 mg) lotion applied to skin surface at t=0, which was repeated for some skin samples at t=12 h and t=24 h; fluid was removed from the receptor chamber at intervals for up to 36 h for analysis by HPLC and replaced by fresh receptor medium	Penetration was inversely proportional to lipophilicity of parabens tested, and increased with repeated applications; penetration 36 h after single application (percentage of applied dose): Methylparaben, 0.057% ± 0.03; Ethylparaben, 0.045% ± 0.01; Propylparaben, 0.028% ± 0.01; Butylparaben, 0.007% ± 0.003; Penetration 12 h after last of 3 repeated applications: Methylparaben, 0.6 ± ± 0.1%; Ethylparaben, 0.3% ± 0.1; Propylparaben, 0.2% ± 0.05; Butylparaben, 0.04% ± 0.01	[41]

CE=Capillary electrophoresis; HPLC=High-performance liquid chromatography; LOD=Level of detection; PBS=Phosphate buffered saline

Table 10. Toxicokinetic Studies-Absorption, Distribution, Metabolism, Excretion (ADME)

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
In Vitro						
Methylparaben Ethylparaben Propylparaben Benzylparaben	Rat (strain not specified)	AFP in rat amniotic fluid	Five to 6 concentrations between 10^{-9} M and 10^{-4} M	Competitive binding to AFP in rat amniotic fluid assayed against 2,4,5,7-[^{3}H]-estrone, with assay tubes containing no "cold" radio-inert test competitor provided the 100% binding level, and 1.5 x 10^{-6} M "cold" competitor maximally competed with 10^{-6} M 2,4,5,7-[^{3}H]-estrone; radioactivity remaining above this standard was considered nonspecific and was subtracted from assay measurements to estimate specific binding	The concentration of Benzylparaben inhibiting the binding of 2,4,5,7-[^{3}H]-estrone to AFP by 50% (IC_{50}) was 0.012 µM; AFP did not exhibit binding affinity for Methylparaben, Ethylparaben, and Propylparaben	47
Butylparaben	Rat (Wistar)	S9 fraction of 5-week old males (n not specified)	Twelve concentrations between about 5 µM and 90 µM	Reactions performed in PBS, pH 7.4, at 37°C in shaking water bath and stopped by adding ice-cold methanol; supernatant was separated by HPLC and formation of *4-Hydoxybenzoic Acid* metabolite was monitored using UV detector at 254 nm; Michaelis-Menten parameters were estimated by Lineweaver-Burk plot (no further details provided)	Butylparaben was biotransformed to *4-Hydoxybenzoic Acid* in the reaction mix with the maximum rate achieved by the system, at saturating substrate concentration (V_{max})=8.8 nmol/min/mg protein and the substrate concentration at which the reaction rate is half of V_{max} (Km)=28.6 mM	48
Butylparaben	Human Rat (Harlan Sprague-Dawley)	Hepatocytes from human subjects (59-year-old woman an 45-year-old man, both non-smokers) and 8 to 12 week old male and female rats	1 µM radiolabeled Butylparaben (phenyl ring-^{14}C(U) – 53.1 mCi/mmol); 10 µM radiolabeled Butylparaben in metabolism studies	The plates were then pre-incubated for 5 min at 37°C and Butylparaben added in acetonitrile (<0.5% final concentration) at t=0; 50 µL aliquots were collected at t=300 min for metabolism studies and at intervals up to t= 300 min for clearance studies for LC-MS/MS analysis	Butylparaben was rapidly cleared in hepatocytes from rats, with little or no sex difference ($t_{1/2}$=3.8 ± 0.3 min and 3.3 ± 0.1 min for hepatocytes from males and females, respectively, corresponding to Cl_{int}=811 ± 53 and 903 ± 28 mL/min/kg); Butylparaben was cleared more slowly in hepatocytes from humans but, again, there was no sex difference ($t_{1/2}$=23.9 ± 1.3 min and 29.6 ± 5.2 min, respectively, corresponding to Cl_{int}=92 ± 5 and 111 ± 22 mL/min/kg); Butylparaben was extensively hydrolyzed to *4-Hydoxybenzoic Acid* as the major metabolite for both sexes and species (92% to 100% in rat, 78% to 84% in human) after 5 h of incubation. The other metabolite observed in human hepatocytes was 4-hydroxyhippuric acid (16% to 22%)	52
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (Sprague-Dawley) Monkey (African green)	Pooled human liver and small intestine microsomes available commercially Rat liver, skin, kidney, pancreas, and small intestine microsomes and blood plasma S9 from COS cells (Monkey-kidney derived, fibroblast like)	100 nmol paraben and tissue microsomes or plasma in final volume of 1 mL 0.1 M K, Na-phosphate buffer (pH 7.4)	Incubation was for 7 min at 37°C, then 10 mg 2,4-dihydroxybenzophenone (internal standard) and 1 mL acetonitrile added; aliquot of the supernatant was collected for analysis of paraben hydrolase activity by HPLC Carboxylesterase activity was determined by measuring deacetylase activities toward 4-nitrophenol acetate and 4-methylumbelliferyl acetate: 4-nitrophenol acetate deacetylase activity measured by spectrophotometry at 405 nm; 4-methylumbelliferyl acetate deacetylase activity measured by fluorophotometry at 329 nm (excitation) and 448 nm (emission)	Rat liver microsomes (RLM) showed the highest activity toward parabens, followed by small-intestinal and lung microsomes; Butylparaben was most effectively hydrolyzed by the RLM, which showed relatively low hydrolytic activity towards parabens with shorter and longer alkyl side chains; In contrast, rat small-intestinal microsomes exhibited relatively higher activity toward longer-side-chain parabens; Rat lung and skin microsomes showed liver-type substrate specificity; Kidney and pancreas microsomes and plasma of rats showed small-intestinal-type substrate specificity; Rat small-intestinal microsomes exhibited higher activity toward longer-side-chain parabens – carboxylase 2 showed a similar activity pattern;	50

Table 10. Toxicokinetic Studies-Absorption, Distribution, Metabolism, Excretion (ADME)

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					In contrast, human liver microsomes showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length; human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Human	Human liver microsomes (pooled from 21 men and women) Blood plasma (pooled from nine 25 to 35 year old men)	164 µM paraben (dissolved in DMSO)	Biotransformation of parabens to yield 4-hydroxybenzoic acid metabolite studied at 37°C in 67 mM PBS (pH 7.4), human plasma, 580 mM albumin solution in phosphate buffer (pH 7.4), and human liver microsomes (100 mg) in 100 mM Tris-HCl buffer (pH 7.4) Glucuronidation of parabens and 4-hydroxybenzoic acid by human liver microsomes and recombinant UDP-glucuronosyltransfeases (UGT) was performed by a modified of the method of Bansal and Gessner (1980)	Methylparaben and Ethylparaben were stable in human plasma, with 95% of the initial concentration remaining after 24-h incubation; Propylparaben, Butylparaben and Benzylparaben concentrations decreased by 50% within 24 h; All parabens tested were rapidly hydrolyzed when incubated with human liver microsomes, depending on the alkyl chain length ($t_{1/2}$=22 min for Methylparaben and 87 min for Butylparaben; Parabens (but not 4-hydroxybenzoic acid) were actively glucuronidated by liver microsomes and mainly by human recombinant UGT1A1, UGT1A8, UGT1A9, UGT2B7, UGT2B15 and UGT2B17	49
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (strain not specified)	HLM, HSM, HLC, and HSC RLM, RSM, RLC, and RSC	100 µM in 50 mM potassium phosphate, pH 7.4	Reactions were initiated with the addition of 100 µM paraben; mixture incubated for 30 min at 37°C; 4-Hydoxybenzoic Acid formation measured by HPLC-analysis of supernatants	Hydrolysis of parabens by HLM was about 10-fold more rapid than by HLC; Metabolism rates were inversely proportional to chain length (the longer the alcohol moiety, the slower the hydrolysis); this trend was also observed for HSM and HSC, but at much lower rates of hydrolysis; Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the ester compared; Paraben hydrolysis rates in rat liver and skin were greater than in human liver and skin; RLM and RSM metabolized parabens 7-fold and 5-fold faster than RLC and RSC, respectively; In contrast to human tissue fractions, hydrolysis rates of the parabens increased as the ester chain length increased in rat tissue. Methylparaben and Propylparaben was the preferred substrate for human tissue fractions and rat tissue fractions, respectively; Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin	51
ANIMAL						
Dermal						
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	n=9/sex/group for the toxicokinetics study and n=3/sex/group for the mass balance study	Single 100 mg/kg bw dosage of radiolabeled (ring-U-^{14}C) paraben, in 60% aqueous ethanol vehicle, applied to the skin	Isotopic mixtures were applied to the interscapular/back region (on an area equivalent to approximately 10% of the total body surface) over a 6-h period; hair at the administration site was clipped before application;	For all 3 parabens, C_{max} (≥693 and ≥614 ng eq/g in males and female, respectively) occurred within 8 h post-gavage, and blood concentrations decreased until the last quantifiable concentration within 24 h; Most of the dosage (≥46.4%) as unabsorbed and recovered in the swabs used for cleaning of the	53

Table 10. Toxicokinetic Studies-Absorption, Distribution, Metabolism, Excretion (ADME)

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				animals wore an Elizabethan collar during the 6-h exposure period Blood samples were taken from the retro-orbital sinus of the toxicokinetic animals pre-dose and then at 0.5, 1, 2, 4, 8, 12, 22, and 24 h after oral dosing; 3 rats/sex/group were sampled each time; Animals were killed after the last sampling; Blood, excreta were collected from all mass balance animals pre-dose and then after the periods 0–6, 6–24, 24–48, 48, 72–96, 96–120, 120–144 and 144–168 h after oral dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection Organs were collected, weighed, and analyzed for radioactivity..	application site at the end of the exposure period; ≤25.8% of the applied radioactivity was found in the urine; urinary excretion was the main route of elimination; radioactivity was eliminated rapidly in the urine with averages ≥11.9% recovered in the first 48 h; ≤0.16 % of the radioactive dose of Methylparaben was found in the skin strips and biopsies from the treated sites after necropsy; for all of the parabens tested, a large part of the radioactivity (≥20.7%) was retained in the carcasses; Metabolic profiling of pooled plasma collected 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of *4-Hydoxybenzoic Acid*	
Butylparaben	Rat (Harlan Sprague-Dawley)	8- to 10-week old males, n=4	Single 10 or 100 mg/kg dosage of radiolabeled Butylparaben [phenyl ring-U-^{14}C] – 53.1 mCi/mmol; 50 µCi dose/animal) in 95% ethanol, applied to the skin	Single dermal dosages (0.5 mL/kg bw) were applied onto a 4 cm^2 (2 cm × 2 cm) area of shaved skin on the backs of the rats; a protective foam appliance was glued onto the skin using medical adhesive, the doses were administered evenly to the dose area, and a non-occlusive cloth cover was attached over the appliance Urine and feces of rats were collected separately for up to 72 h post-exposure; the animals were then killed, blood was collected and the tissues were excised and weighed. The protective appliance was removed, dose-site skin was excised and washed with a series of water-wetted gauzes and appliance.	Absorption of 10 mg/kg and 100 mg/kg Butylparaben 72 h following application was about 52% and 8%, respectively; total absorbed dosage was comparable (5.2 mg and 8 mg for 10 and 100 mg/kg, respectively); authors stated that nonlinearity with increasing dosage indicates saturation of the capacity for dermal absorption; About 21% of the 10 mg/kg dosage remained unabsorbed; about 16% was recovered in the dose-site skin; About 3% and 8% of the 100 mg/kg dosage was absorbed at 24 h and 72 h, respectively; the amount recovered in the dose-site skin increased from 19% at 24 h to 43% at 72 h; Urine was the primary route of elimination, with about 46% of 10 mg/kg recovered in urine and in cage rinse at 72 h; fecal elimination of radioactivity accounted for 1.7%; Tissues contained about 4.3% of the 10 mg/kg dosage; highest concentrations of radiolabel were in bladder, liver and kidney, which contained about twice the concentration of residues found in liver	[52]
			Oral			
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	n=9/sex/group for the toxicokinetics study and n=3/sex/group for the mass balance study	Single 100 mg/kg bw dosage of radiolabeled (ring-U-[^{14}C]) paraben, in 60% aqueous ethanol vehicle, administered by gavage	Blood samples were taken from the retro-orbital sinus of the toxicokinetic animals pre-dose and then at 0.5, 1, 2, 4, 8, 12, 22, and 24 h after oral dosing; 3 rats/sex/group were sampled each time; Rats were killed after the last sampling; Blood, excreta were collected from all mass balance rats pre-dose and then after the periods 0–6, 6–24, 24–48, 48,	For all 3 parabens, C_{max} (≥11432 and ≥21040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h; Mean total cumulative excretion (urine, feces and cage wash) of the administered radioactive dose over a 168-h collection period was complete	[53]

Table 10. Toxicokinetic Studies-Absorption, Distribution, Metabolism, Excretion (ADME)

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				72–96, 96–120, 120–144, and 144–168 h after oral dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection. Organs were collected, weighed, and analyzed for radioactivity.	and amounted to ≥89%; most of the administered dose (≥71%) was eliminated in urine, while ≤3.3% was eliminated in the feces; radioactivity was eliminated rapidly with averages ≥69.6% recovered in the urine during the first 24 h; A small amount of radioactivity (<0.1%) was observed in the collected tissues, and the levels of radioactivity were below the LOQ in the carcasses of most animals; Metabolic profiling of pooled plasma collected at 0.5, 1, 2, 4, and 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of *4-Hydoxybenzoic Acid*	
Butylparaben	Rat (Harlan Sprague-Dawley)	8 to 10 week old males, n=4	Single 10, 100, or 1000 mg/kg dosage of Butylparaben with radiolabeled Butylparaben (phenyl ring-U-[^{14}C]) – 53.1 mCi/mmol; 50 µCi dose/animal) in Cremophor EL, administered by gavage	Urine and feces of rats were collected separately for up to 72 h post-exposure; the animals were then euthanized, blood was collected via cardiac, and the following tissues were excised and weighed: liver, kidney, brain, muscle (hind leg), abdominal skin, adipose (perirenal), spleen, heart, lung, ovaries, uterus, and testes samples were analyzed by liquid scintillation spectroscopy for radioactivity and by HPLC for parabens and potential metabolites (4-hydroxybenzoic acid, HHA, n-butyl-3,4-dihydroxybenzoate, 3,4-dihydroxybenzoic acid, and 3,4-dihydroxybenzoic acid)	Radioactivity was predominantly excreted in urine; rate of urinary excretion was similar across all dosages, with ≥66% recovered in the first 24 h in males, for example; in 72 h, around 80% was recovered in urine and 3% to 6% in feces; Total radioactivity in tissues was low (0.02% - 1.25%) in males at all dosages, decreasing with increasing dosage; Female rats excreted more Butylparaben in urine in the first 4 h after exposure, but there was no sex difference in the total dosage excreted within 24 h. In general, tissue levels at 24 h were considerably higher in female rats; Highest levels in non-gastrointestinal tract tissues were found in kidney and liver, followed by ovaries and uterus; Comparing the disposition Butylparaben in males rats at 24 h with that at 72 h revealed that blood and plasma concentrations dropped about 50% or more levels in tissues such as adipose, muscle and kidney remained unchanged, and levels in liver and skin increased by 44% and 36%, respectively during that interval; Metabolites detected in urine included Butylparaben-glucuronide, Butylparaben-sulfate, hydroxybenzoic acid, hydroxyhippuric acid, and newly discovered metabolites arising from ring hydroxylation followed by glucuronidation and sulfation	[52]
			HUMAN			
			Dermal			
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean=26 years old), n=26	2% (w/w) Butylparaben in cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate	In a 2-week single-blinded study, male subjects were given a whole-body topical application of basic cream 2 mg/cm^2 (control week) and then a cream containing 2% (w/w) of diethyl phthalate (DEP), dibutyl phthalate (DBP) and Butylparaben each (treatment week) daily;	All 26 subjects showed increased excretion of Butylparaben following topical application; Mean total Butylparaben excreted in urine during treatment was 2.6 ± 0.1 mg/24 h; on average, 0.32% of the applied dose was recovered in urine as	[55]

Table 10. Toxicokinetic Studies-Absorption, Distribution, Metabolism, Excretion (ADME)

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				24-h urine samples were collected and analyzed for total and unconjugated Butylparaben by LC-MS/MS	Butylparaben; the concentration peaked in urine 8-12 h after application; on average, 1.5% and 2.1% Butylparaben was excreted as free Butylparaben in urine during the control and treatment week, respectively	
				Oral		
Methylparaben Butylparaben Isobutylparaben	Human	Healthy 31-year old volunteers, n=3 (1 woman and 2 men)	10 mg deuterated (D4-ring-labeled) paraben/dose, dissolved in ethanol and added to a cup of breakfast coffee or tea	Each subject ingested a dose of each paraben, a different paraben each time, with at least 2 weeks between exposures; the first urine samples were collected before exposure and then at 4 13-h intervals for 48 h after exposure for HPLC analysis; ring-deuterated standards included ethyl 4-hydroxybenzoate-2,3,5,6-d4, iso-butyl 4-hydroxybenzoate-2,3,5,6-d4, n-butyl 4-hydroxybenzoate-2,3,5,6-d4, and 4-hydroxybenzoic-2,3,5,6-d4 acid	Free and conjugated parabens and their known, non-specific metabolites, *4-Hydoxybenzoic Acid* and *p*-hydroxyhippuric acid, were detected in the urine samples; new oxidized metabolites with hydroxy groups on the alkyl side chain (3OH-n-butylparaben and 2OH-iso-butylparaben) and species with oxidative modifications on the aromatic ring were discovered; 17.4 %, 6.8 %, 5.6% of the doses of Methylparaben, Isobutylparaben and Butylparaben, respectively, were excreted in the urine; about 16% and 6% of Isobutylparaben and Butylparaben were excreted as 2OH-iso-butylparaben and 3OH-n-butylparaben, respectively; less than 1% was excreted as ring-hydroxylated metabolites; For all parabens tested, 4-Hydoxybenzoic Acid was the major metabolite (57.2% - 63.8%) and urinary *p*-hydroxyhippuric acid ranged from 3.0% - 7.2% of the doses; 80.5% - 85.3% of the doses were excreted as the metabolites detected in this study within 24 h after exposure	[56]

AFP=α-Fetoprotein; Cl_{int}=intrinsic clearance; DMSO=Dimethyl sulfoxide; ESI=Electrospray ionization; GM: geometric mean; HHA=4-hydroxyhippuric acid; HLC=Human liver cytosol; HLM=human liver microsomes; HPLC=High-performance liquid chromatography; HSC=Human skin cytosol; HSM=Human skin microsomes; LC=Liquid chromatography; LOQ=Limit of quantification; MS/MS=Tandem Mass Spectrometry; PBS=Phosphate buffered saline; RLC=Rat liver cytosol; RLM=Rat liver microsomes; RSM=Rat skin microsomes; RSC=Rat skin cytosol; SRM=Selected reaction monitoring; UDP=Uridine 5'-diphospho; UGT-UDP=glucuronosyltransferase

Table 11. Subcutaneous Studies

Ingredient	Animals/Group	Dose/Procedure	Results	Reference
		ACUTE STUDIES		
Isobutylparaben	Mice (# of animals not stated)	NR	LD_{50} was reported to be 2.6 g/kg	[187]
Methylparaben	C57BL/6 Mice (8/group)	125 mg/kg Methylparaben in tricaprylin	Injection sites in the majority of animals developed small, ill-defined soft cysts and small ulcerations that later healed.	[188]
Methylparaben	Mice (# of animals not stated)	up to 333 mg/kg	Doses greater than 165 mg/kg temporarily induced exhaustion, ataxia, and respiratory distress. The acute lethal subcutaneous dose was reported to be greater than 333 mg/kg.	[189]
Methylparaben	Fischer Rats (20/group)	up to 500 mg/kg	No deaths occurred. LD_{50} was reported to be greater than 500 mg/kg.	[190]

Table 11. Subcutaneous Studies

Ingredient	Animals/Group	Dose/Procedure	Results	Reference
Methylparaben, Ethylparaben, Propylparaben, and Butylparaben	Mice (5/group)	NR	The reported LD_{50} values were 1.2, 1.65, 1.65, and 2.5 g/kg, respectively.	[191]
CHRONIC STUDIES				
Methylparaben	Fischer Rats (groups of 80, 60, 40, and 20 animals)	0.6. 1.1, 2, and 3.5 mg/kg administered twice weekly for 52 weeks	Paraben-treated rats had no significant differences in mortality, weight gain, or lesions compared to control rats.	[190]
DART				
Isobutylparaben	Sprague-Dawley Rats (# of animals not stated)	NR	Decrease of plasma corticosterone concentration and increased uterus weight in dams as well as uterine sensitivity to estrogen in adult female offspring was noted.	[17]
Ethylparaben and Butylparaben	Wistar Rats (15/group)	400 mg/kg d Ethylparaben; 200 or 400 mg/kg/d Butylparaben on GD 7-21	No treatment-related effects on testosterone production, anogenital distance, or testicular histopathology. Butylparaben decreased ERβ mRNA expression in fetal ovaries, and mRNA expression of steroidogenic acute regulatory protein and peripheral benzodiazepine receptor in adrenal glands. These effects were not dose-dependent.	[157]
Propylparaben and Butylparaben	Mice (# of animals not stated)	up to 950 mg/kg/d	No effect on number of pups born, litter weights, individual pup weight, or pup survival.	[17]
Methylparaben Ethylparaben Propylparaben Butylparaben Isopropylparaben Isobutylparaben	Female rats (# of animals not stated)	up to 1000 mg/kg/d	At the highest dose level, each of the tested parabens induced or more of the following effects: decreased ovary/kidney weight, increased thyroid gland/adrenal weight, reduced serum estradiol levels, decrease of corporeal lutea, increase in number of cystic follicles, and myometrial hypertrophy. No dose-dependent effects at lower levels.	[17]

NR = Not Reported; GD = Gestation Day

Table 12. Short-Term Toxicity Studies

Test Substance(s)	Species/ Strain	Test Group	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
Animal							
Dermal							
Isopropylparaben Isobutylparaben	Rat (Sprague-Dawley)	5-week old males and females, n=10/sex/ group, 13 groups	50, 100, 300, or 600 mg/kg bw/day Isopropylparaben, Isobutylparaben, or 100, 200, 600 and 1200 mg/kg bw/day of a 1:1 mixture of Isopropylparaben and Isobutylparaben, in 99% ethanol	28 days	Protocol followed current OECD TG 410 for short-term repeated dermal exposure studies; test material was topically applied to shaved dorsal skin and covered with a porous gauze dressing and non-irritating tape, 5 days/week; 8 hematological parameters were evaluated; brains, hearts, kidneys, the large lobe of livers, and sectioned dorsal skin were harvested for histological evaluation; hormone concentrations were measured by ELISA, including concentrations of T3, FSH, estradiol, insulin, T, and TSH	There were no significant changes in body and organ weights in any group; macroscopic and microscopic histopathological examinations revealed mild-to-moderate skin damage in female rats; NOAELs for Isobutylparaben and Isopropylparaben were 600 mg/kg bw/day, and 50 mg/kg bw/day, respectively; a LOAEL for hyperkeratosis of 50 mg/kg bw/day was estimated for the mixture; The relative weight of heart and kidneys increased in a dose dependent manner in male rats treated by paraben mixture; The relative weight of testes showed significant increase in males treated by Isobutylparaben and Isopropylparaben at 600 mg/kg bw/day; Analysis of serum concentrations showed that FSH was dose-dependently decreased in animals treated with ≥200 mg/kg bw/day of the mixture (i.e. ≥100 mg/kg bw/day each of Isopropylparaben and Isobutylparaben combined); No significant change of serum T3, TSH, insulin, E2, or testosterone concentrations in female rats treated by parabens	[58]
Oral							
Propylparaben	Rat (Wistar)	Adult males, n=8/group, 3 groups	100 or 300 mg/kg bw/day, suspended in a few drops of Tween-80 (stock solution) and diluted in distilled water (vehicle)	4 weeks	At the end of the treatment period, blood was collected from the abdominal aorta, liver, kidneys, heart and testes were excised, organ to total body weight ratio was calculated, right lobe of the liver and the left testis were fixed for histological examination and homogenates of the remaining liver and testis were prepared ALT, AST, ALP, and LDH activities were analyzed using ELISA; TP, Alb and creatinine concentrations were measured using commercial assay kits; reduced GSH, lipid peroxides (as MDA) and total NO were determined in liver and testis homogenates by the colorimetric methods and CAT and SOD activities were determined; Serum free T and E2 concentrations were measured by ELISA	Statistically-significant effects included dose-dependent increase in relative liver weights, increases in serum ALT, AST, ALP and LDH activities, and reduced total serum protein and albumin (at both dosage rates) and serum globulin (at 300 mg/kg bw/day) concentrations; Serum urea concentrations and urea/creatinine ratios were statistically-significantly increased (at both dosage rates), as was the serum creatinine concentration (at 300 mg/kg bw/day); Statistically-significant decrease in GSH, CAT and SOD activities, and increase of lipid peroxidation and NO generation (at both dosage rates); Statistically-significant dose-dependent reduction in serum testosterone concentration and T/E2 ratio, and elevation in serum E2;	[59]

Table 12. Short-Term Toxicity Studies

Test Substance(s)	Species/ Strain	Test Group	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
						Livers exhibited presence of dilated congested central and portal veins, focal areas of dilated sinusoids, highly proliferated bile ducts with fibrotic reactions around them, expanded portal areas with edema, multifocal areas of necrotic hepatocytes with inflammatory cells infiltration and severe cytoplasmic vacuolization of hepatocytes (at both dosage rates); Testes exhibited evidence of severe spermatogenic arrest, seminiferous tubules occupied with ill-defined eosinophilic mass structure and giant cells in the lumen, detached spermatogenic lineage, edematous eosinophilic interstitial space with congested blood vessels and a mild loss of Leydig cells population	
Methylparaben	Rats (Wistar)	Females (146 ± 10 g bw), n=10/group	250 mg/kg bw/day, administered in the diet	10 days	Blood samples were collected from the retro-orbital sinuses of the animals on the 10th day of the experiment; plasma was analyzed for total MDA concentrations by HPLC and for 2,3-DHBA by LC-MS/MS	Serum MDA (lipid-peroxidase end-product) and 2,3-DHBA (marker of in vivo hydroxyl radical production) concentrations were statistically-significantly elevated compared with controls ($p<0.01$)	[60]
Butylparaben	Mouse (albino Swiss)	Adult female, n=50, n=10/group, 5 groups	13.33, 20 and 40 mg/kg bw/day, in olive oil by gavage	30 days	Animals were killed on 31st day by cervical dislocation, the liver was excised, a liver sample was homogenized and analyzed for MDA, catalase, GSH, GST, protein, TAA, SOD, GPx, and GR content; Lipid peroxidation in the liver tissue was measured by estimating MDA	All three dosage rates elevated MDA levels in the liver in a statistically-significant ($p < 0.05$), dose-dependent manner TAA levels were reduced by 11.34%, 27.03%, and 41.02% at 13.33, 20 and 40 mg/kg bw/day ($p < 0.05$), respectively; GSH levels were reduced by 22.22%, 44.53% and 55.74% at 13.33, 20 and 40 mg/kg bw/day ($p < 0.05$), respectively; Statistically-significant ($p < 0.05$), dose-dependent reductions in SOD, CAT, GPx, GR, and GST levels were noted in Butylparaben treated mice at all doses	[61]

2,3-DHBA=2,3-dihydroxybenzoic acid; Alb=Albumin; ALP=Alkaline phosphatase; ALT=Serum alanine aminotransferase; AST=Aspartate aminotransferase; BSP=Bromosulfophthalein; ELISA=Enzyme-linked immunosorbent assay; CAT=Catalase; E2=17-ß estradiol; FSH=Follicle-stimulating hormone; GR=Glutathione reductase; GPx=Glutathione peroxidase; GSH=Glutathione; GST=Glutathione transferase; HPLC=High-performance liquid chromatography; ICG=Indocyanine Green; LC-MS/MS=Liquid chromatography-mass spectrometry/mass spectrometry; LDH=Lactate dehydrogenase; LOAEL=Lowest observed adverse effect level; MDA=Malondialdehyde; NO=Nitric oxide; NOAEC=No Observed Adverse Effect Concentration; NOEC=No Observed Effect Concentration; NOAEL=No Observed Adverse Effect Level; OECD TG=Organisation for Economic Co-operation and Development Test Guidelines; SAP=Serum alkaline phosphatase; SOD=Superoxide dismutase; T=Testosterone; T3=Triiodothyronine; TAA=Total ascorbic acid; TP=Total protein; TSH=thyroid-stimulating hormone

Table 13. Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
				Oral		
Butylparaben	Rat (Wistar)	Young adult, pregnant females, n=18/group	0, 10, 100, or 500 mg/kg bw/day in corn oil, by gavage	Dams were dosed once daily from GD7 to the day before expected birth (GD21) and again after birth from PND1 to PND22; one female and one male pup per litter were sacrificed at PD 80–90	Statistically-significant, dose-dependent reductions in anogenital distance in male and female neonates and ovary weight in prepubertal females was noted at 100 and 500 mg/kg bw/day; Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adults were statistically-significantly reduced at all dosage rates from 10 mg/kg bw/day; Testicular CYP19a1 (aromatase) expression was reduced in prepubertal males, but not in adults, at all dosage rates; Prostate histology was altered (reduced epithelial area and the ratio between epithelium and lumen; increased incidence of large acini with cuboidal epithelium) in prepubertal rats at 100 mg/kg bw/day; reduced prostate weight was observed at PND 90 at 500 mg/kg bw/day; Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day; In male offspring, reduction of epididymal sperm count to 76–78% of controls at all doses from 10 mg/kg/day, but same effect size at all doses (no dose-response relationship was observed); No examination of sperm motility; In female offspring, ovary weights were reduced at PND 17, and the effect was statistically significant at 100 and 500 mg/kg bw/day; while at PD 22, ovary weights were slightly higher compared with controls, but not significant; At PND 22, female mammary glands showed a significantly higher number of terminal end buds from 100 mg/kg bw/day, the distance between mammary tissue and lymph node was significantly reduced; No clear effect was seen on mammary glands of adult female offspring;	[62]
Butylparaben	Rat (Wistar)	Pregnant females, n=7 or 8/group, 5 groups	0, 64, 160, 400 and 1000 mg/kg bw/day in corn oil, by gavage	Dams were dosed daily from GD7 to PND21; One male pup from each litter was randomly selected to be sacrificed on PND 21, 35, 49, 90 and 180, respectively.	The body weights on PND 21, 35, and 49 were decreased, with significant differences consistently in 400 and 1000 mg/kg bw/day groups; Weights of the testes in the male offspring were statistically significantly-reduced on PNDs 21 to 90 in the 400 and 1000 mg/kg bw/day groups, weights of the epididymides in these groups were statistically-significantly reduced at all monitoring intervals except PND35, and seminal vesicle weights were reduced on PND21 but increased by PND35; Histologically, the 0 and 160 mg/kg/day dose groups displayed intact basement membranes and clearly structured seminiferous tubules on PND21; in contrast, the 400 and 1000 mg/kg/day dose groups demonstrated reduced and loosely arranged germ cells, and the layers of seminiferous tubules were also reduced; no obvious changes in the Leydig cells in the Butylparaben treatment group, compared with the control group; On PND 90, the number of the caudal epididymal sperm in the offspring was significantly decreased by approximately 36% at 400 and 1000 mg/kg/day ($p < 0.01$), and the daily sperm production values at 1000 mg/kg/day had significantly declined by approximately 55%, compared with those of the control group; Sperm motility was not examined; Butylparaben reduced epididymal cauda sperm counts and daily sperm production in a dose-dependent manner at 400 and 1000 mg/kg bw/day Serum T concentrations were statistically-significantly decreased in males of the 400 and/or 1000 mg/kg bw/day groups, especially on PND49 (>50% decrease in the 1000 mg/kg bw/day group); E2 concentrations were statistically-significantly elevated in males of the 400 and/or 1000 mg/kg bw/day groups, except on PND 180; Serum LH and FSH concentrations in the Butylparaben treated groups were lower on PNDs 21, 35 and 49 but elevated on PND90, compared to controls;	[63]

Table 13. Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
					The results suggested a NOAEL of 160 mg/kg bw/day for Butylparaben for male reproduction and development toxicity	
Butylparaben	Rat (Wistar)	19-21 days old males, n=6/group, 4 groups	50 mg/kg in corn oil, by oral administration	The Butylparaben treatment carried out daily for consecutive 8 weeks; at the end of the treatment period, animals were fasted overnight and then sacrificed	Butylparaben treatment did not alter relative weights of right testis, left testis and cauda, compared to the control group; Butylparaben treatment caused significant elevation in the E2 level, while serum levels of the hormones T, LH, and FSH, as well as ratios of T/E2 and T/LH was decreased; Butylparaben treatment elevated markers of testicular DNA damage in comet assay, including the increase in the tail DNA%, tail length of DNA, and tail moment; The testicular malondialdehyde level was significantly elevated, along with a significant decrease in superoxide dismutase enzyme activity; Histopathological examination showed a reduction in Leydig cells population along with pathological alterations of dilated congested subcapsular blood vessels and the dilation and congestion of interstitial vasculature	[64]
Butylparaben	Rat (Wistar)	Pregnant females, n=7 or 8/group, 5 groups	0, 64, 160, 400, and 1000 mg/kg bw/day in corn oil, by gavage	Dams were dosed daily from GD7 to PND21; One male pup from each litter was randomly selected to be Euthanized; blood and organ samples (e.g., testes, the epididymis and seminal vesicles) were collected on PND 21 and 90	Average body weight of male offspring of the 1000 mg/kg bw/day group was statistically-significantly reduced on PND21 and PND90 ($p< 0.05$); Serum testosterone concentrations were statistically-significantly reduced on PND21 and PND90 ($p< 0.05$) in males of the 1000 mg/kg bw/day group and on PND21 in the 400 mg/kg bw/day group (36% reduction in the 1000 mg/kg bw/day group); Serum E2 concentrations in males of the 400 and 1000 bw/day groups on PND21, and the 1000 mg/kg bw/day group on PND90, were statistically-significantly ($p< 0.01$) higher than the control concentrations (up to 58% elevated on PND21); The expression of StAR, P450scc, SULT1E1, and AR in the testes was statistically-significantly reduced, at both the transcript and protein level, in males of the 400 and/or 1000 mg/kg bw/day groups; CYP19 and ERα expression was statistically-significantly increased and the methylation rate of the ERα promoter was statistically-significantly decreased in males of the 400 and/or 1000 mg/kg bw/day groups	[65]
Butylparaben	Rat (Sprague-Dawley)	3-week old males, n=8	Single 1000 mg/kg bw dosage in 5% ethanol/95% corn oil (vehicle), by gavage	Control animals received the same volume of vehicle (4 mL/kg bw); rats were then killed at 3, 6 and 24 h after dosing, and testes were collected and subjected to histopathological and immunohistochemical examinations	6 h after dosing, vimentin filaments showed shorter projections, concentration near the basal region and disappearance of the apical extensions toward the lumen of the tubules; Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment, there was marked loss of vimentin filaments expression in apical extensions; The staining intensity of actin and α-tubulin was weak in the testes of treated rats, compared with controls, and the α-tubulin staining pattern was characterized by long defined tracts extending along the axes of the Sertoli cells; Primary Sertoli cells exposed to 0. 1, 100, and 1000 nmol/mL Butylparaben for 6 or 24 h in vitro exhibited dose- and time-dependent increase in the numbers of cytoplasmic vacuoles and disruption of vimentin filaments	[66]

Table 13. Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Isopropylparaben Butylparaben Isobutylparaben	Rat (Sprague-Dawley)	Prepubertal (8-week-old) females, N=200, n=10/group, 20 groups	0, 62.5, 250 or 1000 mg/kg bw/day in corn oil (vehicle), by gavage	Prepubertal females were dosed daily with a paraben in corn oil from PND21 to PND40; EE was used as a positive control (1 mg/kg bw/day); all rats were sacrificed at 24 h after the final oral treatment on PND 41	Treatment with Methylparaben (1000 mg/kg bw/day) or Isopropylparaben (250 or 1000 mg/kg bw/day) resulted in a statistically-significant delay in vaginal opening in prepubertal females ($p< 0.05$); in contrast, the positive control (EE) significantly accelerated the date of vaginal opening; In the 1000 mg/kg bw/day groups, there were statistically-significant ($p<0.05$) decreases in ovary weights (Methylparaben or Isopropylparaben) and kidney weights (Ethylparaben, or Isopropylparaben) and increases in adrenal gland weights (Methylparaben, Ethylparaben, or Propylparaben) and thyroid gland weights (Methylparaben); Liver weights increased at all dosage rates of Butylparaben ($p < 0.05$); Histological analysis of the ovaries indicated decrease in the number of corpora lutea, increase in the number of cystic follicles, and thinning of the follicular epithelium; Morphological studies of the uterus revealed myometrial hypertrophy after exposure to 1000 mg/kg bw/day Propylparaben or Isopropylparaben and in animals of all dose groups of Butylparaben and Isobutylparaben; In the 1000 mg/kg bw/day groups, serum estradiol concentrations were statistically-significantly reduced (Ethylparaben or Isopropylparaben) and prolactin concentrations were increased (Methylparaben); Serum concentrations of T4 were statistically-significant reduced after treatment with 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Propylparaben or Isopropylparaben, or 62.5 mg/kg bw/ Isobutylparaben, propyl- and Isopropylparaben; The parabens exhibited affinities for ERα and ERβ (IC_{50}s ranging from 2.07×10^{-6} to 5.55×10^{-5}) in the following order: Isobutylparaben>Butylparaben>Isopropylparaben=Propylparaben>Ethylparaben; IC_{50} for 17β-estradiol was approximately 3×10^{-9}, by comparison	[67]
Butylparaben	Rat (Wistar)	Young adult, pregnant females, n=8/group	0, 100 mg/kg bw/day (vehicle not specified), by gavage	Pregnant females were dosed daily from GD7 to GD21; fetuses were removed on PND21, blood from the fetuses of each litter were pooled (males and females separately) for measurement of plasma insulin, leptin, MCP1, IL-1β, PAI-1 active, IL6, and TNFα concentrations Livers, adrenals and testes were collected from GD21 males for histopathology examination, gene expression analysis, or hormone measurements (estradiol and testosterone)	Butylparaben reduced plasma leptin concentrations in male and female offspring ($p<0.01$); in contrast, no alterations were observed in plasma levels of MCP1, IL-1β, PAI-1 active, IL6, or TNF	[68]

Table 13. Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben	Rat (Sprague-Dawley)	"Nulliparous"/virgin (n=10/group) and "parous" (n=10/group) females	0, 0.105 mg/kg bw/day in olive oil (vehicle), by gavage	Parturition marked LD0 for the F0 females and PND0 for the offspring; F0 females were dosed orally and, thereby, F1 offspring were exposed through lactation After weaning on LD 28, F1 offspring were separated from the F0 females were divided into two groups, "nulliparous" and "parous," and exposed orally PND 181. "Parous" F1 females were mated on PND 97 and exposure continued through pregnancy and delivery of F2 pups and lactation, ending on LD 28; after LD 28, the animals (F1) were separated from their mothers (F0), divided into two groups, "nulliparous" and "parous", and exposed through gavage until the final sacrifice at PND 181	Number of pups born to treated F1 females was statistically-significantly greater than that of controls; F2 pups exhibited statistically-significantly greater mortality at PND 7 and thereafter, compared with controls; All "nonparous" F1 females (treated and controls) exhibited normal mammary-tissue morphology; In treated "parous" F1 females, during lactation, mammary alveoli were not always milk-filled, increase in adipose tissue was noted, and collapsed alveolar and duct structures showed residual secretory content. Whole-mount preparations showed differences in lobular development among control and treated animals, including marked decrease in the size of the lobular structures in all treated F1 females; In treated "parous" F1 females, at PND 181, there were no histopathological differences among treated and control groups	[69]
Propylparaben	Rat (Wistar–Crl:WI [Han])	Lactating females (n=36), each with a litter ≥5 male pups supplied on PND14, n=20 pups/group (10/subgroup)	0, 10, 100, 1000 mg/kg bw/day, 2% suspended in a 1% aqueous hydroxycellulose, by gavage	Juvenile male rats were dosed for 8 weeks starting on PND21; all animals were sacrificed after the treatment	There was no evidence of an effect on the weight of the male reproductive organs, epididymal sperm parameters, hormone concentrations, or histopathology; The highest dosage rate tested (1000 mg/kg/day) was the NOAEL	[70]
Butylparaben	Rat (Sprague-Dawley)	Males, 7-week-old, n=5/group, 4 groups	0, 10, 100 and 1000 mg/kg in corn oil (vehicle), by gavage	Performed in accordance with OECD TG 407 for repeated 28-day oral toxicity studies; 24 h after the last dose, testes, tails and epididymal spermatozoa samples were collected, DNA was extracted, and the DNA samples from each group were pooled, digested (methylation-specific restricted restriction digestion), and analyzed by differential display random amplification of polymorphic DNA (RAPD)	Among 57 RAPD amplicons, six were methylation specific. Densitometric analysis of stained agarose gels revealed that five of these amplicons were elevated 1.4- to 3.8-fold in epididymal sperm DNA in treated vs. control animals, indicating an epigenetic effect on spermatogenic germ cells in adult rats	[192]
Methylparaben Butylparaben	Rat (Wistar –Crl:WI [BR])	Males, 22 days of age, n=16/group, 4 groups	0, 100, 1000 or 10,000 ppm in the diet	Rats were 22 days of age at the start of exposure, which was continued for 56 days; parameters evaluated included organ weights, histopathology of reproductive tissues, sperm production, motility, and morphology; reproductive hormone concentrations (LH, FSH, and T) were measured in blood samples; animals were sacrificed on study days 32, 44 and after final	Methylparaben exposure resulted in a statistically-significantly higher incidence of abnormal sperm in the 1000-ppm ($p \leq 0.01$) and 10,000-ppm ($p \leq 0.05$) exposure groups, mostly sperm with no head in 4% to 5% of sperm, vs. 2.3% in 100-ppm and control groups; 100-ppm Methylparaben in the diet corresponds to 11.2 ± 0.5 mg/kg bw/day; Hormone concentrations were comparable across groups and were not altered from controls, with the following exceptions: Testosterone concentration was statistically-significantly reduced in the 1000-ppm and 10,000-ppm Butylparaben-treated groups after 3 weeks of exposure – removing two rats with aberrantly high testosterone measurement from the control group resulted in a mean control values that were comparable to those of the other groups; T and FSH concentrations were statistically-significantly higher (by 72% and 53%,	[48]

Table 13. Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
				treatment	respectively) in the 10,000-ppm Butylparaben-treated group, compared with the control group; LH concentrations were statistically-significantly lower ($p \leq 0.01$) in the 1000-ppm (by 35%) and 10,000-ppm (by 30%) exposure groups, compared with controls, but only at the 5-week exposure point The authors concluded that none of the parameters evaluated for either paraben showed compound- or dosage-dependent adverse effects, and the NOAEC was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of 1141.1 ± 58.9 and 1087.6 ± 67.8 mg/kg/day for Methylparaben and Butylparaben, respectively	
				Subcutaneous		
Butylparaben	Rat (Wistar)	Male, 2 days of age, N=8, n= 3 or 5/ group, 2 groups	2 mg/kg bw /day in corn oil (vehicle), by subcutaneous injection	Male rats were dosed subcutaneously for 17 days starting on PND2; control group contained 5 rats, and Butylparaben treated group contained 3 rats; parameters evaluated included testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts, and immunoexpression of the water AQP-1(The epithelial cells of the efferent ducts decrease in height coincident with reduced expression of the water channel protein AQP-1; animals that were sampled on day 18 were killed 4 h after injection	No detectable effect on any of the measured reproductive parameters after subcutaneous administration of Butylparaben for 17 days (PND 2-18); the NOEL was 2 mg/kg bw/day.	[155]

AQP-1=channel aquaporin-1; AR=Androgen receptor; CYP19=Aromatase; E2=17β-estradiol; EE=17α-ethynylestradiol; ERα=Estrogen receptor α; FSH=Follicle-stimulating hormone; GD=Gestation day; IL-1β =Interleukin-1beta; IL-6=Interleukin-6; LD=Lactation day; LH=Luteinizing hormone; MCP1=Monocyte chemotactic protein 1; NOAEC=No-observed-adverse-effect-concentration; NOAEL=No-observed-adverse-effect-level; OECD TG=Organisation for Economic Co-operation and Development Test Guideline; P450scc=Cytochrome cholesterol side-chain cleavage enzyme; PAI-1=Plasminogen activator inhibitor type 1; PND=Post-natal day; RAPD=Randomly amplified polymorphic DNA; StAR=Steroidogenic acute regulatory protein; SULT1E1=Estrogen sulfotransferase; T=Testosterone; T4=Tetra-iodothyronine; TNFα=Tumor necrosis factor α

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
In Vitro						
Butylparaben	Mouse (strain not specified)	Murine NIH-3T3-L1 fibroblasts	0, 1, 3, 10, 30, and 100 µM in DMSO (<0.3%)	For the mPPARα/γ transactivation assay, cells were transfected with the luciferase reporter plasmid 4xUAS-TK and either gal4-DBD_mPPARaLBD or gal4-DBD_mPPARcLBD expression vectors; media containing Butylparaben was added and cells incubated for 22 h at 37°C; For analysis of the human PPAR, cells were transfected with expression plasmid for the ligand binding domain of the hPPARα or hPPARγ coupled to Gal4 and a plasmid containing an UAS linked luciferase reporter gene (UAS-TK-luc); For the adipocyte differentiation assay, confluent cells were exposed to induction cocktail for 3 days, the medium was then replaced with differentiation medium with 0.1% DMSO (vehicle) or Butylparaben and the medium changed every 2 days until day 6, when the plates were stained with ORO; rosiglitazone served as a positive control compound; Cytotoxicity was evaluated in parallel experiments not used for Oil Red staining, with resazurin for 3 h followed by measuring fluorescence; To quantify the concentrations of resistin, leptin, and adiponectin in the supernatant from the adipocyte differentiation assay using commercially-available assay kits	Weak activation of mPPARα was seen with the highest concentrations of Butylparaben; Butylparaben activated mPPARγ with a LOEC of 30 µM and a maximal 4-fold induction at 100 µM; The human data for Butylparaben (hPPARα and hPPARγ) were comparable to those obtained with mPPARα and mPPARγ; Butylparaben showed induction of lipid accumulation at 20 µM, and increased leptin, resistin and adiponectin release	[77]
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Chinese hamster	CHO cells, AR-transfected	0, 12 concentrations within the range of 0.025 - 50 µM	Cells were transfected with the expression vector pSVAR0 and the MMTVLUC reporter plasmid; test compounds were added to the cells with or without 0.01 nM of the AR agonist R1881; The principle of concentration addition was applied to predict the effects caused by an equimolar (1:1:1:1:1) of the parabens; concentration-response relationship for the mixture was calculated using data fitted from the concentration-response curves of the individual compounds	Only Isobutylparaben antagonized the AR; the effect was statistically significant at ≥ 25 µM; Butylparaben and Propylparaben inhibited the R1881-induced response, but only at cytotoxic concentrations; The mixture was predicted to antagonize the AR at concentrations ≥ 2 µM	[78]
Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0-200 µM (stock and working solutions in DMSO)	Cells were incubated for 24 h, with or without DHT (1000 pM) in phenol red-free culture medium at 37°C	Butylparaben, tested individually, had no statistically-significant androgen agonistic activity, but exhibited concentration-dependent anti-androgenic activity at >10 µM	[193]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, 10 µM, ethanol vehicle (0.1% final concentration)	BT-474 cells are HER2 negative and ERα-positive; MCF-7 cells are ERα-positive and HER2-negative; SKBR3 cells are HER2-positive and ERα-negative; All cells were grown in phenol red-free culture medium and incubated for 2 h (for RT-PCR and Western blot analysis) or from 1 to 3 h (for chromatin immunoprecipitation analysis), with and without Butylparaben, with and without the HER2 HRG at 27°C	Propylparaben and Butylparaben statistically-significantly, synergistically, elevated c-Myc mRNA expression in BT-474 cells in the presence of HRG; Butylparaben was selected for further study because it was most effective; In BT-474 cells, no increase in c-Myc protein concentrations was observed with Butylparaben or HRG alone; in the presence of HRG with 1 µM and 10 µM Butylparaben, the increase in c-Myc protein concentrations was similar to that induced by 0.01 µM E2 plus HRG; the increase was blocked by ER antagonists ICI 182,780, raloxifene, and tamoxifen; MCF-7 cells treated Butylparaben exhibited a similar enhancement of HRG-induced c-Myc protein expression; no synergistic increase in c-Myc protein concentrations was observed in SKBR3 cells Butylparaben increased the number of BT-474 cells entering S-phase (EC_{50}=0.551 µM); the effect was enhanced in the presence of HRG (EC_{50}=0.024 µM After 1-h treatment with HRG and Butylparaben together, maximal 8-fold enhancement of ERα binding to c-Myc enhancer sequence was observed in BT-474 cells; Butylparaben enhanced binding about 4-fold and HRG <2-fold, by comparison	[79]
Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, 10 nm, and 1 µM, dissolved in DMSO (vehicle)	Cells, stably transformed with MMTV-luciferase, were cultured in Leibovitz's L-15 medium with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin and pre-treated with androgen antagonist flutamide (5 µM) at 37°C; cells then incubated 24 h with and without test compound, and evaluated by means of a cell proliferation assay and an assay for glucocorticoid activity (luciferase-reporter gene)	EC_{50} for glucocorticoid-like activity was 1.75 mM for Butylparaben and 13.01 mM for Propylparaben; Butylparaben and Propylparaben tested separately induced glucocorticoid-like activity at 1 µM, but only Butylparaben induced activity (44% higher than control) at 10 nM	[80]
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, and 25 µM in DMSO (vehicle)	MDA-kb2 cells are stably transformed with the MMTV luciferase neo reporter gene construct, and express high levels of functional endogenous AR and GR, which can both act through the MMTV promoter; cells were cultured and then incubated for 24 h, in the presence or absence of paraben, with and without the AR antagonist flutamide (5 µM), in Leibovitz's L-15 medium supplemented with 10% FBS, with 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C	Butylparaben statistically-significantly enhanced the hydrocortisone-induced GR signal by 85%; Methylparaben, Ethylparaben, and Propylparaben did not; Without hydrocortisone but with flutamide, Ethylparaben, Propylparaben, and Butylparaben increased GR activity by more than 50%, and Methylparaben by more than 20%	[81]
Butylparaben	Human	T47D-KBluc human breast carcinoma cells (ERα and ERβ positive)	0, 3, 10, 30, 60, and 100 µM in DMSO vehicle	Cells were incubated in phenol red-free Dulbecco's Modified Eagle's F-12 containing 10% charcoal stripped FBS, with and without Butylparaben, in the presence or absence of E2 (20 pM), for 24 h at 37°C	Butylparaben exhibited estrogen agonism at all concentrations tested; maximum effect (24% greater than that of E2) was observed at 10 µM; Butylparaben exhibited estrogen antagonism at all concentrations tested in the presence of 30 pM E2; maximum effects at 10 and 30 µM; calculated IC_{50}=59.82 µM	[82]
Methylparaben Ethylparaben Propylparaben	Human	MCF-7 human breast	Range of concentrations tested	Cells prepared as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 5% (v/v) FCS, 10 mg/mL insulin, and 10-8 M E2 at 37°C;	After 14 days of exposure, the EC_{50}s for cellular proliferation ranged from 0.4 - 40 µM, LOECs from 0.1 - 20 µM, and NOECs from 0.05 - 8 µM for the parabens; the parabens, in	[83]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben Isobutylparaben		adenocarcinoma cells	was not specified, ethanol vehicle	incubated with or without paraben or E2 for 7 or 14 days; cellular proliferation was measured using a Coulter counter EC_{100}, EC_{50}, LOEC, and lowest concentration which gave an increase in cell number statistically different ($P<0.05$) from the LOEC were reported	descending order of these values, were Isobutylparaben>Butylparaben> Propylparaben >Ethyl-paraben> Methylparaben; In comparison, corresponding values for E2 were EC_{50}=2 x 10^{-6} µM, LOEC=10^{-6} µM, and 1 x10^{-7} µM; A mixture of all 5 parabens, each at its 7-day NOEC, increased the number of cell doublings above that with any of the parabens tested individually, but lower than with E2	
Propylparaben	Human	MCF-12A and MCF-10A non-transformed, immortalized breast epithelial cells (3D cultures)	10 µM in DMSO vehicle	An in vitro 3D model for breast glandular structure development, using breast epithelial MCF-12A cells cultured in a reconstituted basement membrane matrix (Matrigel); the cells are estrogen-receptor (ERα and ERβ) and GPER competent; cells were cultured, with or without Propylparaben, for 16 days in Matrigel at 37°C	ERα and ERβ were expressed at relatively high levels in MCF-12A cells; MCF-10A cells express no measurable levels of ERα and very low levels of ERβ; Both cell lines expressed the transmembrane GPER MCF-12A cells formed organized acini, with deposition of basement membrane and hollow lumen; treatment with E2 or Propylparaben resulted in deformed acini and filling of the acinar lumen; the ER-inhibitor (ICI 182,780) and/or GPER-inhibitor (G-15) inhibited the Propylparaben-induced effects on acini	[84]
Methylparaben	Human Mouse (FVB)	MCF-7 and MDA-MB-231 human breast adenocarcinoma cells; HCI-7-Luc2 ER+ PDX human breast tumor cells; Normal cells from murine mammary glands of 8-week-old FVB mice	10 nM in ethanol (vehicle control, 0.1%)	Cells were grown in accordance with standard protocols; mammospheres were established, treated with 0.1% ethanol, 10 nM E2, 10 nM Methylparaben, 1 µM tamoxifen or 100 nM fulvestrant on days 4 and 7, and imaged on day 10	10 nM E2 exposure stimulated the proliferation of MCF-7 cells 7-fold after 1 week of exposure; 10 nM Methylparaben did not have this effect, and also failed to increase expression (mRNA) of p52 (TFF1) or progesterone receptor (canonical estrogen-responsive genes) MCF-7 mammospheres treated with Methylparaben exhibited increased expression of ALDH1 (marker of human mammary stem cells) and were larger than control and E2-treated mammospheres; HCI-7-Luc2 and normal murine mammospheres treated with 10 nM Methylparaben were also larger than controls; Methylparaben statistically-significantly increased NANOG, OCT4, and ALDH1 (all of which are stem cell markers) mRNA expression in both MCF-7 and HCI-7-Luc2 mammospheres; Methylparaben also upregulated NANOG protein expression in MCF-7 mammospheres; none of these effects were seen in MDA-MB-231 mammospheres; Neither tamoxifen nor fulvestrant inhibited effects of Methylparaben on MCF-7 mammospheres	[85]
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben 4-Hydroxybenzoic Acid	Mouse (strain not specified) Human	Murine 3T3-L1 fibroblasts Differentiated hADSCs	0, 1, 10, 100 µM in DMSO vehicle	Murine 3T3-L1 cells were grown in DMEM containing 10% calf serum at 37°C until they reached confluence; hADSCs were grown and differentiated according to the supplier's instructions; For the detection of early target genes, Butylparaben or DMSO was added to the media with or without dexamethasone or the differentiation cocktails (cortisone, methylisobutyxanthine, and insulin) For the studies of the antagonists of GR or PPARγ, cells were pretreated with the antagonists of PPARγ (GW9662 and BADGE) or GR (RU-486) or DMSO for	Butylparaben in the presence of differentiation cocktail enhanced 3T3-L1 cell differentiation, as revealed by ORO-stained lipid accumulation, adipocyte morphologies and ORO absorbance; Parabens enhanced differentiation with potencies that increased with the length of the linear alkyl chain (Methylparaben < Ethylparaben < Propylparaben < Butylparaben), and the extension of the linear alkyl chain with an aromatic ring in Benzylparaben further augmented adipogenicity; 4-hydroxybenzoic acid or benzoic acid did not have these effects;	[86]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				1 h before the cells were treated with Butylparaben or DMSO in the presence of the antagonist	In 3T3-L1 cells, the parabens also induced mRNA expression of adipocyte marker genes as well as adiponectin and leptin mRNA, in a concentration-related manner, and activated GR and/or PPARγ; no direct binding to, or modulation of, the ligand binding domain of GR was detected in competitor assays; 50 μM Butylparaben or Benzylparaben, in the presence of differentiation media promoted lipid accumulation in hADSCs as early as day 3 and throughout the differentiation process; on day 14, Benzylparaben showed the most potent adipogenic effects (upregulation of mRNA expression of adipocyte marker gene and lipid-filled adipocyte morphology); 1 μM Butylparaben had the strongest adipogenic effects of the parabens tested, whereas Ethylparaben, Propylparaben, and Benzylparaben had no effect at 1 or 10 μM)	
Butylparaben	Mouse (F1 hybrid (C57BL/6j ×CBA/Caj) Human	Ovaries from immature 13-day-old female mice were used for follicle isolation; Human granulosa cell (hGC) were isolated from blood cells and follicular fluid	10 nM, 100 nM, 1 μM and 10 μM (1.9 ng/ml to 1.9 μg/ml) in DMSO vehicle	After 24 h of incubation to allow cell attachment, the medium was replaced by fresh equilibrated medium containing different concentrations of Butylparaben, DEHP or a mixture of both; The cells were treated with Butylparaben at different concentrations, for 24, 48, 72, or 96 h; Two control groups (control and DMSO) were included in each experiment which consisted of three independent cultures; Progesterone output was measured using commercial progesterone enzyme immunoassay kit	In follicle culture, DEHP and Butylparaben attenuate estradiol output but only when present together; Butylparaben attenuated DEHP induced reduction of progesterone concentrations in the spent media of hGC cultures; No effects on follicular development or survival were noted in the culture systems; DEHP and Butylparaben adversely affect steroidogenesis from the preantral stage onward and the effects of these chemicals are both stage-dependent and modified by co-exposure	[89]
Butylparaben Isobutylparaben	Human	MCF-7 and T47D human breast cancer cells	10 μM in ethanol or DMSO vehicle	MCF-7 and T47D cells were treated at 10 μM with Butylparaben, Isobutylparaben, 3-hydroxy n-butyl 4-hydroxybenzoate (3OH), and 2-hydroxy iso-butyl 4-hydroxybenzoate (2OH) for 2, 4, 6, or 18 h; Cell viability was measured by PrestoBlue assay; GREB1 expression was evaluated by Real-time PCR; ERE–luciferase reporter assay was performed to determine whether the estrogenic activity of the paraben metabolites is mediated by classical estrogen receptor mediated signaling; Computational docking studies were conducted to examine the ligand-binding domain interactions between paraben compounds and human ERα	The 3OH metabolite induced cellular proliferation with EC_{50} of 8.2 μM in MCF-7 cells; The EC_{50} for 3OH in T47D cells could not be reached; The 2OH metabolite induced proliferation with EC_{50} of 2.2 μM and 43.0 μM in MCF-7 and T47D cells, respectively; The EC_{50} for the parental Isobutylparaben and Butylparaben was 0.30 and 1.2 μM in MCF-7 cells, respectively; The expression of GREB1 was induced by these compounds and blocked by co-administration of an ER antagonist (ICI 182, 780), confirming the ER-dependence of these effects; The metabolites promoted significant ER dependent transcriptional activity of an ERE-luciferase reporter construct at 10 and 20 μM for 2OH and 10 μM for 3OH; The expression ofGREB1 was significantly induced in MCF-7 cells treated by 10 μM Butylparaben, Isobutylparaben, 3OH, and 2OH for 2, 4, and 6h; Molecular docking prediction studies showed that the paraben compounds exhibited the potential for favorable ligand-binding domain interactions with human ERα in a manner similar to known x-ray crystal structures of E2 in complex with ERα	[88]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
In Vivo						
Methylparaben	Zebrafish	Embryos, n=10/well	0.1, 1, 10, and 100 ppb in egg water	The collected embryos were segregated for each exposure group in 6-well plates; Exposure groups were maintained in egg water with varying concentrations of Methylparaben, following the guidelines of OECD fish embryo acute toxicity assay; The hatching and heart rate were observed at 48 hpf using a microscope: percentage of hatched embryos was calculated as number of embryos hatched divided by the number of incubated embryos; heart rate was recorded for 30 sec in the embryos at 48 hpf; Novel tank diving and Light-dark preference test: novel tank diving was then assayed in 6-day old larvae using a trapezoid tank; behavioral parameters observed, including latency to reach upper half of the tank, total number of transitions to the upper half, total time spent in the upper half, total erratic movements and total freezing bouts, and natural preference of zebrafish to light or dark compartment; Cortisol assay: a set of 20 larvae per group were taken at 6 dpf; the samples were homogenized and cortisol was estimated using commercially available ELISA kit.	Alterations in heart rate and hatching percentage were observed in embryos exposed to 10 ppb and 100 ppb of Methylparaben; Novel tank diving test indicated that anxiety-like behavior is induced in larvae exposed to 0.1 ppb and 1 ppb of Methylparaben; Methylparaben exposure in zebrafish at sub-lethal concentration inhibited AChE activity and increased cortisol levels	[72]
Methylparaben	Zebrafish	Embryos, n=30 -50 /group	100, 200, 400, 800 and 1000 µM in fish water	Malformations such as coagulation of embryo, lack of somite formation, tail detachment and heart beat were monitored at 24, 48, 72, and 96 hpf; Embryo toxicity assay were carried out in triplicates: in a 24 well plate, 30 embryos were exposed to Methylparaben for 8hpf; Non-lethal malformations like heartbeat, hatching rate, pericardial edema and bent spine were observed under the microscope and vitellogenin I gene expression was analyzed by qRT-PCR	With increasing concentrations of Methylparaben 200 µM, 400 µM and 800 µM, the heart rate decreased to 36, 33 and 22 beats per 20s respectively, while Control larvae showed an average heart rate of 42 beats; A deceleration in the hatching rate was observed with increasing concentration of Methylparaben, with 80% of embryos hatching in 100 µM, 55% in 200 µM , 40% in 400 µM and 10% in 800 µM; Defects including pericardial edema blood cell accumulation and bent spine were observed in all the treated concentration, except at 100 µM; The 96 hpf LC_{50} of Methylparaben was calculated to be 428 µM (0.065 mg/L); In larval zebrafish exposed to 100 µM (0.015 mg/L) for 96 hpf, expression of vitellogenin I was significantly upregulated	[73]
ANIMAL						
Oral						
Benzylparaben	Rat (Sprague-Dawley and Wistar)	Immature females, n=13 - 14/group	0, 0.0064, 0.032, 0.16, 0.8, 4, and 20 mg/kg bw/day by gavage, in peanut oil (vehicle)	Rats were exposed to Benzylparaben for 3 days, beginning on PND 21; on PND 24, the rats were weighed and killed, and uteri dissected and weighed	Relative uterine weights (ratios of uterine weights to final body weights) of Sprague-Dawley rats increased after treatment with ≥5 µg/kg bw/day E2, but Wistar rats given up to 100 µg/kg bw/day E2 showed no obvious effect; 400 µg/kg bw/day E2 increased relative uterine weight in Sprague-Dawley rats by 281% and in Wistar rats by 83%;	[93]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					Relative uterine weights were elevated in Sprague-Dawley rats after treatment with ≥0.16 mg/kg bw/day ($p<0.05$) in a dose-dependent manner; relative uterine weights increased by 3%, 7%, 19%, 24%, 27%, 31%, and 36% in the 0.0064, 0.032, 0.16, 0.8, 4, 20 and mg/kg bw/day groups, respectively The Wistar rats were not tested for sensitivity to Benzylparaben in this study	
Methylparaben Ethylparaben	Rat (Sprague-Dawley)	Immature females (PND 20); n=6 - 9/ group (n=17 in one of the control groups)	0, 0.8, 4, and 20 mg/kg bw/day (20 mg/kg bw/day when tested with 10 mg/kg bw/day fulvestrant) in peanut oil, by gavage	Rats were exposed to a paraben for 3 days, beginning on PND 21; rats were then weighed and sacrificed, and uteri dissected and weighed, and relative uterine weights calculated, except for 1 group that was transferred on PND 23 to individual metabolic cages in which only pure water was available, ad libitum, and from which urine was collected for 24 h and analyzed for Methylparaben and Ethylparaben concentrations; Relative expressions of estrogen-responsive genes in the uteri were evaluated by quantitative real-time RT-PCR	LOELs for increased relative uterine weight after treatment with Methylparaben and Ethylparaben were 20 and 4 mg/kg bw/day, respectively; NOELs for Methylparaben and Ethylparaben were 4 and 0.8 mg/kg bw/day, respectively; The uterotrophic effects of 25 µg/kg bw/day E2 or 20 mg/kg bw/day Methylparaben or Ethylparaben were antagonized by 10 mg/kg bw/day fulvestrant; Expression of icabp, itmap1, CaBP-9k, and/or Pgr biomarker genes were elevated in a concentration-dependent manner after treatment with 4 or 20 mg/kg bw/day Methylparaben or Ethylparaben; Mean urinary concentrations of the Methylparaben and Ethylparaben increased in a dose-dependent manner, from 491 to 17,635 ng/mL for Methylparaben and 376 to 11,906 ng/mL for Ethylparaben in rats that received 0.8 to 20 mg/kg/day Methylparaben or Ethylparaben	[94]
Ethylparaben Propylparaben	Mouse (C57BL/6J)	Ovariectomized females, 8 weeks of age, n=6/group, 11 groups	0, 1000 mg/kg bw/day in corn oil, by gavage	Study was performed in compliance with OECD TG 440 (Uterotrophic Bioassay in Rodents); mice were dosed daily for 7 consecutive days; 6 µg/kg bw/day E2 was given orally as the positive control in the test for agonism, and subcutaneously 15 min after administration of the test compound in the test for antagonism; 24 h after the last treatment, the animals were killed, and uteri were excised and weighed	Ethylparaben and Propylparaben were negative for estrogen agonism and antagonism	[95]
Butylparaben	Rat (Sprague-Dawley)	3-week old males, n=8	0, 1000 mg/kg, single oral dosage in 5% ethanol/95% corn oil vehicle	Rats were killed 3, 6, or 24 h after administration of Butylparaben; testes were collected for histopathological examination, in situ terminal deoxynucleotidyl transferase-mediated TUNEL assay, and analysis using transmission electron microscopy	Histopathologic examination revealed progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules and reduction and/or disappearance of tubular lumen 3 h after Butylparaben treatment; Sertoli cells and spermatogonia with few spermatocytes remained within the seminiferous tubules were observed at 6 h; thin seminiferous epithelia and wide tubular lumen were found at 24 h; TUNEL assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h, and declined at 24 h, though still substantially greater than in the controls; Apoptotic spermatogenic cells were found in semi-thin sections of the testes to be more frequently in treated rats, compared with controls; Apoptotic cells were rounded-up and sur-rounded by empty space, sometimes appearing to be separate from neighboring cells; transmission electron	[71]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					microscopy revealed condensed chromatin and shrinkage of cytoplasm and nucleus of apoptotic spermatocytes.	
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	Female rats (8-week old), n=6/group, 8 groups	100 mg/kg/day in the diet	Rats were orally exposed to 100 mg/kg bw/day for 5 weeks; Ovarian follicle development and steroid synthesis were investigated through real-time PCR and histological analyses; A disruptor of ovarian small pre-antral follicle 4-vinylcyclohexene diepoxide (VCD, 40 mg/kg bw/day), was used to induce premature ovarian failure (POF)	Propylparaben and Butylparaben treatment prolonged diestrus phases and shortened the interval of the estrous cycle, whereas Methylparaben treatment did not; No effect on number of primary follicles, and secondary follicles showed a decrease in total number in all treated groups; Propylparaben and Butylparaben decreased mRNA level of folliculogenesis-related genes (*Foxl2, Kitl* and *Amh*); All three Parabens induced an increase in FSH levels in serum, which implied impairment of ovarian function	[90]
Methylparaben	Rat (Sprague-Dawley)	Female rats (n= 3-10/group, 12 groups)	0.105 mg/kg /day, by gavage	Rats were orally exposed across several key developmental stages including perinatal (GD1–GD20, n=10 or PND1–PND21, n=10), prepubertal (PND21–PND42, n=5) and pubertal (PND42-PND63, n=5) windows as well as long-term exposures from birth to lactation (PND1–PND146, n=3)	Perinatal Methylparaben exposure decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad; Pubertal Methylparaben exposure elevated the amounts of glandular tissue, visible as a higher degree of branching relative to the total gland area; Long-term Methylparaben treatment from birth to lactation did not result in significant histological changes; In the pubertal window, expression alterations in 993 genes enriched in pathways including cholesterol synthesis and adipogenesis were observed	[91]
Methylparaben	Gerbils	Male and female adults (3-month old) n=16/group, 4 groups	500 mg/kg/day in 0.2 mL of 1% hydroxyethyl-cellulose, orally	8 control males and 8 control females received daily oral doses of 1% hydroxyethyl-cellulose for 21 days; 24 males and 24 females were randomly distributed in three groups that received daily oral doses of Methylparaben at 500 mg/kg (in 0.2 mL of 1% hydroxyethyl-cellulose) for 3, 7, and 21 days; After treatment, the body, ovary, testis, and prostatic complex (urethral segment, ventral, dorsolateral, and dorsal prostate lobes in males, and urethral segment plus prostatic tissue in females) were weighed; Various biometrical, morphological, and immunohistochemical analyses were performed	Methylparaben caused morphological changes in gerbil prostates in all experimental groups; Animals displayed similar alterations such as prostate epithelial hyperplasia, increased cell proliferation, and a higher frequency of AR-positive cells; The Skene's paraurethral glands of the female gerbil showed additional changes such as stromal inflammatory infiltration, intraepithelial neoplasia foci, and an increase in AR-positive frequency	[92]

Table 14. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
HUMAN						
Dermal						
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean= 26 years old), n=26	2% (w/w) Butylparaben in cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate	Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week, followed by daily application of cream with test substances for 1 week; concentrations of the following hormones were measured in blood serum (as well as the serum concentrations of Butylparaben): FSH, LH, T, estradiol, inhibin B, TSH, FT4, T3, and T4; Application of cream and blood sampling were done at same time every day at 0, 24, 96 and 120 h	Minor differences in serum inhibin B, LH, E2, T4, FT4, and TSH concentrations were observed during the treatment week, compared with the control week; the differences could not be attributed to the treatment because they were also seen at t=0, when treatment had not yet started	[42]

AR=Androgen receptor; CHO=Chinese hamster ovary; DEHP= di-(2-ethylhexyl) phthalate; DHT=5α-dihydrotestosterone; DMEM=Dulbecco's modified Eagle's medium; DMSO=Dimethyl sulfoxide; E2=17β-estradiol; EC_{100}=Lowest concentration from maximal stimulation of proliferation; EC_{50}=Concentration for half maximal stimulation of proliferation; E2: Estradiol; ER=Estrogen receptor; ERE=Estrogen-response element; FBS=Fetal bovine serum; FCS=Fetal calf serum; FSH=Follicle stimulating hormone; FT4=Free thyroxine; GD=gestation day; GPER=G-protein coupled estrogen receptor 1; GR=Glucocorticoid receptor; GREB1=Estrogen-inducible gene; hADSC=Human adipose-derived stem cells; HER2=Human epidermal growth factor receptor; hGC=Human granulosa cell; hpf= post fertilization; HRG=Ligand heregulin; LH=Luteinizing hormone; LNOEC=Lowest no observed effects concentration; LOEC=Lowest observed effect concentration; MMTV=Murine mammalian tumor virus; mPPAR=Murine peroxisome proliferator-activated receptor; NOEL=No observed effects level; OECD TG=Organisation for Economic Co-operation and Development Test Guidelines; ORO=Oil red O; PDX=Patient-derived xenograft; PND=Post-natal day; PPAR=Peroxisome proliferator-activated receptor; POF=premature ovarian failure; RT-PCR=Real time-polymerase chain reaction; T=Testosterone; T3=Total triiodothyroxine; T4=Total thyroxine; TSH=Thyroid stimulating hormone; TUNEL=Transferase uridyl nick end labeling

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	US NHANES, 2686 urine samples, male and female participates ≥ 6 years of age	Aggregate exposures (undefined sources)	-Annual survey conducted by CDC between 2005 and 2014; -Three age groups (6-11years, 12-19 years, 20 years and older), total 13,076 subjects: 2005-2006, n= 2448; 2007-2008, n= 2604; 2009-2010, n= 2749; 2011-2012, n= 2489; 2013-2014, n= 2686; -NHANES includes household interviews, standardized physical examinations, and collection of urine specimens for parabens exposure examination via HPLC-MS/MS analysis; -Urine samples were treated to free conjugated paraben in urine, thus representing a total concentration	- The median urine concentration was similar across the two sampling periods of 2011-2012 and 2013-2014 for the three parabens with Methylparaben at much higher concentrations than Propylparaben and Butylparaben; - The median urine concentration of the three parabens was decreased in the 2011-2014 sampling period comparing to the 2005-2010 sampling period; - For the 2013–2014 sampling period, Methylparaben in urine was 48.1 µg/L (95th percentile: 819 µg/L), and Propylparaben in urine was 5.74 µg/L (95th percentile: 224 µg/L); - For Butylparaben, the median concentration in urine was below the limit of detection (0.1 µg/L) for all groups in the 2011–2014 reporting period; - In females, the median concentration of Ethylparaben in the 2013–2014 reporting period was 1.6 µg/L (95th percentile: 145 µg/L) while males were below the limit of detection (95th percentile: 34 µg/L); - The reported median concentration in male urine for Methylparaben (24.4 µg/L) and Propylparaben (1.7 µg/L) was lower than that for females (Methylparaben: 73.9 µg/L; Propylparaben: 13.5 µg/L)	[99]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	US NHANES, 3529 adults	Aggregate exposures (undefined sources)	- Mouthwash use was estimated from the Oral Health questionnaire; responses were recoded as follows: "Always" (reported use 7 out of the last 7 days); "Sometimes" (reported use 1–6 out of the last 7 days); or "Never" (reported use 0 out of the last 7 days); - Sunscreen use was estimated from the Dermatology questionnaire, with a subset of participants ages 20–59; responses were coded as "Always"; "Sometimes" (reported use Most of the time, Sometimes, or Rarely); and "Never"; - A panel of phthalate metabolites and environmental phenols were measured in urine samples using HPLC-MS/MS and on-line solid phase extraction (SPE) coupled to HPLC-isotope dilution MS/MS; - For phthalate analysis, urine samples first underwent enzymatic deconjugation from glucuronidated forms; - Levels below LOD were replaced with the LOD divided by the square root of 2; - Urinary creatinine concentrations, indicative of urine dilution, were assessed using an enzymatic reaction and measurement with a Hitachi Modular P Chemistry Analyzer	Mouthwash use: -The distribution of use was: "Always" use (n=973, 34.3%); "Sometimes" use (n=654, 23.1%); and "Never" use (n=1209, 42.6%); - Compared to "Never" use, individuals with daily use had significantly elevated urinary concentrations of Methylparaben and Propylparaben (30 and 39%, respectively); - Associations with mouthwash use were generally stronger in men compared to women Sunscreen use: - The distribution of use was: "Always" use (n=296, 12.1%); "Sometimes" use (n=1051, 42.9%); "Never" use (n=1101, 45.0%); - Compared to "Never" use, individuals who reported "Always" had significantly higher urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben, (92, 102, and 151% higher, respectively); - Associations between exposure biomarkers and sunscreen use were stronger in women compared to men	[100]
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben Benzylparaben	Human	80 pregnant women (age 18 years or older)) at the Ottawa Hospital, Canada	Aggregate exposures (undefined sources)	- Prior to 20 weeks of pregnancy, 80 women collected all their urine from two 24 h periods on a weekday and/or a weekend day as multiple spot urine samples; a subset of women (n = 31) who provided multiple spot urine samples (n = 542) collected over two 24-h periods; - Women were instructed to keep the urine cool at all times and samples were delivered to hospital within 36 h; - Breast milk samples were collected at the woman's home 2-3 months after delivery (n =56); - Women recorded the date and time of the sample collection, which breast they collected it from, the time since the last feed from that breast and the name of any creams, lotions, or cleansers used on their breast; - At the same time as the urine collection, women were asked to record their activities, food consumption, and PCP use throughout the day; the PCP content of the diaries were manually categorized into the 16 mutually exclusive categories; - Five parabens were measured in urine and breast milk samples by HPLC-MS/MS analysis	- Women who used lotions in the past 24 h had significantly higher geometric mean paraben concentrations (80 - 110%) in their urine than women who reported no use in the past 24 h; -Women who used shampoo, conditioner, and cosmetics also showed 70.80% higher Butylparaben concentrations in their urine; - There was 100%, 72%, 96%, and 90% detection of Methylparaben, Butylparaben, Propylparaben, and Ethylparaben in urine respectively; Lower detection rates were seen for Isobutylparaben (39%) and Benzylparaben (41%); - All parabens with >70% detection (Methylparaben, Ethylparaben, Butylparaben, and Propylparaben) were significantly and strongly correlated with each other with Spearman correlation coefficients ranging from 0.48 (Methylparaben and Ethylparaben) to 0.86 (Propylparaben and Methylparaben); -Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben; - There was <1% detection for Butylparaben, Benzylparaben and Isobutylparaben - GM concentrations of parabens in urine samples: Methylparaben 30.02 µg/L (95th percentile: 403.26 µg/L), Ethylparaben 2.43 µg/L (95th percentile: 84.16 µg/L), Propylparaben 4.6 µg/L (95th percentile: 111.18 µg/L), Butylparaben 0.15 µg/L (95th percentile:	[108]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					12.20µg/L), No GM concentrations for Isobutylparaben and Benzylparaben ; - GM concentrations of parabens in breast milk samples: Methylparaben 0.0672 µg/L (95th percentile: 6.792 µg/L), Ethylparaben 0.0023 µg/L (95th percentile: 0.614 µg/L), Propylparaben 0.0277 µg/L (95th percentile: 1.32 µg/L); No GM concentrations for Butylparaben, Isobutylparaben and Benzylparaben	
Methylparaben Ethyl paraben Propylparaben Butylparaben	Human	100 Latina girls (14-18 years old) living in Salinas, California	Each girl was provided with small (2–4 oz) containers of shampoo, conditioner, body wash, and moisturizing lotion; a bar of hand soap; a container of liquid; and roll-on deodorant	- Participants enrolled in the Health and Environmental Research on Makeup of Salinas Adolescents (HERMOSA) Study which was a youth empowerment intervention study examining strategies to reduce PCP chemical exposure to adolescent girls; - Girls participating in the study were provided with low-chemical PCPs and asked to refrain from using their regular products for 3 days; - Each girl was allowed to choose four items from among liquid or powder foundation, mascara, eyeliner, lipstick/lip gloss/lip balm, and sunscreen; - Participants were asked to avoid using any personal care products or cosmetics other than those provided by the study; the replacement personal care products provided to participants were selected to be free of parabens; - Pre- and post-intervention urine samples were analyzed for parabens using HPLC-MS/MS	- Methylparaben and Propylparaben concentrations decreased by 43.9% (95% CI: –61.3, –18.8) and 45.4% (95% CI: –63.7, –17.9, respectively; - The GM concentration of Methylparaben decreased from 77.4 to 43.2 µg/L; - The proportion of girls with detectable concentrations of Methylparaben decreased non significantly from 93% to 87%, and decreases in concentrations were observed in 61% of girls; - The GM concentration of Propylparaben decreased from 22.6 to 12.3 µg/L, with decreases observed in 63% of girls; - The proportion of girls with detectable concentrations of Propylparaben also decreased between pre- and post-intervention (90% vs 87%), but not significantly; - Unexpectedly, Ethylparaben and Butylparaben concentrations both increased over the course of the intervention period, with Butyl paraben increasing by 101.7% (95% CI: 35.5, 203.2) and Ethylparaben increasing by a nonsignificant 47.3% (95% CI: –0.7, 118.4); however, concentrations of both Ethylparaben and Butylparaben were low overall and not detected in almost half the samples; - The absolute changes in concentrations were small for both Butylparaben (preintervention GM = 0.8 µg/L vs. postintervention GM = 1.7 µg/L) and Ethyl paraben (preintervention GM = 2.9 µg/L vs. postintervention GM = 4.2 µg/L)	[101]
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	100 Latina girls (14-18 years old) living in Salinas, California	Aggregate exposures; participants reported using specific makeup , including foundation, blush, and mascara every day	- Participants enrolled in the HERMOSA Study; -Evaluated the relationship between recent self-reported PCPs use and concentrations for urinary metabolites of parabens and other endocrine disruptors in 100 Latina adolescents; -The analysis focused on use of a comprehensive list of personal care products, including face products, oral hygiene, soap, nail and hair products, and feminine care products; - Urine samples were subjected to HPLC-MS/MS analysis; - GMs were compared across categories and calculated a P value for trend using one-way ANOVA and linear regression;	- Urinary concentrations of Methylparaben and Propylparaben were detected in over 90% of participants; - Detection frequencies were below 49% for Butylparaben and 55% for Ethylparaben, so these two analytes were not included in final statistical analyses; - Girls who reported using makeup every day vs. rarely/never had higher urinary concentrations of Methylparaben (120.5 ng/ mL vs. 13.4 ng/mL, $P < 0.01$), and Propylparaben (60.4 ng/mL vs. 2.9 ng/mL, $P < 0.01$); - Girls who reported recent use of specific makeup products, including foundation, blush, and mascara, had higher urinary concentrations of Methylparaben, and Propylparaben;	[102]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				- Urinary concentrations of Methylparaben and Propylparaben were compared in girls who used products every day, 2–6 times per week, once a week, and rarely/never; - GM urinary concentrations of Methylparaben and Propylparaben metabolites were compared by frequency of use of make-up, fragrance, and moisturizer	- Both Methylparaben and Propylparaben urinary concentrations were positively associated with use of foundation (Methylparaben: 52.1%, Propylparaben: 69.3%), blush (Methylparaben: 34.0%, Propylparaben: 44.9%), mascara (Methylparaben: 64.3%, Propylparaben: 76.3%), any eye makeup (Methylparaben: 58.0%, Propylparaben : 84.3%), and any makeup (Methylparaben: 77.9%, Propylparaben:75.7%); - Propylparaben urinary concentrations were negatively associated with lip gloss use (−51.1%); - Concentrations also varied by frequency of fragrance use for Methylparaben (112.1 ng/mL vs. 23.7 ng/mL, P_{trend} = 0.04); and by frequency of moisturizer use for Methylparaben (123.8 ng/mL vs. 69.4 ng/mL, P_{trend} = 0.01). - Girls who used 20 or more products today and yesterday had higher levels of the Propylparaben compared to girls who used fewer than nine products today or yesterday (33.4 ng/mL vs. 6.1 ng/mL, P_{trend} = 0.04).	
Methylparaben Propylparaben	Human	18 females (21-25 years old) from the Federal University of Alfenas-MG located in Minas Gerais, Brazil	Using lipstick containing parabens for 5 days, lipstick used/day was 0.001 mg/kg/day ± 0.05	- In phase 1, the women used paraben-containing products according to their routine - In phase 2, the women used donated lipstick containing Methylparaben and Propylparaben for 5 days in conjunction with the routine use of paraben-containing products - In phase 3, the women routinely used paraben-containing products while abstaining from lipstick for five days, and blood (15mL) was collected for HPLC-MS/MS analysis	- In phase 2, total paraben levels were significantly higher than phases 1 and 3; - The median concentration ± average deviation was 2.14 ng/mL ± 3.24 ng/mL in phase 2, comparing to 1.06 ng/mL ± 0.80 ng/mL in phase 1 and 1.27 ng/mL ± 0.79 ng/mL in phase 3;the values represent total parabens concentrations (Methylparaben plus Propylparaben) in serum; - Statistically significant difference was demonstrated between serum parabens in women who used lipstick containing Methylparaben and Propylparaben (p = 0.0005 and 0.0016, respectively); - A strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202)	[103]
Methylparaben Propylparaben	Human	Human serum samples from 5 males and 11 females (n=16)	Aggregate exposures (undefined sources)	16 commercially available serum samples collected between 1998 and 2003 were purchased from Tennessee Blood Services in Memphis; To determine the concentrations of the free plus conjugated species of the parabens, the enzyme solution, containing β-glucuronidase/sulfatase in ammonium acetate buffer , and radio-labeled standards were added into the serum; Six phenols concentrations in the serum sample, including bisphenol A, benzophenone-3, triclosan, 2,5-dichlorophenol, Methylparaben and Propylparaben, were measured by on-line SPE coupled to HPLC - MS/MS	The mean paraben concentrations in serum are 42.6 µg/L and 7.4 µg/L for Methylparaben and Propylparaben, respectively; The free concentration of Methylparaben and Propylparaben in the serum is 2.2 µg/L and 0.5 µg/L, respectively, indicating that parabens that are not hydrolyzed to 4-Hydroxybenzoic Acid are rapidly conjugated; The conjugated species of Methylparaben and Propylparaben are more stable than their corresponding urinary conjugates	[104]
Methylparaben Ethylparaben Propylparaben	Human	Female breast cancer patients undergoing	Aggregate exposures (undefined sources)	Human breast tissue was collected from 40 mastectomies for primary breast cancer in England between 2005 and 2008; concentrations of parabens were measured (HPLC-	One or more paraben ester was detected 99% of the tissue samples and all 5 esters were detected in 60% of the samples; Median concentrations in the 160 tissue	[105]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben Isobutylparaben		radical mastectomy, n=40		MS/MS) in breast tissue samples excised from four serial locations (quadrants) across the breast, from axilla to sternum	samples were highest for Propylparaben (16.8 ng/g tissue) and Methylparaben (16.6 ng/g tissue), lower for Butylparaben (5.8 ng/g tissue) and Ethylparaben (3.4 ng/g tissue, and least for Isobutylparaben (2.1 ng/g tissue); Maximum concentrations ranged from 95.4 ng Butylparaben/g tissue to 5103 ng Methylparaben/g tissue; Propylparaben concentrations were statistically significantly higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Human	Human placentas collected from healthy mothers after delivery (singleton term pregnancies) at St. Hospital Joan de Déu (Barcelona), n=12	Aggregate exposures (undefined sources)	Placental tissue was obtained from the maternal side, each placenta sectioned transversally, and three fragments of about 1 cm^3 of tissue near the umbilical cord insertion were biopsied after removal of amniotic and chorionic layers; analytes were extracted from the samples and separated by a chromatographic procedure developed by the authors; MS/MS detection was performed in negative ESI under SRM mode for improved selectivity and sensitivity	Methylparaben, Butylparaben, and Benzylparaben were detected in all samples; The highest measured concentration was 11.77 ng Methylparaben/g tissue	[106]
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	Human ovarian tumor samples were obtained from Yong Loo Lin School of Medicine, National University of Singapore, n=30	Aggregate exposures (undefined sources)	15 ovarian malignant tissues and 15 benign tissues were analyzed; technique involves the simultaneous use of MASE and micro-solid SPE, in tandem with HPLC/UV analysis for the determination of parabens concentration; ovarian tissues were not spiked with parabens; the mass fractions of parabens present in human ovarian tissues were then calculated	-The tissue mass fractions of Methylparaben and Propylparaben were higher than Propylparaben and Butylparaben; -The tissue mass fractions of four parabens in all the ovarian cancer tissues are at least twice as much as those present in the benign tissues; -The method detection limits for parabens ranged from 0.005 to 0.0244 ng/g	[107]
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben heptylparaben 4-Hydroxybenzoic Acid	Human	Human adipose fat samples collected from Wadsworth Center, New York City, n = 20	Aggregate exposures (undefined sources)	Human adipose fat samples were collected from volunteers who underwent liposuction surgery between 2003 and 2004; tissues were spiked with methanol solution containing isotope labeled internal standards and analyzed by HPLC-MS/MS for the presence of parabens as well as several environmental phenols and aromatic compounds	-Among the six parabens analyzed, Ethylparaben and Propylparaben were more frequently detected than the other parabens, at a detection frequency of 60% and 50%, and a GM concentration of 0.90 and 0.49 ng/g, respectively; -4-Hydroxybenzoic Acid was detected in almost all samples, at concentrations as high as 17,400 ng/g; -The GM concentration of the sum of six parabens and 4-Hydroxybenzoic Acid ($C_{\Sigma parabens}$) in adipose fat was 3420 ng/g; - Among the 20 samples analyzed, high $C_{\Sigma parabens}$ ($>10^5$ ng/g) were found in 5 females and 2 males, indicating high exposure to parabens by some individuals; -No gender-related difference in $C_{\Sigma parabens}$ was found, and the age related difference between the two age groups (18–33 yr and 34–58 yr) was equivocal; -Paraben concentrations in adipose fat samples of Caucasian volunteers (GM: 7050 ng/g) were higher than those of African Americans (GM: 3440 ng/g); - The authors stated it should be noted that high concentrations of 4-Hydroxybenzoic acid (log K_{ow} = 1.39)	[109]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					found in adipose samples could be an artifact from the reaction of paraben esters with $NaHCO_3$ solution used in liposuction procedures (i.e., alkaline hydrolysis), thus further studies are warranted	
Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben heptylparaben 4-Hydroxybenzoic Acid	Human	Human urine samples collected from 40 children (17 males and 23 females, 3−10 yr) in Albany, 70 Chinese children (38 males and 32 females, 9−10 yr), and 26 Chinese adults (15 males and 11 females, most of 22−30 yr) in Shanghai and Tianjin, China.	Aggregate exposures (undefined sources)	Urine samples were spiked with methanol solution containing isotope labeled internal standards and analyzed by HPLC-MS/MS for the presence of parabens and their metabolite, 4-Hydroxybenzoic Acid	- Parabens were present predominantly (>90%) as conjugated species in urine; - Among the six parabens analyzed, Methylparaben and Propylparaben were the predominant compounds, which accounted for 57−98% and 1.4−12%, respectively, of the total concentrations; - The median concentration of Methylparaben and Propylparaben in US adults was 43.9 and 9.1 ng/mL, respectively; - The median concentrations of the sum of Six parabens in urine from US children were 54.6 ng/mL; - The GM concentrations of 4-Hydroxybenzoic Acid in urine from US children were 752 ng/mL for girls and 628 ng/mL for boys, which were 2 - 3 times lower than the concentrations determined for Chinese children	[110]
Methylparaben Ethylparaben Propylparaben Isopropylparaben Butylparaben Isobutylparaben Benzylparaben	Human	Human adipose tissue collected from San Cecilio University hospital and Santa Ana Hospital in Sprain (n=144, 88 males and 56 females)	Aggregate exposures (undefined sources)	114 adipose tissue samples were collected from participants of GraMo cohort study; The participants were recruited between July 2003 and June 2004 among patients undergoing non-cancer-related surgery and at two public hospitals in Southern Spain; Study inclusion criteria were age over 16 years, absence of diagnosed hormone-related disease or cancer and residence in one of the two study areas for ≥10 years; Adipose tissue samples were intraoperatively collected and stored in aliquots at −80 °C until analysis; Main tissue sources were pelvic waist (46.5%), front abdominal wall (44.4%), and limbs (9.0%); Samples were spiked with isotope labeled internal standard stock solution and subjected to HPLC-MS/MS for the presence of parabens as well as several environmental phenols; Spearman correlation tests were performed, followed by stepwise multivariable linear regression analyses to assess determinants of the exposure	- Detection frequencies and median concentrations were: Methylparaben (100.0%, 0.40 ng/g tissue), Ethylparaben (20.1%,<LOD), - Propylparaben (54.2%, 0.06 ng/g tissue), Isopropylparaben (0, <LOD), Butylparaben (5.6%, <LOD), Isobutylparaben (2.1%, <LOD) and Benzylparaben (0, <LOD); - Isopropylparaben and Benzylparaben were not detected in any of the samples; - Isobutylparaben concentrations above LOD were recorded in 8 and 3 of the 144 samples; - Older participants showed higher concentrations of Methylparaben; the author stated that the positive association of Methylparaben with age might be a consequence of a lower metabolic activity in older individuals, which may delay the metabolism and clearance of these chemicals; - Methylparaben, Ethylparaben, Propylparaben and bisphenol-A levels were significantly and positively correlated; - A wide variability in exposure levels was found among participants, with some samples showing 10 to 50-fold higher levels than the median level in the population	[111]
Methylparaben Propylparaben Butylparaben	Human	Human urine samples from US NHANES program, male and female participates ≥ 20 years of age(men, n=1399; women, n= 1350)	Aggregate exposures (undefined sources)	-A PBPK model for Methylparaben, Propylparaben, and Butylparaben were developed which were parameterized through a combination of quantitative QSAR for tissue solubility and quantitative IVIVE for hydrolysis in portals of entry including intestine, skin, and liver; -The human paraben PBPK model was then used to estimate the plasma free paraben concentration in adults consistent with 95th percentile urine concentration reported in US NHANES program (2009 - 2010 collection	- For the 2009 - 2010 sampling period, the estimated plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 70 kg male was 0.73, 0.21 and 0.052 µg/L, respectively; -The estimated plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 60 kg female was 1.19, 0.54 and 0.58 µg/L, respectively; - In vitro estrogenicity assay reported parabens concentration resulting in a 10% change from	[57]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				period); - The model assume that 4-Hydroxybenzoic Acid and the conjugated metabolites were exclusively excreted in urine; - The EC10 used in this assessment were generated from two assays, ERLUX (reporter gene) and E-Screen (cell proliferation), which were used to assess estrogenicity of the parabens; - In vitro metabolic parameters (nmol/min/ mg microsomal protein) were converted to an intrinsic clearance (Cl_{int}) expressed in terms of L/h-mg protein; The Cl_{int} was then scaled to the whole tissue based on the amount of microsomal protein per gram of tissue; - An in vitro based cumulative MOS was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the free plasma paraben concentrations predicted by the model to be associated with the 95th percentile urine concentrations reported in NHANES (2009–2010 collection period)	control (EC10): Methylparaben, 1162-1238 µg/L; Propylparaben, 180-234 µg/L; Butylparaben 96.5-111 µg/L -Based on human paraben PBPK model, the calculated cumulative MOS for adult females was 108, whereas the cumulative MOS for males was 444	
Methylparaben Propylparaben Butylparaben	Human	400 men (18 - 55 year old) at the Massachusetts General Hospital Fertility Center	Aggregate exposures (undefined sources)	- This was a prospective cohort study, enrolled couples seeking fertility treatment; - At each visit, men completed a questionnaire on PCPs use within the past 24h and at what time they last used each PCP prior to the collection of each urine sample; - PCPs included deodorants, shampoo, conditioner/crème rinse, hairspray/hair gel, combined other hair care products (including mousse, hair bleach, relaxer, perm, and straightener), shaving cream, aftershave, cologne/perfume, mouthwash, bar soap, liquid soap/body wash, hand sanitizer, hand/body lotion, and suntan/sunblock lotion; - Urine samples were collected at each men's visit. The analytical technique for quantification of the urinary biomarkers involved enzymatic deconjugation of the urinary metabolites, followed by solid phase extraction and HPLC-MS/MS analysis	- The EARTH study examined the association between PCP use and urinary concentrations of parabens in men; - The largest percent increase for parabens was associated with the use of suntan/sunblock lotion (66–156%) and hand/body lotion (79–147%); - A subset of 10 PCPs that were used within 6 h of urine collection contributed to at least 70% of the weighted score and predicted elevated urinary concentrations of Methylparaben, Propylparaben, and Butylparaben (788%, 1333%, and 254% higher, respectively); - GM concentrations of Methylparaben, Propylparaben, and Butylparaben in urine were 28, 2.86, and 0.26 µg/L, respectively; in comparison, the concentrations of Methylparaben and Propylparaben, in urine reported in US NHANES program (2011 - 2012 collection period) were 23.2 and 2.44 µg/L, respectively (Butylparaben < LOD of 0.1 µg/L); - Self-reported PCP use among men was associated with higher urinary concentrations of three parabens (Methylparaben, Propylparaben, and Butylparaben)	112,113
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben 4-Hydroxybenzoic Acid heptylparaben	Human	143 healthy, premenopausal women (aged 18 - 44)	Aggregate exposures (undefined sources)	- Participants were free of known chronic health conditions, and not using hormonal contraception who were recruited at the University at Buffalo research center from 2005 to 2007; - Participants attended up to 8 clinic visits for up to two menstrual cycles of study; urine samples were selected at key menstrual cycle phases; - Reproductive hormones levels timed to key periods of variability across the menstrual cycle were measured, including E2, progesterone, LH and FSH;	- All individuals had levels of Methylparaben and 4-Hydroxybenzoic Acid above the LOD; - Benzylparaben and heptylparaben were below the LOD for > 45% and were excluded in the analyses; - In a single-chemical model, 4-Hydroxybenzoic Acid was associated with increased FSH 0.07 (95% CI: 0.01, 0.13); parabens were not associated with LH; - The paraben factor was significantly associated with increased E2 0.21 (95% CI: 0.15, 0.28) as well as increased progesterone 0.32 (95% CI: 0.23, 0.41)	114

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				- Urine samples were spiked with ^{13}C-labelled and analyzed by HPLC-MS/MS; the LOD was 1 mg/dL; - Using the hierarchical principal component analysis approach, paraben factor consists of Methylparaben, Ethylparaben, Propylparaben and Butylparaben		
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	1003 pregnant women (aged 18 - 40)	Aggregate exposures	- Participants enrolled in the PROTECT project, were recruited at seven prenatal clinics and hospitals throughout Northern Puerto Rico during 2010–2016 (14 ± 2 weeks of gestation); - The questionnaire was administered at each urine sample collection to gather data on self-reported product use: bar soap, cologne/perfume, colored cosmetics, conditioner, deodorant, fingernail polish, hair cream, hairspray/hair gel, laundry products, liquid soap, lotion, mouthwash, other hair products, shampoo, and shaving cream; - The questionnaire contained yes/no questions about the use of different products in the 48-h preceding urine sample collection, in addition to questions on the usual frequency (not at all,<once/month, 1–3 times/month, once/week, few times/week, every day); - The participants were also asked to report the specific brand of the product; - Urine samples were analyzed by solid-phase extraction HPLC-MS/MS; - The LODs were 1.0 µg/L for Methylparaben, and 0.1 µg/L for Propylparaben and Butylparaben; all paraben concentrations were adjusted for SG	- Detectable paraben concentrations among pregnant women were prevalent; Median concentrations of Butylparaben among Puerto Rican women were 2 fold greater than women in US NHANES program, while Methylparaben, Ethylparaben and Propylparaben were lower; - There was correlation between the four parabens, particularly between methylparaben and propylparaben (Spearman r =0.78); - Trends were observed for increasing concentration of four parabens with increasing age categories; - Decreasing temporal trends were observed for all parabens in the study population from 2011 to 2016; - Exposure to parabens varied by location, sex, age, race, and ethnicity; - GM concentration at first visit for Butylparaben was statistically higher than later visits in the study; - Higher paraben concentrations were found among women who reported using cosmetics and lotion	[115]
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	482 pregnant women (130 preterm birth cases and 352 controls)	Aggregate exposures (undefined sources)	- Participants enrolled in the LIFECODES prospective birth cohort at the Brigham and Women's Hospital in Boston between 2006 and 2008; - Participants were 18 years of age or older and their pregnancy was <15 weeks gestation at the initial study visit; - Participants attended up to four study visits: visit 1 (4.71–19.1 weeks), visit 2 (14.9–32.1 weeks), visit 3 (22.9–36.3 weeks), and visit 4 (33.1–38.3 weeks); - Exposure biomarkers were quantified using isotope dilution LC-MS/MS; - Cytokines in plasma were measured using the MilliplexMAP High Sensitivity Human Cytokine Magnetic Bead Panel and had an LOD of 0.128 ng/mL	- Methylparaben and Propylparaben had overall detection rates above 75%, whereas the overall detection rates of Ethylparaben and Butylparaben were 59.5% and 68.4% respectively; - Compared to the White participants, African-American participants had 211 ng/mL higher median concentration of Methylparaben ($p< 0.001$), and 35.4 ng/mL higher median concentration of Propylparabe ($p< 0.001$); - Compared to older age groups, participants under the age of 25 had 0.64–0.91 ng/mL lower median concentrations of Butylparaben (P-trend = 0.001); - An interquartile range increase in Methylparaben (359 ng/mL) was positively associated with a 6.69% increase in IL-6 (95% CI: 0.02, 13.8); - An interquartile range increase increase in Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in interleukin 1β (95% CI: −14.1, −0.86); - It is difficult to make conclusions about the magnitude by which parabens contribute towards inflammatory processes during pregnancy due to the complexity of receptor signaling in immune cells	[116]
Methylparaben	Human	602 pregnant women (aged 18-40 years) in	Aggregate exposures	- Participates at 14 ± 2 weeks gestation enrolled in PROTECT project between 2012 and 2017;	-Methylparaben and Propylparaben were strongly correlated [Spearman correlation of 0.8 ($p < 0.001$)];	[117]

Table 15. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Ethylparaben Propylparaben Butylparaben		North Puerto Rico	(undefined sources)	- Spot urine samples were collected at three visits (Visit 1: 16–20; Visit 2: 20–24; Visit 3: 24–28 gestation weeks); - Urinary paraben concentrations were analyzed by online solid phase extraction HPLC-MS/MS, and adjusted for SG; - Progesterone, SHBG, testosterone, T3, T4, FT4 and TSH were measured in serum using a chemiluminescence immunoassay (ADVIA Centaur® CP Immunoassay System); Estriol and CRH were measured in serum using an enzyme immunoassay; - The ratio of progesterone to estriol (Prog/Estriol Ratio), and the ratio of T3 and T4 (T3/T4 ratio) were calculated; - The LODs were 0.1 µg/L for Butylparaben and Propylparaben, as well as 1 µg/L for Methylparaben and Ethylparaben;	- Ethylparaben and Butylparaben showed moderate correlation with Methylparaben and Propylparaben with Spearman correlations between 0.33–0.47 (p values < 0.001); - Butylparaben, Methylparaben and Propylparaben were associated with decreases in SHBG [(%Δ: -5.27; 95% CI: -9.4, − 1.14); (%Δ: -3.53; 95% CI: -7.37, 0.31); (%Δ: -3.74; 95% CI: -7.76, 0.27)]; - Methylparaben was associated with decreases in reproductive hormones, including an 8% decrease in estriol, a suggestive 3% increase in the progesterone/estriol ratio, and a suggestive decrease in testosterone at 16–20 weeks [(%Δ: -7.76; 95% CI: -15.4, 0.61); (%Δ: 3.14; 95% CI: -2.95, 9.61); (%Δ: -6.77; 95% CI: -13.13, 0.29), respectively]; - Propylparaben was associated with a 9–10% increase in progesterone and estriol at 24–28 weeks [(%Δ: 9.67; 95% CI: -1.30, 21.85); (%Δ: 8.92; 95% CI: -1.56, 20.52)]; - A decrease in thyroid hormones in relation to Methylparaben and Propylparaben, and a decrease in TSH in association with Methylparaben, particularly at 16–20 weeks (%Δ:-11.69; 95% CI: -21.97, − 0.06)	

CDC=Centers for Disease Control and Prevention; CRH=corticotropin-releasing hormone; EARTH=Environment and Reproductive Health; E2= 17β-estradiol; EC= Effective concentration; FSH=Follicle stimulating hormone; FT4= free thyroxine; GM= geometric mean; HPLC-MS/MS= High-performance liquid chromatography tandem mass spectrometry; IVIVE=in vitro to in vivo extrapolation; LH= Luteinizing hormone; LOD= limit of detection; NHANES= National Health and Nutrition Examination Survey; PBPK= Physiologically based pharmacokinetic; PROTECT= Puerto Rico Testsite for Exploring Contamination Threats; QSAR=quantitative structure–activity relationship; SHBG=sex hormone-binding globulin; SPE=solid phase extraction; T3= total triiodothyronine; T4=total thyroxine; TSH=thyroid-stimulating hormone; MASE=microwave-assisted solvent extraction

Table 16. Contact dermatitis studies on paraben mixture (Data collected by ESSCA between 2009 and 2012 from 12 European Countries).[125]

Allergen	Con (mg/cm^2)	Test No.	% (+)	%(++/+++)	% (doubtful/irritant)	% (pos.)	% (pos.std.)*	95% CI
Paraben mix (Overall)	16	52586	0.47	0.26	1.78	0.7	0.7	(0.63–0.77)
Paraben mix (TRUE-Test®)	1	2362	0.21	0.17	0.27	0.38	0.35	(0.12–0.59)

Note: %(pos.std.), proportion of positives, directly age- and sex-standardized; Reactions designated as either +, ++ or +++ were classified as positive (allergic);
TRUE-Test®, combined with an additional set of allergens using investigator-loaded chambers and petrolatum- or water-based allergens to achieve a better coverage of the desired range of allergens and concordance with the European baseline series (EBS)

Table 17. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			Prospective Studies			
Methylparaben Propylparaben Butylparaben	245 women who completed ≥1 IVF cycle and provided ≥1 urine sample/IVF cycle between November 2004 and April 2012 at the Massachusetts General Hospital (MGH) Fertility Center	Subjects recruited from 11/2004 to 4/2012	- Subjects provided up to two spot urine samples per IVF cycle; first collected between Day 3 and Day 9 of the gonadotrophin phase, second collected on day of oocyte retrieval - Urinary concentrations of total parabens were measured by HPLC-MS/MS - Clinical information was abstracted from the patient electronic medical records - Serum concentrations of FSH and E2 were measured - Each subject was assigned an infertility diagnosis by a physician - Subjects underwent one of three controlled ovarian stimulation IVF treatment protocols, after completing a cycle of oral contraceptives - Embryologists determined the total number of oocytes retrieved per cycle and classified them - Oocytes underwent either conventional IVF or ICSI, and embryologists determined fertilization rate 17-20 h after insemination - Embryo quality was classified based on morphology and number of blastomeres, ranging from 1 (best) to 5 (worst) on day 2 and 3 - In women who underwent an embryo transfer, implantation was assessed and pregnancy was confirmed by ultrasound at 6 weeks - Live birth was defined as birth of a neonate on or after 24 weeks gestation - Exposures were categorized into quartiles of urinary concentrations; the lowest quartile used as the reference group - Associations between urinary concentrations and demographics and baseline reproductive characteristics were evaluated using Kruskal-Wallis and Chi-squared tests - Multivariable generalized linear mixed models were used to evaluate associations between concentrations and IVF outcomes - Poisson distributions and log link functions were specified for oocyte counts, and a binomial distributions and logit link functions for embryo quality, fertilization rates, and clinical outcomes (implantation, clinical pregnancy and live birth) - Potential confounders considered include factors previously related to IVF outcomes in this or other studies and factors associated with paraben exposure and IVF outcomes in this study - Final models were adjusted for age, BMI, race (white vs nonwhite), smoking status (never vs ever), and infertility diagnosis (male factor, female factor, unexplained) <u>Limitations</u> - Study design may not allow extrapolation of the findings to the general population - Misclassification of paraben exposure based on concentrations from spot urine samples is possible	Urinary paraben concentrations were not associated with IVF outcomes; Geometric means of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben were 133, 24 and 1.5 µg/L, respectively; The urinary concentrations were not associated with total or mature oocyte counts, proportion of high embryo quality, fertilization rates, implantation rats, clinical pregnancy, or live births	None of the ORs calculated for total oocyte yield, metaphase II oocyte yield, >1 best embryo quality, and fertilization rate in the 2nd, 3rd, and 4th quartiles of Methylparaben, Propylparaben, and Butylparaben urinary concentrations were statistically-significantly different from those of the 1st quartile, adjusted or unadjusted	126

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	11,311 pregnant women (19-45 year old) in Wuhan city, China	Subjects recruited from 09/2012 to 10/2014	- Concentrations of parabens were measured by UPLC-MS/MS in maternal urine collected before delivery; - Gestational age was calculated based on the date of last menstrual period or assessed by ultrasound data; - General linear models were used to analyze the associations of maternal parabens exposure levels with birth weight and birth length <u>Limitations:</u> - Urinary paraben concentrations measured at one spot time may not reflect prenatal paraben exposure levels and thus cause exposure misclassification; - The sum of the isomers (n-Propylparaben vs. Isopropylparaben, n-Butylparaben vs. Isobutylparaben) were measured in this study (they couldn't be separated by the detection method)	- Methylparaben, Ethylparaben and Propylparaben were detected in 98.3%, 70.9% and 96.4% of the urine samples, respectively; - Butylparaben and Benzylparaben were detected in 15.0% and 2.3%, respectively, and thus were excluded from further statistical analyses; - The SG-adjusted GM and medians of Methylparaben, Ethylparaben and Propylparaben were 5.41 ng/mL (4.20 ng/mL), 0.11 ng/mL (0.09 ng/mL), and 0.94 ng/mL (0.71 ng/mL); - For overall infants, no significant associations were found between maternal urinary parabens and length of infants at birth; - Sex stratification analysis indicated a significant association between urinary Methylparaben and birth length in boys; - No significant associations were observed between urinary parabens and birth length in girls; - Boys in the medium and highest Methylparaben tertiles had a 0.30 (95% CI: 0.01, 0.58) cm and 0.30 (95% CI: 0.01, 0.58) cm longer birth length compared to boys in the lowest tertile, respectively;		[127]
Methylparaben Ethylparaben Propylparaben Butylparaben	922 pregnant women older than 18 years (18 ± 2 weeks gestation) in northern Puerto Rico	2011-2017	- Each woman participated in three study visits: visit 1 was targeted at 16–20 weeks gestation; visit 2 at 20–24 weeks gestation; and visit 3 at 24–28 weeks gestation; - Concentrations of parabens were measured by HPLC-MS/MS in urine samples collected during the three study visits; - Individual paraben concentrations were adjusted for SG; - The gestational age for complete pregnancies was calculated according to the American Congress of Gynecologists (ACOG) recommendations; - Birthweight values extracted from medical records were converted to gestational age and sex specific z-scores, calculated according to the INTERGROWTH-21st standards; - Infants were considered SGA if they fell below the 10th percentile of birthweight z-scores, while infants were considered large for gestational age (LGA) if they fell above the 90% percentile of birthweight z-scores; - Multiple linear regression models were conducted to regress gestational age and birth weight z-scores against woman's log average urinary concentrations of parabens; - Logistic regression models were conducted to calculate odds of preterm birth, SGA and LGA	- Ethylparaben were detected in less than 50% of the samples; - Average Methylparaben and Propylparaben concentrations were strongly correlated (Spearman correlation=0.78, p <0.001); - Propylparaben was moderately correlated with Butylparaben and Ethylparaben (Spearman correlation=0.42, p <0.001); - A protective effect of parabens on SGA was observed <u>Change in Gestational Age Days per IQR Increase in Paraben Concentrations</u>	<u>β Coefficient</u> Methylparaben **1.63(0.37, 2.89)** Ethylparaben − 0.11 (−0.44, 0.23) Propylparaben **2.06 (0.63, 3.48)** Butylparaben 0.60 (−1.23, 2.42)	[121]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			Limitations: - Data collected at three time points may not be sufficient to understand the effects of the measured biomarkers on gestational age; - The variation of concentrations of the exposure biomarkers over time may introduce potential bias, stemming from random measurement error; - Given the multiple comparisons conducted, there is a possibility of chance findings due to Type I error	OR per IQR Increase in Paraben Concentrations Methylparaben Ethylparaben Propylparaben Butylparaben	 **0.66 (0.47, 0.93)** 1.57 (0.86, 2.89) **0.61 (0.41, 0.91)** **0.50 (0.28, 0.88)**	
Methylparaben Ethylparaben Propylparaben Butylparaben	346 infants born to 346 mothers (average age of 34.8 year old) and 184 (average age of 35.7 year old) fathers at the Massachusetts General Hospital Fertility Center	2005 – 2016	- Urine samples were collected before the index pregnancy in both men and women to estimate mean preconception urinary Butylparaben, Propylparaben, Methylparaben, or Ethylparaben concentrations; - Mean maternal prenatal urinary parabens concentrations were estimated by averaging trimester-specific urine samples; - Birth weight and head circumference were abstracted from delivery records; - The association of natural log-paraben concentrations with birth outcomes were estimated using multivariable linear regression models, adjusting for known confounders, such as paternal and maternal age, BMI, smoking, education, and status of in-vitro fertilization based treatment Limitations: - Inherent limitations in measuring exposure in spot urine samples	- None of the maternal preconception parabens concentrations were associated with birth weight; - Maternal preconception Methylparaben concentration was associated with a decreased head circumference of 0.27 cm (95% CI: −0.54, 0), while no associations were observed between other parabens and head circumference; - Prenatal Propylparaben concentration showed a sexually-dimorphic pattern: boys had a 67 g (95% CI: −133, −2) decrease in birth weight compared with only a 2 g (95% CI: −62, 58) decrease among girls		113,194
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Males partners (≥18 years) of 501 couples from16 counties in Michigan and Texas, who discontinued contraception for purposes of becoming pregnant, no physician-diagnosed infertility and couple off contraception for ≤2 months	2005- 2009	-In-person interviews with male partners ascertained lifestyle and reproductive history followed by measuring BMI and a baseline urine sample collection; -After two days of abstinence, male participants provided a baseline semen sample and a second sample 1 month later; - Labeled internal standards were spiked into all samples; concentrations of free parabens were measured in urine samples by UPLC-ESI–MS/MS; limit of quantification ranged from 0.05 to 5.00 ng/mL; - Sperm concentration was assessed using the IVOS system and the IDENT stain, sperm viability was determined by hypo-osmotic swelling (HOS assay), sperm motility was assessed using the HTM-IVOS computer assisted semen analysis system, and Sperm morphometry was conducted using the IVOS METRIX system; - 35 semen parameters were quantified: sperm concentration), semen volume, total sperm count, straw distance, hypoosmotic swollen average path velocity, straight line velocity, curvilinear velocity, amplitude head displacement, beat cross frequency, straightness, linearity, percent motility; length, area, width, perimeter, elongation factor, and acrosome area of head, strict criteria, traditional normal (%), amorphous (%), round (%),pyriform (%), bicephalic (%), taper (%), megalo head (%), micro head(%), neck and midpiece abnormalities (%), coiled tail (%), other tail	Median urinary parabens concentrations among 419 males who both provided urine and semen samples (IQR): Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben Significant associations between urinary parabens concentrations and semen quality parameters: Sperm concentration ($\times 10^6$/mL) Methylparaben Ethylparaben Propylparaben Butylparaben	**Urinary con (ng/mL)** 6.51 (2.16, 26.4) 0.36 (0.17, 1.24) 1.39 (0.49, 5.52) 0.03 (0.01, 0.17) 0.02 (0.00, 0.04) **β Coefficient** −1.91 (−8.03, 4.21) **−6.96 (−12.8, −1.08)** −2.38 (−8.45, 3.69) **−6.89 (−12.9, −0.85)**	129,130

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			abnormalities (%), cytoplasmic droplet (%), immature sperm(n); DNA fragmentation index (%), and high DNA stainability (%); - Significance was assessed using the Chi-Square and Wilcoxon non-parametric tests for categorical and continuous covariates, median and accompanying interquartile ranges (IQRs) of urinary paraben concentrations were calculated; - Urinary concentrations of parabens were modeled individually for each semen parameter and adjusted based age, urinary creatinine, BMI, and race (non-Hispanic White, non-Hispanic Black, Hispanic, other) Limitations: - An observational study design: reliance on a single spot urine, uncorrected comparisons, and potential for residual confounding; - Only 339 men provided sufficient semen samples for the quantification of parabens in seminal plasma	Total count (× 10^6/mL concentration x volume) Methylparaben Ethylparaben Propylparaben Butylparaben Percent motility (%) Methylparaben Ethylparaben Propylparaben Butylparaben Significant associations between seminal plasma parabens concentrations and semen mobility parameters: Percent motility (%) Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben - Inverse associations were observed between urinary concentration increase of Ethylparaben and Butylparaben and sperm count; - Inverse associations were observed between urinary concentration increase of Methylparaben and Ethylparaben and percent motile sperm; - Butylparaben was associated with reductions in most sperm motility parameters: including average path velocity, straight-line velocity, curvilinear velocity, beat cross frequency, percent straightness, and percent linearity; -Hydroxylated paraben metabolites (methyl-protocatechuic acid and ethyl-protocatechuic acid) significantly positively associated with sperm morphology (enhanced semen quality); -Seminal plasma concentrations of Ethylparaben and Benzylparaben were associated with an increased percentage of sperm motility, while urinary concentrations were negatively associated with Ethylparaben	−14.6 (−35.3, 6.05) −18.7 (−38.7, 1.30) −6.67 (−27.3, 13.9) −11.1 (−31.7, 9.46) **−1.56 (−2.87, −0.26)** **−1.5 (−2.76, −0.24)** −1.03 (−2.33, 0.27) −0.95 (−2.25, 0.35) 0.05 (-0.23, 0.34) **0.28 (0.00, 0.56)** -0.13 (-0.42, 0.15) 0.15 (-0.18, 0.49) **0.32 (0.04, 0.60)**	
Methylparaben Propylparaben	936 men of couples seeking infertility treatment at the	2000-2017	- Self-reported demographic, nutritional and reproductive characteristics were collected using standardized questionnaires;	-Decreasing trends were observed for sperm concentration, count, total motility and morphologically normal sperm;		[131]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
	Massachusetts General Hospital		- Urinary concentrations of parabens was quantified by isotope-dilution MS/MS; - Semen samples were analyzed for volume, sperm concentration, count, motility and morphology following WHO guidelines; -Estimate the differences in semen parameters over time by fitting generalized linear mixed models with random intercepts and adjust for abstinence time; - Adjust for demographic, nutritional and environmental factors Limitations: -It is uncertain whether the outcomes from an infertility clinic population can be generalized to men in the general population and in non-Western countries; - Lack of data on all potential predictors, i.e., demographic, nutritional and environmental factors, in all study participants over the study period, which resulted in deficiency of evaluating potential contributors to the trends in semen quality	- Urinary concentrations of parabens remained stable over the study period; - However, the observed trends in sperm sperm concentration and total count were not substantially affected by including parabens in the model		
Methylparaben Ethylparaben Propylparaben Butylparaben	482 pregnant women (130 women delivered preterm <37 weeks gestation and 352 women who delivered after 37 weeks gestation) at the Brigham and Women's Hospital in Boston	2006-2008	- Participants attended four study visits during their pregnancy: visit 1 (4.71–19.1 weeks), visit 2 (14.9–32.1 weeks), visit 3 (22.9–36.3 weeks), and visit 4 (33.1–38.3 weeks); - Demographic and health-related information were collected at the first visit; - Physical examinations were conducted during each visit and both urine and plasma samples were collected; - Parabens were quantified by isotope dilution LC-MS/MS; - Inflammatory biomarkers were measured by ELISA, including pro-inflammatory markers CRP, IL-1β, IL-6, and TNF-α as well as an anti-inflammatory marker IL-10 Limitations: - Unable to assess causality between exposure and inflammatory markers; - The four cytokines measured represented only a fraction of the cytokine repertoire within the maternal immune system; - The study focused on characterizing single pollutant associations for specific toxicants	- An interquartile range increase in Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in IL-1β (95% CI: −14.1, −0.86); - However, the association between Ethylparaben and IL-1β differed across study visits, becoming positive by visit 4; - A greater inverse association between Butylparaben and IL-1β among preterm birth cases compared to controls		[116]
Methylparaben Propylparaben Butylparaben	338 children (159 boys and 179 girls) in the Center for the Health Assessment of Mothers and Children of Salinas	Pregnant women were recruited in 1999–2000	- Mothers were interviewed at two time points during pregnancy (mean: 14.0 and 26.9 weeks' gestation) and when their children were 9 years old; - Information collected during pregnancy included maternal age, marital status, race/ethnicity, country of birth, years in the USA, educational attainment, household income and the number of people in the household. - Timing of puberty was assessed by clinical Tanner staging: Children were examined every 9 months between 9 and 13 years of age (i.e. at age 9 (n = 312), 9¾ (n = 268), 10½ (n = 300), 11¼ (n = 275), 12 (n = 301) and 12¾ (n = 264);	- With peripubertal exposure in girls at age 9, associations of earlier thelarche (mean shift = −1.1 months, 95% CI: −2.1, −0.0), pubarche (mean shift = −1.5 months, 95% CI: −2.5, −0.4), and menarche (mean shift = −0.9, 95% CI: −1.6, −0.1) were observed with each doubling of urinary concentrations of Methylparaben, and earlier pubarche (mean shift = −0.8, 95% CI: −1.6, −0.1) with each doubling of Propyl paraben concentrations; - In boys, no prenatal parabens were associated with pubertal timing; with peripubertal concentrations,		[132]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Spot urine samples were collected from mothers at the time of the two pregnancy interviews (prenatal samples) and from the children at the 9-year-old visit (peripubertal samples); - Urinary concentrations of three parabens were quantified by isotope dilution LC-MS/MS; - LOD was 1.0 ng/mL for Methylparaben, 0.2 ng/mL for Propylparaben and Butylparaben; - For prenatal exposure, average of the creatinine-corrected concentrations were used in the two pregnancy urine samples, while for peripubertal exposure, single creatinine-corrected concentration was quantified in children's urine; - Parametric accelerated failure time (AFT) models were conducted to determine the association of urinary biomarker concentrations with timing of pubertal onset using the Stata intcens program Limitations: - Given parabens are quickly metabolized, urinary parabens typically reflect exposure in the past 24–48 h and can not accurately reflect usual exposure; - One or two urinary measurements are not sufficient to characterize usual parabens exposure over the prenatal and peripubertal periods; - Because the study participants lives in an agricultural community, potential confounding factors exist, such as pesticides exposure; - The study population was limited to Latino children of low socioeconomic status and may not be widely generalizable; - Unable to assess causality between peripubertal measurements and parabens exposure: children going through puberty early are more likely to use PCPs	an association of earlier gonadarche with each doubling of Propylparaben (mean shift = −1.0 months, 95% CI: −1.8, −0.1) was observed; - Butylparaben was detected in<40% of samples and was not included in the analyses; - In prenatal urine samples collected in pregnancy, the GM concentrations of Methylparaben and Propylparaben were 36.4, and 34.5 ng/g creatinine, respectively; - In peripubertal urine samples collected at 9 years of age, the GM concentrations of Methylparaben and Propylparaben were 44.9, 4.9 ng/g creatinine, respectively		
Methylparaben Propylparaben Butylparaben	241 pregnant women (between 18 and 45 years) from the Massachusetts General Hospital Fertility Center in Boston	2005-2015	- Used data on women who had completed at least one in vitro fertilization cycle, and provided at least one urinary sample during 1st or/and 2nd trimester; - Blood glucose levels were assessed as a continuous outcome during the 2nd trimester of pregnancy (median: 27 weeks gestation) through a 1-h non-fasting, 50-g GLT used as the first step in screening for GDM; - Women with glucose levels >140 mg/dL as having abnormal GLT; - Urine samples were collected during the 1st and 2nd trimesters of pregnancy (median: 7 and 21 gestation weeks, respectively); - When two urine samples were available (about 80% of measurements), the geometric mean of the SG-adjusted concentrations was used as a measure of trimester-specific urinary paraben; - All models were adjusted for the following confounders: maternal age, pre-pregnancy BMI, total physical activity, race, smoking status, education level, infertility diagnosis, number of fetuses, previous IVF, previous intrauterine insemination; - The LODs were 1.0 μg/L for Methylparaben, and 0.2 μg/L for Propylparaben and Butylparaben; all paraben concentrations were adjusted for SG;	- 1st trimester Butylparaben and Propylparaben urinary concentrations were associated with glucose levels in a pregnancy cohort of women at high risk of GDM Association between Pregnancy Glucose Levels and the 1st trimester Parabens Mixture (4th vs. 1st quartiles) Methylparaben Propylparaben Butylparaben Association between Pregnancy Glucose Levels and the 2nd trimester Parabens Mixture (4th vs. 1st quartiles) Methylparaben Propylparaben Butylparaben	β Coefficient (Adjusted) 13.1 (-7.9, 34.0) ***-22.3 (-43.2, -1.4)*** **12.5 (0.9, 24.2)** β Coefficient (Adjusted) -4.8 (-19.8,10.3) 1.2 (-13.6,16.0) **11.2(0.2,22.3)**	133

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Methylparaben, Butylparaben, and Propylparaben were evaluated separately or simultaneously as a chemical mixture; linear regression models or BKMR method were applied Limitations - Only evaluated continuous glucose levels; - The analysis did not include other chemicals that may be associated with glucose levels, e.g., phthalates			
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	850 pregnant women (between 20 to 44 years)-infant pairs at Wuhan Women and Children Medical and Healthcare Center in Hubei Province, China	2014-2015	- Maternal urine samples collected at the first, second, and third trimesters during pregnancy; - Paraben concentrations were analyzed by UPLC–MS/MS; the LODs were 0.01 ng/mL for Ethylparaben and Benzylparaben and 0.05 ng/mL for Methylparaben, Propylparaben and Butylparaben; - Urinary paraben concentration was adjusted for the SG; - Birth and early childhood weights and heights were normalized to z-scores by applying WHO child growth standards specified by sex and age Limitations: - Pregnancy exposure is limited by low to moderate interclass correlation coefficients, indicating the temporal variability of paraben concentrations throughout pregnancy; - The information regarding collection conditions of urine samples, e.g., the hour of sampling and time since last void, were not considered in the analyses; - Without collecting data on lactational or other sources of paraben exposure during early childhood, which may also influence growth during childhood	- Results suggested negative associations between prenatal paraben exposure and fetal and childhood growth; - The third trimester may be the window of susceptibility <u>Association of Urinary Paraben Concentrations with Weight Z-score at Birth (All, n=850)</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <u>Association of Urinary Paraben Concentrations with Weight Z-score at Birth (Male, n=446)</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	<u>β Coefficient</u> -1.83% (-4.75%, 1.09%) ***-2.82% (-5.11%,- 0.53%)*** -1.51% (-3.84%, 0.82%) 0.14% (-13.11%, 13.40%) -0.65% (-19.24%, 7.13%) <u>β Coefficient</u> -0.47%(-4.58%, 3.65%) ***-3.61% (-6.74%,- 0.48%)*** -0.70% (-3.90%, 2.51%) -0.81% (-19.12%, 17.49%) -5.29% (-24.02%, 13.43%)	134
Methylparaben Ethylparaben Propylparaben Butylparaben	473 mother–son pairs from the EDEN cohort study, the obstetrical departments of the university hospitals of Nancy and Poitiers, France	2003-2006	- Placental and birth weight were obtained at birth from hospital maternity records; - Concentrations of parabens were measured in a single spot urine sample collected during pregnancy; - All paraben concentrations were adjusted by creatinine Limitations: -The high frequency of missing placental weight led to an underrepresentation of mother–son pairs; - A delay in the weighing of the placenta after delivery may lead to a lower weight estimate; - Missed other placental characteristics, such as placental diameter, thickness, shape, and vascularization, etc.	- A positive association between the sum of parabens and placental weight β=7.12 (95% CI: 0.41, 13.9), p=0.04		135
Methylparaben Ethylparaben Propylparaben	1087 pregnant women at Wuhan Women and Children Medical Care	2014-2015	- The random spot urine samples were collected between 8 and 16 weeks of gestation (on average 13 weeks);	- A total of 103 (9.5%) women were diagnosed with GDM;		136

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Butylparaben Benzylparaben	Center in Wuhan, China		- Only included the first delivery records for women who had two separate deliveries; - Standard face-to-face interviews were conducted to collect retrospective information about sociodemographic characteristics (maternal age and education) and lifestyle habits during pregnancy (smoking, passive smoking, and alcohol consumption); - Paraben concentrations were analyzed by HPLC–MS/MS; The LODs were 0.01 ng/mL for Ethylparaben and Benzylparaben, and 0.05 ng/mL for Methylparaben, Propylparaben and Butylparaben; - Urinary paraben concentration was adjusted for the SG; the total concentrations of parabens Σparabens = [1×Methylparaben+16.7×Ethylparaben+83.3×Propylparaben+250×Butylparaben]; Benzylparaben was excluded for the calculations due to the low detection rate; - GDM was assessed by 75-g OGTT; women were diagnosed with GDM according to the IADPSG recommendations Limitations: - The interviews were conducted at delivery, which was after the diagnosis of GDM and might resulted in recall bias; - The information on the family history of diabetes was self-reported, and thus pregnant women with a family history of diabetes and type 2 diabetes may not be totally excluded; - Information on food consumption was not collected, which may be related to GDM risk or paraben levels; - The paraben concentrations measured at one spot time may not accurately reflect paraben exposure	- The detection rate of urinary Methylparaben, Ethylparaben and Propylparaben is >90%, while Butylparaben and Benzylparaben were detected in less than 50% urine samples; - There was no evidence of associations between urinary Methylparaben or Propylparaben and GDM; - After adjustment for potential confounders, including maternal age, education, maternal pre-pregnancy BMI, parity, and cadmium levels, urinary Ethylparaben was associated with GDM Ethylparaben < 0.24 μg/L 0.24-0.54 μg/L 0.54-1.93μg/L ≥ 1.93 μg/L **p_{trend} =0.051**		
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	478 mother-child pairs at Wuhan Women and Children Medical Care Center in Wuhan, China	2014-2015	- Three spot urine samples collected in the first (13.0 ± 1.2 weeks), second (23.6 ± 3.4 weeks) and third (36.1 ± 3.3 weeks) trimester during pregnancy; - Paraben concentrations were analyzed by UPLC–MS/MS and adjusted for the SG; - At the age of around 24 months, the participating children were given the BSID assessments, which provided two main scales: the MDI to assess cognition, language and social development, and the PDI to assess gross (crawling, sitting, walking) and fine (isolation of fingers, grasping) motor skills; - The paraben sum (Σparabens) was calculated by the sum of molar concentrations of five parabens; - To examine windows of vulnerability to exposure during pregnancy, generalized estimating equations were used to examine the relationships of parabens concentrations over trimesters with BSID results to jointly evaluate the exposure-outcome relationships at each trimester; - All models were adjusted for the following confounders: maternal education (≤ high school, college, or ≥ bachelor's degree), child sex, passive smoking during pregnancy as well as maternal age and pre-pregnancy BMI	- Butylparaben and Benzylparaben were detected less frequently (< 50%) of urine samples and were not included in the statistical analysis; - In the adjusted models, each 2-fold increase in average prenatal paraben concentration was significantly associated with lower MDI scores among girls [**−1.08 (95% CI: −2.10, −0.06)** and **−1.51 (95% CI: −2.69, −0.32**) for Methylparaben and Σparabens, respectively]; - The association was not statistically significant among boys; - In trimester-specific analyses, increasing parabens was associated with lower girls' MDI only in the second trimester; - The results suggested that prenatal exposure to parabens may be associated with impairment in child cognitive abilities at 2 years		137

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Propylparaben Butylparaben	392 mother-child pairs in the Salinas Valley, California	1999–2000	- Participants were enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, examining the effects of environmental exposures in an agricultural community; - Parabens were measured in urine collected twice during pregnancy from 392 women; - Interviews were conducted with the mothers in English or Spanish using structured questionnaires at two times during pregnancy (mean 13 and 26 weeks gestation); - Mothers were also interviewed at delivery, and when the child was six months, one year, two years, three and a half years, five years, and seven years old; - Children were classified as having "probable asthma" at age seven if they were currently taking asthma medication or had two or more of the following criteria: any current respiratory symptom, doctor diagnosis of asthma at any age, or a positive bronchodilator test - Paraben concentrations were analyzed by solid phase extraction coupled with isotope dilution HPLC-MS/MS and adjusted for the SG; - Intracellular Th1 and Th2 cytokines were detected in unfrozen pediatric whole blood, collected at ages two, five, and seven, using flow cytometry; - Bayesian Model Averaging (BMA) was analized to determine which chemical exposures to include in the fully adjusted regression models. Limitations: - The concentrations of biomarkers in this study have relatively low intraclass correlation coefficients, demonstrating relatively high variability in exposures	-Methylparaben and Propylparaben were detected in over 95% of samples, while Butylparaben was not (detected in 66.5% of early pregnancy samples and 71.0% of late pregnancy samples); - 37 children (11%) were categorized as having probable asthma, 87 (25%) as having inhalant allergies; - In fully adjusted models, Methylparaben was associated with lower Th1% (RR: −3.35, 95% CI: −6.58, −0.02) and Th2% at borderline significance (RR: −4.45, 95% CI: −8.77, 0.08); - In fully adjusted models, Propylparaben was associated with decreased odds of probable asthma (OR: 0.86, 95% CI: 0.74, 0.99)		[27,102]
Methylparaben Ethylparaben Propylparaben Butylparaben	480 pregnant women at Brigham and Women's Hospital in Boston	Subjects recruited from 10/2006 and to 09/2008	- Study includes 130 cases of preterm birth (defined as delivery before 37 weeks gestation) and 350 random controls; - At the firststudy visit (median 9.7 weeks gestation), participants completed demographic questionnaires to provide information e.g., race/ethnicity, tobacco and alcohol use, in addition to providing urine and blood samples for biomarker analysis; - During the three subsequent visits (median 17.9 weeks, 26.0 weeks, and 35.0 weeks), additional biological samples were collected as well as clinically relevant pregnancy Characteristics; - All gestational age dating was validated by first trimester ultrasound measurements; - Urine samples underwent enzymatic deconjugation, solid phase extraction, and analysis with a triple quadrupole MS; Urinary paraben concentrations were adjusted by SG; - Associations between parabens and preterm birth were estimated using multivariate logistic regression Limitations:	- Of 130 cases of preterm birth, there were 75 cases of spontaneous preterm birth (characterized by spontaneous preterm labor and/or preterm premature rupture of membranes), and 37 cases of placental preterm birth (characterized by preeclampsia and/or intrauterine growth restriction); - Methylparaben was detected in the most samples (> 99%), whereas Ethylparaben was not detected in 40.5% of samples (LOD=1ng/mL); - Compared to concentrations in pregnant women from the NHANES (2005–2010), higher median concentrations for Methylparaben (151 ng/mL, NHANES: 84.7 ng/mL) and Propylparaben (37 ng/mL; NHANES: 20.6 ng/mL) were observed;		[116]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Study does not contain data on dietary patterns, a confounder for paraben exposure and preterm birth; - Study does not includes data on direct socioeconomic metrics such as household income, which can be an important predictor of environmental exposures; - Urinary measurements are reflective of recent exposures, which may cause non-differential measurement error. and preterm birth; - The study does not account for co-exposure to other toxicants that are responsive for birth outcomes, such as heavy metals and persistent organic pollutants;	- Ethylparaben was associated with increased risk for placental preterm birth OR=1.47 (95% CI: 1.14 – 1.91);		
Methylparaben Propylparaben	420 women (18-45 years) undergoing IVF treatment at the Massachusetts General Hospital Fertility Center	2006-2017	- Participants were women enrolled in the EARTH Study, who completed at least one IVF cycle (n = 648 cycles); - Women provided one (23%) or two (77%) spot urine samples per IVF cycle: Visit 1 (between Day 3 and Day 9 of the gonadotrophin phase), and Visit 2 (on the day of oocyte or on day of embryo transfer); - Parabens were measured by online solid-phase extraction coupled with isotope dilution HPLC-MS/MS, and adjusted for SG; -FSH was measured in a blood sample collected on the third day of the menstrual cycle by automated electrochemiluminescence immunoassay; - Infertility diagnosis was coded according to SART standard, including male and female infertility factors, and idiopathic infertility; - Women underwent one of three controlled ovarian stimulation IVF treatment protocols on day 3 of induced menses after completing a cycle of oral contraceptives: (1) luteal phase GnRH-agonist protocol, (2) follicular phase GnRH-agonist/Flare protocol, or (3) GnRH-antagonist protocol; - All clinical outcomes (i.e. implantation, clinical pregnancy and live birth) were assessed identically for fresh, cryo-thaw, and donor-egg recipient cycles Limitations: - It is not applicable to generalize the findings to couples from the overall population; - Exposure misclassification is possible given the short biological half-lives of parabens; - Other EDCs (e.g., phenols, phthalates) were not measured, which may resulted in residual confounding; - Study did not consider male partner's exposure	- The detection frequencies for urinary concentrations of Methylparaben and Propylparaben were above 98%; - Methylparaben and Propylparaben) concentrations are highly correlated (Spearman r = 0.86); - Urinary paraben were not associated with the IVF outcomes examined		[126]
Methylparaben Ethylparaben Propylparaben Butylparaben	252 adolescents at St. Luke's Hospital in Massachusetts	2008-2014	- Data collected from NBC project, in which mother-infant pairs were recruited after delivery from 1993 to 1998; - Between 2008 and 2014, in-person neurodevelopmental testing was done on NBC participants at 15 years of age; - Of 252 NBC adolescents, 144 (70%) provided two urine samples and the rest collected only one sample;	-LODs were 1µg/L for Methylparaben and Ethylparaben and 0.1 µg/L for Propylparaben and Butylparaben; - Urinary concentrations of ∑Parabens were not associated with BSI, externalizing and internalizing behaviors;		[138]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Urinary parabens were measured by online solid phase extraction coupled with HPLC-MS/MS; - A summary measure for total paraben exposure (∑Parabens), was created as the molar sum of the four parabens; - Participants' teachers completed the Behavior Assessment System for Children Second Edition -Teacher Rating Scale (BASC-2 TRS) a median of approximately 2.5 months (IQR: 4.5 months) after urine was collected; - Possible non-linear relationships between urinary paraben concentrations and behavioral outcomes were examined using restricted cubic splines	-A two-fold increase in urine ∑Parabens concentration was not associated with BASC-2 scores: Adaptive Skills β= − 1.44 (95%CI: −4.53, 1.64) and Developmental Social Disorders β= 0.13 (95%CI: −0.38, 0.65);		
Methylparaben Ethylparaben Propylparaben Butylparaben	152 pregnant women in Europe	2014-2015	- Participates enrolled in HELIX project,: 52 from Barcelona (Spain), 46 from Grenoble (France) and 55 from Oslo (Norway); - The women collected 2–3 urines per day during one week in the second trimester and one week in the third trimester; - Blood pressure measurement was performed at the end of each week using the OMRON 705-CPII automated oscillometry; -Parabens were quantified by UPLC-MS/MS	- Significant decreases in diastolic blood pressure were associated with exposure to parabens including Methylparaben, Ethylparaben, and Butylparaben in the second trimester (β = −0.62 mmHg; 95%CI: −1.16, −0.08 per doubling of Methylparaben concentrations); - Significant interactions were observed between maternal BMI and exposure to Ethylparaben during the 2nd trimester: the decrease in systolic and/or diastolic BP reported above were only observed among overweight/obese women (i.e., BMI > 25 kg/m2; $p_{interaction} < 0.05$);		[139]
			Retrospective Studies			
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	185 pregnant women (18 to 45 years of age) recruited from Brooklyn's Prenatal Clinic and their singleton infants	Subjects recruited from 10/2007 to 12/2009	- Random spot urine specimens were provided once per participant during last 4 months of pregnancy; - Convenience subset of the subjects were followed to delivery, when umbilical cord blood was collected; - Maternal urinary concentrations were measured; - Random subset of umbilical-blood plasma samples were analyzed for free and total parabens; - Questionnaire was used to gather demographic; - Neonate outcome data were from patient charts; - Urinary biomarker concentrations were corrected for creatinine levels and were log-transformed; - Non-detect values were treated as the MDL divided by the square root of 2; - Covariates were selected if they achieved $p < 0.05$ in Spearman correlations or Chi-square tests in relation to biomarker concentrations or birth outcomes; - Measures of birth outcomes (body length, gestational age at birth, birth weight, and head circumference) were analyzed using linear models;	In regression models adjusting for confounders, adverse exposure-outcome associations observed between Butylparaben concentrations and increased odds of PTB, decreased gestational age at birth and birth weight, and decreased body length (Propylparaben), and between Benzylparaben concentrations and protective effects on PTB ($p<0.05$). No associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated <u>Low Birth Weight and Maternal Urine Concentrations</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <u>Low Birth Weight and Cord Blood Concentrations</u>	**<u>OR</u>** 0.83 (0.37-1.87) 1.18 (0.74-1.89) 0.92 (0.44-1.94) 1.45 (0.88-2.39) NA	[140]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Multiple linear regression analysis was used to evaluate concentration-outcome associations adjusted for maternal age, nativity, neonate gender, and alcohol and tobacco use; additional adjustments were made for confounders independently associated with outcomes or which changed the magnitude of effects by ≥ 5%; -Relationships between concentrations and dichotomous outcomes were analyzed by logistic regression <u>Limitations</u>: - Maternal urine was used as a proxy for fetal exposure, except where neonate cord blood plasma was available; - Timing of sampling may have biased results; product use contributing to exposure may differ over the course of the pregnancy; - Multiple urine levels may be more appropriate to capture variability and characterize exposures; - No correction was made for conducting multiple data comparisons; - Small size and homogeneity of the participant population the limit generalizability of the results	Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben Preterm Birth and Maternal Urine Concentrations Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparabe <u>Preterm Birth and Cord Blood Concentrations</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparabe	NA 1.89 (0.62-5.81) 1.52 (0.66-3.45) 10.27 (0.68-156.07 0.18 (0.01-2.63) 0.78 (0.40-1.54 1.15 (0.78-1.69) 1.27 (0.67-2.43) 1.42 (0.93-2.16) NA NA 2.65 (0.83-8.48) 1.86 (0.84-4.08) 60.77 (2.60-1417.93) 0.03 (0.01-0.44)	
Methylparaben Ethylparaben Propylparaben Butylparaben	520 mother-son pairs with complete data on prenatal (3 ultrasound measurement), neonatal (biometry), and postnatal growth up to 3 years of age (≥4 weight/height measurements and clinical exam), recruited before the end of gestation week 28 from Poitiers and Nancy University hospitals (France)	Subjects recruited from 4/2003 to 3/2006	- Biparietal diameter was measured by ultrasound during gestation weeks 12.6, 22, and 32.6 (on average); - Fetal head circumference, abdominal circumference, and femur length were assessed during the last 2 ultrasound examinations; - Fetal weights were estimated from measures of abdominal circumferences, femur lengths, head circumferences, and biparietal diameter; - Weight and length at birth were extracted from hospital records; - Infants were weighed and measured at 1 and 3 years of age; - Mothers were mailed questionnaires at 4, 8, 12, 24, and 36 months about the boys' weight and height measures; - Jenss nonlinear model was used to evaluate growth and predict weight and height at 6, 12, 24, and 36 months; - Head circumference was assessed within 4 days after birth and at 3 years; - Abdominal circumference was measured at 3 years; - Urine samples were collected between gestation weeks 22 and 29 - Total paraben concentration was calculated by summing molar concentrations of the 4 parabens; - Non-detects were replaced by the lowest instrumental reading value divided by the square root of 2; - Concentrations were standardized for collection conditions, including creatinine concentrations; - Cross-sectional analyses and linear regression models with a random effect variable corresponding to the mother-son pair were used to study associations between concentrations and growth parameters; - Models for prenatal and postnatal growth were adjusted for maternal and paternal height, pre-pregnancy weight, maternal active	No statistically-significant associations were found between maternal urinary paraben concentrations during pregnancy and prenatal or postnatal growth of male newborns. However, maternal urinary concentrations during pregnancy appeared to be positively associated with body weights: <u>Body Weight at Birth</u> Methylparaben Ethylparaben Propylparaben Butylparaben <u>Body Weight at 6 Months</u> Methylparaben Ethylparaben Propylparaben Butylparaben <u>Body Weight at 12 Months</u> Methylparaben Ethylparaben Propylparaben Butylparaben <u>Body Weight at 24 Months</u> Methylparaben	**β Coefficient** 36.0 (-12.4-84.4) 49.9 (-2.21-102) 48.0 (-3.64-99.6) 50.1 (-5.69-106) 85.3 (-16.5-187) 17.8 (-92.9-129) 80.1 (-27.4-188) 55.8 (-62.0-174) 81.2 (-45.4-208) 2.60 (-135-140) 79.1 (-54.9-213) 54.5 (-91.1-200) 128 (-31.88-287) 45.3 (-128-219)	[141]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			and passive smoking during pregnancy, maternal education, recruitment center, and parity; - Model for head circumference was also adjusted for number of days between birth and assessment of head circumference - Analyses of postnatal growth were additionally adjusted for breastfeeding duration; - Effect estimates were reported for an increase by 1 IQR of ln-transformed standardized concentrations <u>Limitations:</u> - Use of only 1 urine sample to assess paraben concentrations increases the chances of exposure misclassification - Use of estimates of caloric intake (rather than specific food usually eaten) increases the chance of confounding by differences in eating behavior.	Ethylparaben Propylparaben Butylparaben <u>Body Weight at 36 Months</u> Methylparaben Ethylparaben Propylparaben Butylparaben β coefficients calculated for Ethylparaben and Butylparaben, body weights estimated at the 3rd ultrasound examination, were 13.00 (-13.1-39.1) and 23.5 (-3.96-50.9), respectively; coefficients for all other parameters were < 7.5 with CIs spanning across negative and positive values	116 (-53.3-285) 111 (-71.2-294) 193 (-3.88-389) 113 (-101-327) 159 (-49.4-368) 179 (-45.3-404)	
Methylparaben Ethylparaben Propylparaben Butylparaben	28 boys diagnosed with cryptorchidism and/or hypospadias at San Cecilio University Hospital of Granada: 19 cryptorchidism cases (n=9 unilateral, 6 bilateral), 12 hypospadias cases, 1 case with both disorders; 51 matched controls	Subjects recruited from 10/2000 to 7/2002	- This was a case-control study nested within a prospective birth cohort study of risk factors for male urogenital malformations; - All boys in the cohort were examined at birth and those diagnosed with cryptorchidism and/or hypospadias were re-examined at 1month of age; -Information on potential confounding variables related to parents, pregnancy/delivery and activities were gathered from structured interviews with the mother within 48 h after delivery; - There was a larger proportion of mothers reporting historical (pre-pregnancy) use of oral contraceptives in the selected versus non-selected cases (21% vs. 53%, p=0.034), although not in the selected versus non-selected controls (37% vs.42%, p=0.686) - Placentas were collected immediately after delivery and analyzed by UPLC–MS/MS; - Crude and adjusted ORs and corresponding 95% CIs were calculated by conditional logistic regression; - Concentrations of parabens were used as independent variables and analyzed both as continuous variables and in tertiles, with the first tertile as the reference group; - Concentrations below the LOQ were assigned a value of half of the LOQ; - Potential confounding variables were selected if they were statistically-significantly associated with outcomes in bivariate analyses or changed the β coefficient by >20% in the multivariable analysis; - Only maternal age and newborn birthweight had a substantial effect on results; - In the bivariate analyses, differences between groups were tested with Pearson's chi-square test or Fisher's exact test, when appropriate <u>Limitations:</u>	Methylparaben <0.4 ng/g 0.44-1.91 ng/g 1.96-11.69 ng/g Concentration as continuous variable Ethylparaben <LOD 0.07-0.89 ng/g 0.91-5.49 ng/g Concentration as continuous variable Propylparaben <LOD 0.06-1.15 ng/g 1.16-5.52 ng/g Concentration as continuous variable Butylparaben <0.08 ng/g 0.16-0.74 ng/g 0.79-1.60 ng/g Concentration as continuous variable Methylparaben <0.4 ng/g 0.44-1.91 ng/g 1.96-11.69 ng/g Concentration as continuous variable Ethylparaben <LOD	**OR (unadjusted)** 1.00 1.00 (0.32-3.09) **3.18 (0.88-11.48)** 1.17 (0.94-1.46) 1.00 0.29 (0.08=1.06) 1.51 (0.44-5.15) 1.07 (0.74-1.55) 1.00 1.23 (0.30-5.04) **4.72 (1.08-20.65)** **1.90 (1.12-3.22)** **OR (adjusted)** 1.00 2.29 (0.65-8.05) 2.31 (0.72-7.46) 2.27 (0.8-6.42) OR (adjusted) 1.00 1.04 (0.33-3.26) 3.24 (0.83-12.69) 1.17 (0.93-1.48) 1.00	[142]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Relatively small sample size prevented adjustment for some potential confounders, such as the type of delivery, fetal; presentation, weeks of gestation, child length, head size, presence of other malformations and season of birth; - Exposure assessment made in term placentas may have resulted in exposure misclassification; - Cryptorchidism and hypospadias grouped together for statistical analysis discounts the fact that these conditions are related to inset mechanisms occurring at different critical stages in gestation	0.07-0.89 ng/g 0.91-5.49 ng/g Concentration as continuous variable Propylparaben <LOD 0.06-1.15 ng/g 1.16-5.52 ng/g Concentration as continuous variable Butylparaben <0.08 ng/g 0.16-0.74 ng/g 0.79-1.60 ng/g Concentration as continuous variable	0.26 (0.07-1.00) 1.25 (0.34-4.60) 1.00 (0.68-1.47) 1.00 1.39 (0.33-5.91) 6.42 (1.16-35.47) 2.16 (1.16-4.01) 1.00 2.26 (0.62-8.21) 2.11 (0.62-7.16) 2.07 (0.71-6.06)	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	436 3-year old children recruited from Sheyang Maternal and Child Health Care Centre (China)	Subjects recruited between 7/2012 and 4/2013	- Questionnaire survey was administered to each child's caregiver by trained interviewers, covering sociodemographics, living environment and lifestyles; - Pregnancy and maternal health information was obtained from medical records and questionnaires; - Spot urine sample was collected from each child, and urinary paraben concentrations were measured by LVI-GC-MS/MS; - EDI_{urine} of parabens was calculated based on urinary concentrations and a steady-state toxicokinetic model; - Anthropometry measurements were compared with sex-specific WHO child growth standards, and age- and sex-standardized z scores were calculated; - Generalized linear models were used to examine associations between SG-adjusted concentrations and body growth outcomes; - Individual paraben concentrations and the $P_{parabens}$ were adjusted for SG; - Analyses of quartiles of $P_{parabens}$ were conducted separately - Urinary concentrations were log transformed for univariate and multivariate analyses; - Associations between concentrations and sociodemographic characteristics were examined using a Wilcoxon rank-sum or Kruskal-Wallis rank sum test; - Log-transformed concentrations were assessed using Pearson correlation coefficients; - Concentrations below LOD were substituted with LOD divided by the square root of two; - Covariates considered included: maternal and paternal BMI, child's sex, maternal education, family income, habitation in town, suburb or countryside, feeding pattern, smoking status, time spent outdoors, sampling season, and birth outcome; - Potential confounders that were separately include: urinary bisphenol A, triclosan, and benzophenone-3 concentrations	Weight z Score (Boys) Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben ∑Parabens Height z Score (Boys) Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben ∑Parabens All β coefficients calculated for girls and all other β coefficients for boys were not statistically significant	**β Coefficient** 0.08 (-0.06-0.23) **0.16 (0.03-0.28)** 0.00 (-0.16-0.17) 0.12 (-0.09-0.32) -0.04 (-0.18-0.10) 0.17 (-0.04-0.39) 0.11 (-0.02-0.26) **0.15 (0.03-0.27)** 0.05 (-0.11-0.21) 0.14 (-0.06-0.34) 0.08 (-0.06-0.21 **0.23 (0.03-0.43)**	[143]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			Limitations: - Spot urine samples may cause exposure misclassification; - Specific diet information was not sufficiently obtained and evaluated			
Methylparaben Propylparaben Butylparaben	Female participants of a prospective fertility study at the MGH Fertility Center, undergoing infertility evaluation, n=109 to 142, depending parameter measured	2004-2010	- Subjects had at least one hormonal or ultrasonographic marker of ovarian reserve measured and contributed at least one urine sample; - Clinical information was abstracted from medical records; - Intravenous blood sample was drawn on the 3rd day of the; menstrual cycle, and the serum was analyzed for FSH - AFC and OV were measured for both ovaries using transvaginal ultrasound; - Each patient was given an infertility exam and diagnosis by a physician at the MGH Fertility Center; - Demographic data were collected using a nurse-administered questionnaire at entry into the study; - Convenience spot urine sample was collected at recruitment and at subsequent visits during infertility treatment cycles; - Paraben concentrations were measured by HPLC-MS/MS; - Distribution of exposures was summarized using the median, IQR, and range of urinary paraben concentrations; - Urinary concentrations below LOD were assigned a value equal to the LOD divided by the square root of two; - Concentrations were corrected for SG; - Spearman's rank correlation coefficients (r_S) were calculated for markers of ovarian reserve, age, and BMI; - Multivariable linear regression was used to estimate associations between within-person paraben concentrations (divided into tertiles) and day-3 FSH and OV; OV was ln-transformed before all regression analyses; - Poisson regression was used to estimate associations between within-person paraben concentrations (tertiles) and AFC; - Covariates considered included age at time of outcome and BMI determinations at study entry into the study; - MPC in outcome from the lowest tertile of paraben concentrations was calculated for both OV and AFC; - Secondary analysis combined concentrations of parabens using two methods: an EEQ factor approach, and summation of concentrations; - Multivariable linear regression was used to evaluate association between EEQ (parabens) and Σ(parabens) with day-3 FSH and OV; Limitations: - Time period of collection of the urine samples was up to 3 years before the outcome measure; - Relatively small sample size; - Not all subjects had all three of the outcome measures;	Methylparaben Tertile 1 (5.13-132 µg/L) Tertile 2 (145-377 µg/L) Tertile 3 (381-2,428 µg/L) p_{trend} =0.31 Propylparaben Tertile 1 (<LOD-25.2 µg/L) Tertile 2 (26.3-81.8 µg/L) Tertile 3 (87.8-727 µg/L) p_{trend} =0.07 Butylparaben Tertile 1 (<LOD-0.73 µg/L) Tertile 2 (0.75-5.12 µg/L) Tertile 3 (5.44-177 µg/L) p_{trend} =0.86 All MPCs and p_{trens} calculated for AFC and OV were not statistically significant	**MPC in AFC** 0 (Reference) -6.8 (-23.5-13.7) -10.6 (-28.2-11.2) 0 (Reference) -5.0(-23.7-18.4) -16.3 (-30.8-1.3) 0 (Reference) -4.8 (-22.5-16.8) -2.0 (-21.0-21.6)	144

Table 17. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Inclusion of high proportion of Caucasian and older women and sole inclusion of women from a fertility clinic undergoing in vitro fertilization or intrauterine insemination (all with varied SART diagnoses) may limit generalizability of findings			
Methylparaben Propylparaben Butylparaben	194 male partners (18 to 55 years old; mean = 36.7 years of age) of subfertile couples seeking treatment from the Vincent Memorial Obstetrics and Gynecology Service, Andrology Laboratory, Massachusetts General Hospital (MGH)	2000-2004	- A single spot urine sample was collected on day of each subject's clinic visit; 2nd and 3rd samples were collected from a subset of men at subsequent visits; - Concentrations of total (free + conjugated) parabens were measured in urine samples by HPLC-MS/MS; - One nonfasting blood sample was drawn on the same day and time as the first urine sample; - Serum testosterone, E2, sex-hormone-binding globulin, inhibin B, FSH, LH, prolactin, free thyroxine (T4), total triiodothyronine (T3), and TSH were measured; - Free androgen index (FAI), testosterone:LH ratio, FSH:inhibin B and E2:testosterone ratios were calculated; - Semen quality parameters and motion characteristics were measured: sperm concentration, motility, and motion parameters; - Total sperm count was calculated and sperm morphology was assessed; - Sperm damage was assessed by comet assay: comet extent, tail distributed moment (TDM), and percent DNA located in the tail (Tail%) were determined; - Multivariable linear regression was used to explore relationships between urinary paraben concentrations and hormone levels, semen quality parameters, and sperm DNA damage measures; - Distribution of sperm count, sperm concentration, FSH, LH, SHBG, prolactin, TSH, all calculated hormone ratios, and paraben concentrations were ln-transformed for statistical analyses; - Paraben concentrations < LOD were assigned values of LOD/2 - Inclusion of covariates in the multivariable models was based on statistical and biologic considerations; - Age and BMI were modeled as continuous variables; abstinence period was treated as an ordinal categorical variable; - Race, smoking status, and timing of the clinic visit by season and time of day were considered for inclusion as dichotomous variables; - Covariates with $p < 0.2$ in their relationship with one or more paraben or ≥ 1 outcome measure in preliminary bivariate analyses were included in a "full" model; - Covariates with $p > 0.15$ in full models for all measures within the three sets of outcomes (hormone levels, semen quality, sperm DNA damage) were removed from the final models Limitations: - Urine samples were collected weeks or months after, rather than before, serum and semen samples were collected; - Only a single blood or semen sample was available for assessment of hormone levels, semen quality, and sperm DNA damage;	Comet Tail % Butylparaben <0.2 µg/L 0.2-0.6 µ/L >0.6 µg/L **P_{trend}=0.03** No other comparisons were statistically significant in this study	**β Coefficient (adjusted)** 0 6.81 (-1.80-15.4) 8.23 (-0.41-16.9)	145

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Cross-sectional design restricts the ability to draw conclusions about causal relationships; - Relatively small sample size provided low statistical power			
			Cross-sectional Studies			
Methylparaben Ethylparaben Propylparaben Butylparaben, Isobutylparaben	315 men who attended the infertility clinic for diagnostic purposes in Lodz, Poland	2008-2011	- Semen samples were analyzed for sperm concentration, motility, and motion parameters using a computer-aided semen analysis (Hamilton-Thorne Version 10HTM-IVOS); - Three principal parameters for the vigor and pattern of sperm motion were examined: straight-line velocity, curvilinear velocity, and linearity; - Sperm morphology was quantified using strict Kruger criteria to classify men as having normal or below normal morphology; - Sperm chromatin structure assay was performed using flow cytometry to assess sperm DNA damage; - Levels of follicle-stimulating hormone, testosterone, and estradiol were determined in human plasma using a Chemiluminescent Microparticle Immunoassay Limitations: - A single urine sample was used to assess parabens exposure, to describe the level of reproductive hormones, and to assess semen quality; - Temporal reliability was less for concentrations of urinary metabolites of parabens than for phthalate; - As conducted among men recruited through an infertility clinic, the study is limited to generalize the results to the general population; - As a large number of analyses were performed, some of the observations could be chance findings due to multiple testing	- The statistically significant associations were found between urinary parabens concentrations and an increase the percentage of sperm with abnormal morphology and percentage of sperm with high DNA stainability; - Neither categories of urinary concentrations of parabens nor continuous concentrations of parabens were associated with the level of reproductive hormones; - Urinary concentrations of Methylparaben and Propylparaben were not related to any of the examined semen quality parameters, sperm DNA damage, or the level of reproductive hormones Percentile of Exposure Ethylparaben Morphology ≤ 25th >75th Butylparaben Morphology ≤ 25th >75th Isobutylparaben High DNA stainability ≤ 25th >75th	**β Coefficient (adjusted)** Reference 1.97 (0.05-12.16) Reference 9.51 (0.80-18.21) Reference *3.52 (1.02-16.03)*	***P*** 0.048 0.03 *0.03*	146
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	27 healthy pregnant women aged 33 ± 4.1 years in Czech Republic	Subjects recruited between 10/2016 and 01/2017	- 5 parabens and 15 steroids including estrogens, corticoids, androgens and immunomodulatory ones in maternal and cord plasma were measured by liquid chromatography - tandem mass spectrometry methods; - Samples of venous blood from the mothers were taken from the cubital vein during the 37th week of pregnancy, and at birth, a sample of mixed cord blood was taken Limitations: - Sample size is small	- Multiple regression models showed that in cord blood, Methylparaben (β=−0.027, p=0.027), Propylparaben (β=−0.025, p=0.03) and the sum of all measured parabens (β=−0.037, p=0.015) were inversely associated with T levels; - No influence of parabens on estrogen levels were observed			147

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Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	215 healthy unselected young university students (18–23 years old) in Southern Spain (Murcia Region).	2010-2011	- All men provided a urine, blood and semen sample on a single day; - Urinary paraben concentrations were measured by DLLME and UHPLC-MS/MS; - Semen quality was evaluated by measuring volume, sperm concentration, total sperm count, motility and morphology following WHO guidelines; - Serum samples were analyzed for reproductive hormones, including follicle-stimulating hormone, luteinizing hormone, testosterone, inhibin B and estradiol using immunoassays; - Associations between urinary concentrations of parabens and semen quality parameters and reproductive hormone levels were examined using linear regression, adjusting for potential covariates Limitations: - As with all observational studies, causal inference is limited. Residual confounding should always be considered and low statistical power might have played a role in the null findings; - Both urinary parabens and our outcomes were based on a single blood serum, urine or semen sample; - Exposure measurement error or misclassification cannot be ruled out	- Taking into account important covariates, urinary concentrations of parabens or their molar sum were not significantly associated with any semen parameters or any of the reproductive hormone levels; - 94% of the men had detectable urinary concentrations of parabens	Relative to men in the lowest quartile of sum of urinary paraben concentrations, the adjusted difference (95% CI) of sperm count for men in the 2nd, 3rd, and 4th quartiles were 4.1% (-37.1-45.3), -1.6% (-41.9-38.8), and -9.8% (-52.5-32.8), respectively (P-trend = 0.55)	[148]
Methylparaben Ethylparaben Propylparaben Butylparaben	42 men (36.8 ± 5.4 years old) of couples who visited a gynecology clinic in Tokyo for infertility consultation	2010	- Urinary parabens analysis was carried out by HPLC MS/MS; - LODs were 0.24, 0.021, 0.065 and 0.0090 ng/mL for Methylparaben, Ethylparaben, Propylparaben and Butylparaben, respectively; - Recoveries of the internal standards were 34–44% for the 4 parabens; - Specific gravity (SG)- and creatinine-adjusted urinary concentrations of parabens were measured; Limitations: - Sample size was small (n = 42); - The subjects of this study included people had normal semen quality and those who did not, therefore the association between exposure and effects might be obscured; -The level of parabens exposure was assessed by the parabens concentrations in a single spot urine, not representing long-term exposure level	- The relative contribution of Methylparaben, Ethylparaben, Propylparaben and Butylparaben to estrogen-equivalent total paraben (ETP, sum of the individual concentrations of the 4 parabens) was 12, 12, 38 and 38%, respectively; - Average semen volume, sperm concentration and sperm motility of the present subjects were similar to the levels of fertile Japanese men; - Significantly positive relationship between semen volume and urinary Ethylparaben was observed; - No significant association was found between semen parameters (semen volume, sperm concentration and motility) and urinary paraben concentrations in multiple regression analyses and logistic regression analyses		[149]

Table 17. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 subsample of US NHANES participants n=185 adolescent males (ages 12 to 19) males, 171 adolescent females, 785 adult (ages ≥20) males, and 708 adult females	2007-2008	- Stratified multistage probability sample of civilian US population was surveyed via household interviews, physical exams, and collection of medical histories and biologic specimens; - Urinary parabens concentrations were measured; - Spot urine samples were analyzed by HPLC-MS/MS; - LOD values were estimated as 3 x standard deviation as concentrations approached zero; - Serum thyroid measures included free and total T3 and T4, thyroglobulin, and TSH (or thyrotropin); - Potential confounders considered: age, sex, BMI, urinary creatinine levels, race/ethnicity, poverty income ratio , education, serum cotinine levels and alcohol intake; - Variables used as the basis for creation of sample weights, including race/ethnicity, PIR, and education, were not included in final models to avoid over-adjustment; - Following ln-transformation of the remaining variables with log-normal distributions, Pearson correlations, one-way ANOVA, and t-tests were used to evaluate potential confounders; - Covariates were adjusted for in the final models if there were statistically-significantly associated with one exposure or outcome variable based on a priori evidence or the analysis, and if they altered parameter estimates of the main effects by more than 10%; - Final regression models included age, sex, BMI, and urinary creatinine; - Concentrations of urinary parabens below the LOD were replaced with values equal to the LOD divided by the square root of two; - Parabens were analyzed on a creatinine-adjusted basis for univariate and bivariate analyses; unadjusted urinary concentrations were used in regression models with urinary creatinine included as a covariate; - Final multivariate linear regression models included serum thyroid concentrations (continuous variable) as the dependent variable and an individual urinary Methylparaben and Propylparaben concentration (continuous) as a predictor, along with age (continuous), sex (dichotomous), BMI (continuous), and ln-transformed urinary creatinine (continuous) <u>Limitations</u>: - Causality cannot be established because NHANES is an observational, cross-sectional study; - Exposures were evaluated based on spot urine measurements; - Spot urine samples served as the basis for estimating exposures, so time of sample collection could be a source of intra-individual variability and the concentrations may not accurately represent average body burdens	<u>Adults, Total T4 (µg/dL)</u> Methylparaben Ethylparaben Propylparaben Butylparaben <u>Adult Females, ln-Free T3 (pg/mL)</u> Methylparaben Ethylparaben Propylparaben Butylparaben <u>Adult Females, ln-Free T4 (ng/mL)</u> Methylparaben Ethylparaben Propylparaben Butylparaben <u>Adult Females, T4 (µg/dL)</u> Methylparaben Ethylparaben Propylparaben Butylparaben All other β coefficients calculated were not statistically significant	<u>β Coefficient</u> -0.04 (-0.12-0.03) ***-0.5 (-0.10 - -0.002)*** -0.19 (-0.46-0.07) ***-0.20 (-0.36 - -0.03)*** 0.005 (-0.01-0.000) ***-0.006 (-0.001- -0.0001)*** ***-0.02 (-0.04- -0.002)*** ***-0.02 (-0.03- -0.002)*** ***-0.01 (-003- -0.000)*** ***-0.01 (-0.02- -0.003)*** -0.02 (-0.05-0.01) ***-0.04 (-0.07- -0.004)*** -0.09 (-0.26-0.08) -0.08 (-0.20-0.05) -0.30 (-0.65-0.06) ***-0.36 (-0.57- -0.16)***	[150]

Table 17. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 sub-sample of the US NHANES participants ≥6 years of age, n=860 (450 males, 410 females)	2005-2006	- Sociodemographic data, urinary paraben levels, total and specific IgE levels, respiratory disease and medical condition questionnaire data were included in the dataset; - Urinary parabens levels were collected; - Subject answered the following questions: Has a doctor or other health professional ever told you that you have asthma? In the past 12 months, have you had wheezing or whistling in your chest? - Atopic asthma was defined as having doctor-diagnosed asthma in addition to at least 1 positive aeroallergen-specific IgE level; - Nonatopic asthma was defined as having doctor-diagnosed asthma with negative specific IgE test results; - Atopic wheeze was defined as having a history of wheezing in the past 12 months in addition to at least 1 positive aeroallergen-specific IgE level; - Nonatopic wheeze was defined as having a history of wheezing in the past 12 months with negative specific IgE test results; - Parabens were measured in urine samples by HPLC-MS/MS; - Serum total IgE levels and aeroallergen-specific IgE levels were measured, including IgE specific for cat, dog, mouse, rat, Dermatophagoides, cockroach, ragweed, thistle, rye, Bermuda, oak, birch, *Alternaria* species, and *Aspergillus* species; - Food-specific IgE levels measured were for milk, egg, peanut, and shrimp; - Subjects were considered to have aeroallergen or food sensitization if the specific IgE level was ≥0.35 kU/L; - Urinary paraben concentrations were divided into tertiles or dichotomized when 50% or fewer of the subjects had detectable levels (as was the case for Butylparaben); - Linear regression was used to determine whether mean urinary concentrations varied by race/ethnicity; - Logistic and linear regression were used to determine associations between paraben concentrations and food and aeroallergen sensitization, atopic and nonatopic asthma and wheeze, and total IgE levels; - Test for trend was performed by using the variable for tertiles of the paraben concentrations; - Multivariate models were adjusted for age, sex, race/ethnicity, urinary creatinine level, and PIR <u>Limitations</u>: -Data are drawn from a cross-sectional study, which introduces the possibility of reverse causation (i.e., subjects with allergy might use more products containing parabens); - Use of allergen sensitization as an outcome was limited by lack of clinical correlation of allergic disease; - Urinary paraben levels were used as biomarkers of exposure, which might not reflect actual exposure	<u>Aeroallergen and Food Sensitization (males and females)</u> Methylparaben Tertile 1 Tertile 2 Tertile 3 P_{trend}=0.4 Propylparaben Tertile 1 Tertile 2 Tertile 3 **P_{trend}=0.04** Propylparaben Tertile 1 Tertile 2 Tertile 3 P_{trend}=0.2 Butylparaben Tertile 1 Tertile 2 P_{trend}=0.9 <u>Nonatopic Asthma (males and females)</u> Methylparaben Tertile 1 Tertile 2 Tertile 3 **P_{trend}=0.04** <u>Nonatopic Wheeze (males and females)</u> Tertile 1 Tertile 2 Tertile 3 P_{trend}=0.47 In addition, the OR and p_{trend} calculated for Propylparaben concentrations and aeroallergen and food sensitization in males were statistically significant The ORs and p_{trend}s calculated for all other comparisons were not statistically significant	<u>**OR (unadjusted)**</u> 1.0 (Reference) 1.11 (0.82-1.47) **1.74 (1.02-3.11)** 1 (Reference) 1.35 (1.00-1.82) 1.74 (0.98-3.08 **OR (adjusted)** 1.0 (Reference) **1.51 (1.15-1.99)** **2.04 (1.12-3.74)** 1 (Reference) **1.55 (1.02-2.33)** 1.0 (Reference) 0.43 (0.47-3.73) **0.25 (0.07-0.90)** 1.0 (Reference) 0.51 (0.18-1.46) **0.23 (0.05-0.99)**	[151]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	696 pregnant women at the Women and Children's Medical Care Center of Wuhan City in Hubei province, China	2012-2014	- GDM was diagnosed on the basis of the fasting plasma glucose level after overnight fasting and 1 h and 2 h plasma glucose levels after having 75-g OGTTs; the cut-off values were 5.1, 10.0 and 8.5 mmol/L, respectively; - Face-to-face interviews were conducted within 3 days before or after delivery to collect information on lifestyle habits and sociodemographic characteristics; - Prepregnancy BMI was calculated as self-reported weight before pregnancy divided by the square of height; Participants were classified into underweight, normal weight and overweight/obese by prepregnancy BMI based on the criteria for Asian populations by the WHO; the cut-off values for underweight and overweight/obese were 18.5 and 23.0 kg/m2, respectively; - Urinary paraben concentrations were analyzed with UPLC-MS/MS Limitations: - Only one measurement of parabens before delivery, while GDM was diagnosed in the middle of pregnancy; - The urine samples were collected within three days of delivery and the exact time of sample collection was not recorded; - One spot urine sample was sufficient to capture the exposure profiles during a period of time; - Die and exercise information of the pregnant women was limited, both of which were important factors associated with GDM; - Weighting coefficients in the calculation equation of summed estrogenic activity were derived from in vitro experiments, which cause biases when applied into human studies; - Limited number of overweight/obese pregnant women in the study population	- No statistically significant association between parabens and GDM was found in the overall population; - Among the overweight/obese pregnant women, significant non-linear associations of Propylparaben and the summed estrogenic activity of parabens with GDM were found, with adjusted ORs of 3.47 (95% CI: 1.28, 9.42) and 2.87 (95% CI: 1.07, 7.73) for GDM in the second tertile of urinary Propylparaben (0.17–0.93 ng/mL) and the summed estrogen activity, respectively		[11]

Table 17. **Epidemiological studies of parabens**

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	450 children with asthma and 4023 children with asthma prevalence (between 6 and 19 years) from US NHANE Survey	2005-2014	- Paraben exposure measurements were conducted on a random one-third subsample of participants 6 years of age and older; - Urinary paraben concentration was adjusted for the creatinine; LODs were 1.0 µg/L for Methylparaben and Ethylparaben and 0.2 µg/L for Propylparaben and Butylparaben; - Participants or their caregivers completed a questionnaire relevant to medical conditions of asthma; for current asthma, the comparison group was children who never received an asthma diagnosis or who reported formerly having asthma; - Logistic regression models were analyzed to examine associations between urinary paraben biomarker concentrations and each outcome of interest Limitations: - Cause-effect relationship between paraben exposures and outcomes of interest cannot be elucidated through cross-sectional design; - Paraben concentrations only reflected recent rather than long-term exposures; - Analyses were limited by the variables available in this national survey	- An increased prevalence odds of reporting emergency department visits was observed for every 10-fold increase in Methylparaben and Propylparaben concentrations among boys with asthma 2.61 (95% CI, 1.40-4.85) and 2.18 (95% CI, 1.22-3.89), respectively; - Associations remained after adjusting for other phenolic compounds previously linked to respiratory outcomes (e.g., triclosan, bisphenol A, and 2,5-dichlorophenol); - No other dimorphic effects of exposure by sex were observed		[12]
Methylparaben Ethylparaben Propylparaben Butylparaben	1693 Black women aged 23–34 years residing in Detroit, Michigan	2010-2012	- Participants had an intact uterus, no prior diagnosis of uterine leiomyomata (fibroids), cancer, or autoimmune disease; - Paraben concentrations were analyzed by solid phase extraction coupled with isotope dilution HPLC-MS/MS and adjusted for the creatinine; - BMI was calculated based on technician-measured weight and height; Limitations: - Samples are from a single urban area of the U.S., which may not represent locations where other Black women reside; - Did not consider use of personal care products as sources of exposure, with the exception of sunscreen use; -Did not assess dietary factors as potential correlates; - Study was based on self-reported variables, thus misclassification could have resulted in bias	- Methylparaben and Propyl paraben were strongly correlated with one another (r = 0.80); - Median concentrations of Methylparaben, Propylparaben, Ethylparaben and Butylparaben were116.8, 16.8, 2.36, and 0.09µg/g creatinine, respectively; - Methylparaben concentrations were 30.7% lower for BMI ≥ 35 vs. < 25 kg/m^2 (95% CI: −48.0%, −7.7%), and Butylparaben concentrations were 30.6% lower for BMI ≥ 35 vs. < 25 kg/m^2 (95% CI: −49.6%, −4.6%)		[154]

Table 17. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylpraben	156 men under 45 years in Lodz, Poland	2008-2011	- Semen samples were obtained at the clinic via masturbation; - Sperm aneuploidy was measured by multicolor FISH analysis using DNA probes specific for chromosomes 13, 18, 21, X and Y and the slides were viewed by fluorescence microscopy; - Parabens were isolated by liquid –liquid extraction with hexane-tert-butyl methyl ether mixture and further cleaned-up using dispersive solid phase extraction; after evaporation, residue was derivatized with a mixture of N,O-bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane ; derivated extract was subjected to GC-MS/MS; - 28 % of examined men were smokers, and most of the study men drank 1–3 drinks per week (51.3 %); - Duration of couple's infertility last from 1 to 2 years (37.8 %) and from 2 to 3 years (30.8 %); - Past diseases which may have impact on semen quality was reported by 14 % of participants; - The sexual abstinence before the semen analysis last mostly 3–7 days (71.8 %) Limitations: - The men in this study were from a fertility clinic but not the general population; - The availability of only a semen sample for the assessment of sperm aneuploidy, which may also vary over time	- GM concentrations of Methylparaben, Ethylparaben, Propylparaben, Butylparaben and Isobutylparaben were 14.1, 1, 4.3, 0.3, and 0.4 µg/l, respectively; - Examined parabens were highly correlated: Methylparaben with Ethylparaben, Propylparaben, Isobutylparaben, and Butylparaben with Isobutylparaben ($p < 0.0001$) and Butylparaben with Methylparaben) and Ethylparaben ($p = 0.013$, $p = 0.033$, respectively) and Isobutylparaben with Ethylparaben ($p = 0.012$); - No correlations were found between Propylparaben and Butylparaben, Isobutylparaben and Ethylparaben (Spearman correlation coefficient = 0.07, 0.08, 0.09, respectively); - The positive association was observed between the urinary level of Butylparaben and XY18 disomy ($p = 0.045$) and the urinary level of Propylparaben and disomy of chromosome 13 ($p = 0.007$); - The increase in sperm disomy of chromosome 21 (2121) with increasing level of BP in urine was noticed only in crude analysis, whereas in the adjusted analysis this association was not statistically significant ($p = 0.08$); - The urinary concentration of Methylparaben, Propylparaben, Butylparaben, and isobutylparaben were not significantly associated with any of the examined sperm chromosome disomy		[146]

*** Bolded text** was used to highlight statistically significant increases; ***Italicized text*** was used to highlight statistically significant decreases

AFC=Anthral follicle count; ANOVA=Analysis of variance procedures; BKMR=Bayesian kernel machine regression; BMI=Body mass index; BSI=Behavioral Symptoms Index ; BSID= the Bayley Scales of Infant Development; CASA=computer-aided semen analysis; CI=Confidence interval; DLLME=dispersive liquid–liquid micro extraction; EARTH=Environment and Reproductive Health; E2=Estradiol; EDI=Estimated daily intake; EDEN = Etude des Déterminants pré et postnatals du développement et de la santé de l'Enfant; EEQ=Estrogen equivalency; FSH=Follicle stimulating hormone; GDM= Gestational diabetes mellitus; GLT=Glucose loading test; GM: Geometric mean; GnRH=Gonadotropin-releasing hormone; HELIX =the Human Early-Life Exposome project; HPLC-MS/MS=High-performance liquid chromatography-mass spectrometry/mass spectrometry; ICSI=Intracytoplasmic sperm injection; IADPSG=International Association of Diabetes and Pregnancy Study Groups; IQR=Interquartile range; IVF=In vitro fertilization; LOD=Limit of detection; LOQ=Limit of quantification; LVI-GC-MS/MS=Large volume-injection gas chromatography with tandem mass spectrometry; MDI= Mental developmental index; MDL=Method detection limit; MGH=Massachusetts General Hospital; MPC=Mean percent change; NA=Not applicable; NHANES=National Health and Nutrition Examination Survey; NBC=New Bedford Cohort; OR=Odds ratio; OV=Ovarian volume; PDI=Psychomotor development index; PFR: Placental–to–birth weight ratio; $P_{parabens}$=Sum molar concentrations of the parabens; PIR=Poverty income ratio; PTB=Preterm birth; SART= Society for Assisted Reproductive Technology; SART: Society for Assisted Reproductive Technology; SG=Specific gravity; OGTTs=Oral glucose tolerance tests; UPLC-MS/MS=Ultra-high-performance liquid chromatography-tandem mass spectrometry; WHO=World Health Organization

Table 18. Global exposure estimate for parabens illustrated using the survey data for Butylparaben[25,158,159]

Type of Exposure[159]	Product[158]	Daily Use[158] (g/day)	Cumulative Exposure (g/day)	Maximum use concentration of Butylparaben[25]	Maximum exposure estimate of Butylparaben (g/day)	Butylparaben Exposure (mg/kg/day) assuming 60 kg person
Oral	Toothpaste	0.14	2.36	0.2% (Lipstick)	0.0047	0.079
	Mouthwash	2.16				
	Lipstick	0.06				
Eye products	Eye makeup	0.02	0.05	0.5% (Mascara)	0.00025	0.0042
	Mascara	0.025				
	Eyeliner	0.005				
Non rinse-off products	Face cream	1.54	13.93	0.24% (Moisturizing products)	0.0334	0.54
	Hand cream	2.16				
	Liquid Foundation	0.51				
	Body lotion	7.82				
	Deodorant	1.50				
	Hair styling products	0.40				
Rinse-off products	Make-up remover	0.50	1.04	0.33% (Skin cleansing)	0.0034	0.04
	Hand wash soap	0.20				
	Shower gel	0.19				
	Shampoo	0.11				
	Conditioner	0.04				
Total		17.4			0.042	0.6632

REFERENCES

1. Nikitakis J, Kowcz A. Web-Based Cosmetic Ingredient Dictionary and Handbook. http://webdictionary.personalcarecouncil.org/jsp/IngredientSearchPage.jsp. Washington, DC: Personal Care Products Council. Last Updated: 2019.

2. Andersen F, (ed). Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products. *Int J Toxicol* 2008;27(Suppl. 4):1-82.

3. European Chemicals Agency (ECHA). Sodium 4-(methyoxycarbonyl)phenolate (Sodium Methylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/5580/1. Last Updated: 12/27/2015. Accessed: 3/4/2017.

4. European Chemicals Agency (ECHA). Ethyl 4-hydroxybenzoate (Ethylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/13843/1. Last Updated: 12/29/2015. Accessed: 3/4/2017.

5. European Chemicals Agency (ECHA). Propyl 4-hydroxybenzoate (Propylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/13890. Last Updated: 12/17/2015. Accessed: 1/19/2017.

6. European Chemicals Agency (ECHA). Sodium 4-propoxycarbonylphenoxide (Sodium Propylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/17005/1. Last Updated: 3/18/2016. Accessed: 6/8/2017.

7. European Chemicals Agency (ECHA). Benzyl 4-hydroxybenzoate (Benzylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/17658/1. Last Updated: 8/17/2016. Accessed: 2/27/2017.

8. European Chemicals Agency (ECHA). Methyl 4-hydroxybenzoate. https://echa.europa.eu/registration-dossier/-/registered-dossier/14310/4/6. Last Updated: 8/3/2018. Accessed: 9/26/2018.

9. European Chemicals Agency (ECHA). Butyl 4-hydroxybenzoate. https://echa.europa.eu/registration-dossier/-/registered-dossier/25335/4/6. Last Updated: 8/8/2018. Accessed: 9/29/2018.

10. European Chemicals Agency (ECHA). Propyl 4-hydroxybenzoate. https://echa.europa.eu/registration-dossier/-/registered-dossier/13890/4/6. Last Updated: 8/3/2018. Accessed: 9/29/2018.

11. European Chemicals Agency (ECHA). Hydroxybenzoic acid. https://echa.europa.eu/registration-dossier/-/registered-dossier/15944/4/6. Last Updated: 5/12/2018. Accessed: 9/27/2018.

12. Joint FAO/WHO Expert Committee on Food Additives (JECFA), . Evaluation of certain food additives and contaminants. Rome, Italy2007. 940. http://apps.who.int/iris/bitstream/10665/43592/1/WHO_TRS_940_eng.pdf. Accessed 1/10/2017. Pages1-104.

13. Scientific Committee on Consumer Products (SCCP). Extended opinion on parabens, underarm cosmetics and breast cancer. 2005. SCCP/0874/05. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_00d.pdf. Accessed 1/12/2012. Pages1-8.

14. Scientific Committee on Consumer Products (SCCP). Extended opinion on the safety evaluation of parabens. 2005. SCCP0873/05. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_019.pdf. Accessed 1/7/2012. Pages1-11.

15. Scientific Committee on Consumer Products (SCCP). Opinion on parabens, COLIPA No P82. 2006. SCCP/1017/06. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_074.pdf. Accessed 2/10/2012. Pages1-19.

16. Scientific Committee on Consumer Safety (SCCS). Opinion on parabens, COLIPA No P82. 2008. SCCP/1183/08. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_138.pdf. Accessed 2/19/2012. Pages1-13.

17. Scientific Committee on Consumer Safety (SCCS). Opinion on parabens, COLIPA No P82. 2011. SCCS/1348/10. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_041.pdf. Accessed 2/13/2012. Pages1-36.

18. Scientific Committee on Consumer Safety (SCCS). Clarification on Opinion SCCS/1348/10 in light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age. 2011. SCCS/1446/11. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_069.pdf. Accessed 2/23/2012. Pages1-51.

19. Scientific Committee on Consumer Safety (SCCS). Opinion on parabens; Updated request for a scientific opinion on propyl- and butylparaben, COLIPA No P82. 2013. SCCS/1514/13. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_132.pdf. Pages1-50.

20. US National Center for Biotechnology Information. PubChem Compound Database; CID=7456: Methyl 4-hydroxybenzoate. *Open Chemistry Database.* 2017. https://pubchem.ncbi.nlm.nih.gov/compound/7456. Accessed 1/10/2017.

21. European Chemicals Agency (ECHA). Isopropyl 4-hydroxybenzoate (Isopropylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/19482/1. Last Updated: 6/3/2017. Accessed: 9/28/2018.

22. European Chemicals Agency (ECHA). Isobutyl 4-hydroxybenzoate (Isobutylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/17752. Last Updated: 8/8/2018. Accessed: 9/28/2018.

23. European Chemicals Agency (ECHA). Sodium 4-ethoxycarbonylphenoxide (Sodium Ethylparaben). https://echa.europa.eu/registration-dossier/-/registered-dossier/16994. Last Updated: 2018. Accessed: 2/8/2018.

24. Iijima T, Yamaguchi T. The improved Kolbe-Schmitt reaction using supercritical carbon dioxide. *Tetrahedron Letters* 2007;48(30):5309-5311.

25. Personal Care Products Council. 2016. Concentration of Use by FDA Product Category: Parabens. (Unpublished data submitted by Personal Care Products Council on December 12, 2016.)

26. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD 2019 2019. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 3, 2019; received February 13, 2019.)

27. Bremmer H, Prud'homme de Lodder L, van Engelen J. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 2006 2006. RIVM 320104001/2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed 8/24/2011. Pages1-77.

28. Johnsen M. The Influence of Particle Size. *Spray Technology and Marketing* 2004;14(11):24-27.

29. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett* 2011;205(2):97-104.

30. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). 2015. Cosmetic Powder Exposure. (Unpublished data submitted by the Personal Care Products Council.)

31. Aylott R, Byrne G, Middleton J, Roberts M. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci* 1979;1(3):177-186.

32. Russell R, Merz R, Sherman W, Sivertson J. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol* 1979;17(2):117-122.

33. European Union (EU). Commission Regulation (EU) No 358/2014 of 9 April 2015 amending Annexes II and V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. 2014. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32014R0358. Accessed 2/25/2017. (Official Journal of the European Union.)

34. European Union (EU). Commission Regulation (EU) No 1004/2014 of 18 September 2014 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. 2014. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32014R1004. Accessed 2/25/2017. (Official Journal of the European Union.)

35. US Food and Drug Administration (FDA). Inactive Ingredient Search for Approved Drug Products; *FDA Database*. https://www.accessdata.fda.gov/scripts/cder/iig/index.Cfm. Washington, DC: FDA. Last Updated: 7/12/2018. Accessed: 7/30/2018.

36. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Human health tier II assessment for parabens. 2016. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1714#recommendation. Accessed 3/24/2017.

37. Pazourekova S, Hojerova J, Klimova Z, Lucova M. Dermal absorption and hydrolysis of methylparaben in different vehicles through intact and damaged skin: using a pig-ear model in vitro. *Food Chem Toxicol* 2013;59:754-765.

38. Caon T, Costa A, Leal de Oliveira M, Micke G, Simoes C. Evaluation of the transdermal permeation of different paraben combinations through a pig ear skin model. *Int J Pharm* 2010;391(1-2):1-6.

39. Pedersen S, Marra F, Nicoli S, Santi P. In vitro skin permeation and retention of parabens from cosmetic formulations. *Int J Cosmet Sci* 2007;29(5):361-367.

40. Seo J, Kim S, Kim B. In vitro skin absorption tests of three types of parabens using a Franz diffusion cell. *J Expo Sci Environ Epidemiol* 2016;27(3):320-325.

41. El Hussein S, Muret P, Berard M, Makki S, Humbert P. Assessment of principal parabens used in cosmetics after their passage through human epidermis-dermis layers (ex-vivo study). *Exp Dermatol* 2007;16(10):830-836.

42. Janjua N, Mortensen G, Andersson A, Kongshoj B, Skakkebaek N, Wulf H. Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. *Environ Sci Technol* 2007;41(15):5564-5570.

43. Romonchuk W. Mechanism of enhanced dermal permeation of 4-cyanophenol and methyl paraben from saturated aqueous solutions containing both solutes. *Skin Pharmacol Physiol* 2010;23(3):152-163.

44. Elder R, (ed). Final report on the safety assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. *J Am Coll Toxicol* 1984;3(5):147-209.

45. Elder R, (ed). Final report on the safety assessment of Benzylparaben. *J Am Coll Toxicol* 1986;5(5):301-307.

46. Andersen F, (ed). Final report on the safety assessment of Isobutylparaben and Isopropylparaben. *J Am Coll Toxicol* 1995;14(5):364-372.

47. Hong H, Branham W, Dial S, et al. Rat α-fetoprotein binding affinities of a large set of structurally diverse chemicals elucidated the relationships between structures and binding affinities. *Chem Res Toxicol* 2012;25(11):2553-2566.

48. Hoberman A, Schreur D, Leazer T, et al. Lack of effect of butylparaben and methylparaben on the reproductive system in male rats. *Birth Defects Res B Dev Reprod Toxicol* 2008;83(2):123-133.

49. Abbas S, Greige-Gerges H, Karam N, Piet M, Netter P, Magdalou J. Metabolism of parabens (4-hydroxybenzoic acid esters) by hepatic esterases and UDP-glucuronosyltransferases in man. *Drug Metab Pharmacokinet* 2010;25(6):568-577.

50. Ozaki H, Sugihara K, Watanabe Y, et al. Comparative study of the hydrolytic metabolism of methyl-, ethyl-, propyl-, butyl-, heptyl- and dodecylparaben by microsomes of various rat and human tissues. *Xenobiotica* 2013;43(12):1064-1072.

51. Harville H, Voorman R, Prusakiewicz J. Comparison of paraben stability in human and rat skin. *Drug Metab Lett* 2007;1(1):17-21.

52. Mathews J, Brown S, Patel P, et al. Metabolism and disposition of [^{14}C]n-butyl-p-hydroxybenzoate in male and female Harlan Sprague Dawley rats following oral administration and dermal application. *Xenobiotica* 2013;43(2):169-181.

53. Aubert N, Ameller T, Legrand J. Systemic exposure to parabens: pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration. *Food Chem Toxicol* 2012;50(3-4):445-454.

54. Roberts GK, Waidyanatha S, Kissling GE, et al. Exposure to butyl paraben during gestation and lactation in Hsd:Sprague dawley SD rats via dosed feed. *Toxicol Rep* 2016;3:774-783.

55. Janjua N, Frederiksen H, Skakkebaek N, Wulf H, Andersson A. Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. *Int J Androl* 2008;31(2):118-130.

56. Moos R, Angerer J, Dierkes G, Bruening T, Koch H. Metabolism and elimination of methyl, iso- and n-butyl paraben in human urine after single oral dosage. *Arch Toxicol* 2016;90(11):2699-2709.

57. Campbell JL, Yoon M, Clewell HJ. A case study on quantitative in vitro to in vivo extrapolation for environmental esters: Methyl-, propyl- and butylparaben. *Toxicology* 2015;332:67-76.

58. Kim M, Kwack S, Lim S, et al. Toxicological evaluation of isopropylparaben and isobutylparaben mixture in Sprague-Dawley rats following 28 days of dermal exposure. *Regul Toxicol Pharmacol* 2015;73(2):544-551.

59. Salem A, Said M, Badawi M, Abd Rabo M. Subchronic toxicity of propyl paraben in adult male rats. *Egypt J Biochem Mol Biol* 2013;31(1):1-20.

60. Popa D, Kiss B, Vlase L, et al. Study of oxidative stress induction after exposure to bisphenol A and methylparaben in rats. *Farmacia (Bucharest, Rom)* 2011;59(4):539-549.

61. Shah K, Verma R. Butyl p-hydroxybenzoic acid induces oxidative stress in mice liver--an in vivo study. *Acta Pol Pharm* 2011;68(6):875-879.

62. Boberg J, Axelstad M, Svingen T, et al. Multiple Endocrine Disrupting Effects in Rats Perinatally Exposed to Butylparaben. *Toxicol Sci* 2016;152(1):244-256.

63. Zhang L, Dong L, Ding S, et al. Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed E(2) levels in male rat offspring. *Environ Toxicol Pharmacol* 2014;37(2):705-717.

64. Riad MA, Abd-Rabo MM, Abd El Aziz SA, El Behairy AM, Badawy MM. Reproductive toxic impact of subchronic treatment with combined butylparaben and triclosan in weanling male rats. *J Biochem Mol Toxicol* 2018;32(3):e22037.

65. Zhang L, Ding S, Qiao P, et al. n-Butylparaben induces male reproductive disorders via regulation of estradiol and estrogen receptors. *J Appl Toxicol* 2016;36(9):1223-1234.

66. Alam M, Kurohmaru M. Disruption of Sertoli cell vimentin filaments in prepubertal rats: an acute effect of butylparaben in vivo and in vitro. *Acta Histochem* 2014;116(5):682-687.

67. Vo T, Yoo Y, Choi K, Jeung E. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. *Reprod Toxicol* 2010;29(3):306-316.

68. Boberg J, Metzdorff S, Wortziger R, et al. Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. *Toxicology* 2008;250(2-3):75-81.

69. Manservisi F, Gopalakrishnan K, Tibaldi E, et al. Effect of maternal exposure to endocrine disrupting chemicals on reproduction and mammary gland development in female Sprague-Dawley rats. *Reprod Toxicol* 2015;54:110-119.

70. Gazin V, Marsden E, Marguerite F. Oral propylparaben administration to juvenile male Wistar rats did not induce toxicity in reproductive organs. *Toxicol Sci* 2013;136(2):392-401.

71. Alam M, Ohsako S, Kanai Y, Kurohmaru M. Single administration of butylparaben induces spermatogenic cell apoptosis in prepubertal rats. *Acta Histochem* 2014;116(3):474-480.

72. Luzeena RG, Divya SK, Lite C, Santosh W, Barathi S. Transient exposure of methylparaben to zebrafish (Danio rerio) embryos altered cortisol level, acetylcholinesterase activity and induced anxiety-like behaviour. *Gen Comp Endocrinol* 2018.

73. Dambal VY, Selvan KP, Lite C, Barathi S, Santosh W. Developmental toxicity and induction of vitellogenin in embryo-larval stages of zebrafish (Danio rerio) exposed to methyl Paraben. *Ecotoxicol Environ Saf* 2017;141:113-118.

74. Samarasinghe SVAC, Krishnan K, Naidu R, et al. Parabens generate reactive oxygen species in human spermatozoa. *Andrology* 2018;6(4):532-541.

75. Perez Martin J, Peropadre A, Herrero O, Fernandez F, Labrador V, Hazen M. Oxidative DNA damage contributes to the toxic activity of propylparaben in mammalian cells. *Mutat Res* 2010;702(1):86-91.

76. Tayama S, Nakagawa Y, Tayama K. Genotoxic effects of environmental estrogen-like compounds in CHO-K1 cells. *Mutat Res* 2008;649(1-2):114-125.

77. Taxvig C, Dreisig K, Boberg J, et al. Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARgamma activation. *Mol Cell Endocrinol* 2012;361(1-2):106-115.

78. Kjaerstad M, Taxvig C, Andersen H, Nellemann C. Mixture effects of endocrine disrupting compounds in vitro. *Int J Androl* 2010;33(2):425-433.

79. Pan S, Yuan C, Tagmount A, et al. Parabens and human epidermal growth factor receptor ligand cross-talk in breast cancer cells. *Environ Health Perspect* 2016;124(5):563-569.

80. Klopcic I, Kolsek K, Dolenc M. Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. *Toxicol Lett* 2015;232(2):376-383.

81. Kolsek K, Gobec M, Mlinaric Rascan I, Sollner Dolenc M. Screening of bisphenol A, triclosan and paraben analogues as modulators of the glucocorticoid and androgen receptor activities. *Toxicol In Vitro* 2015;29(1):8-15.

82. Pop A, Kiss B, Drugan T, Cherfan J, Loghin F. In vitro estrogenic/anti-estrogenic effects of certain food additives and cosmetic preservatives. *Farmacia (Bucharest, Rom)* 2014;62(5):863-873.

83. Charles A, Darbre P. Combinations of parabens at concentrations measured in human breast tissue can increase proliferation of MCF-7 human breast cancer cells. *J Appl Toxicol* 2013;33(5):390-398.

84. Marchese S, Silva E. Disruption of 3D MCF-12A breast cell cultures by estrogens--an in vitro model for ER-mediated changes indicative of hormonal carcinogenesis. *PLoS One* 2012;7(10):e45767.

85. Lillo M, Nichols C, Perry C, et al. Methylparaben stimulates tumor initiating cells in ER+ breast cancer models. *J Appl Toxicol* 2016;37(4):417-425.

86. Hu P, Chen X, Whitener R, et al. Effects of parabens on adipocyte differentiation. *Toxicol Sci* 2013;131(1):56-70.

87. US Environmental Protection Agency (EPA). Endocrine Disruptor Screening Program (EDSP) in the 21st Century. www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-21st-century. Washington, DC: EPA. Last Updated: 2018. Accessed: 3/8/2018.

88. Gonzalez TL, Moos RK, Gersch CL, et al. Metabolites of n-Butylparaben and iso-Butylparaben Exhibit Estrogenic Properties in MCF-7 and T47D Human Breast Cancer Cell Lines. *Toxicol Sci* 2018;164(1):50-59.

89. Guerra M, Furlong H, Kempinas W, Foster W. Effects of in vitro exposure to butylparaben and di-(2 ethylhexyl) phthalate, alone or in combination, on ovarian function. *J Appl Toxicol* 2016;36(9):1235-1245.

90. Lee J, Lee M, Ahn C, Kang H, Tran D, Jeung E. Parabens Accelerate Ovarian Dysfunction in a 4-Vinylcyclohexene Diepoxide-Induced Ovarian Failure Model. *Int J Environ Res Public Health* 2017;14(2):161-174.

91. Gopalakrishnan K, Teitelbaum S, Lambertini L, et al. Changes in mammary histology and transcriptome profiles by low-dose exposure to environmental phenols at critical windows of development. *Environ Res* 2017;152:233-243.

92. Costa JR, Campos MS, Lima RF, et al. Endocrine-disrupting effects of methylparaben on the adult gerbil prostate. *Environ Toxicol* 2017;32(6):1801-1812.

93. Hu Y, Zhang Z, Sun L, et al. The estrogenic effects of benzylparaben at low doses based on uterotrophic assay in immature SD rats. *Food Chem Toxicol* 2013;53:69-74.

94. Sun L, Yu T, Guo J, et al. The estrogenicity of methylparaben and ethylparaben at doses close to the acceptable daily intake in immature Sprague-Dawley rats. *Sci Rep* 2016;6(25173):1-6.

95. Ohta R, Takagi A, Ohmukai H, et al. Ovariectomized mouse uterotrophic assay of 36 chemicals. *J Toxicol Sci* 2012;37(5):879-889.

96. Khanna S, Darbre P. Parabens enable suspension growth of MCF-10A immortalized, non-transformed human breast epithelial cells. *J Appl Toxicol* 2013;33(5):378-382.

97. Goodson WI, Luciani M, Sayeed S, Jaffee I, Moore D, Dairkee S. Activation of the mTOR pathway by low levels of xenoestrogens in breast epithelial cells from high-risk women. *Carcinogenesis* 2011;32(11):1724-1733.

98. Yang C, Lim W, Bazer F, Song G. Butylparaben promotes apoptosis in human trophoblast cells through increased oxidative stress-induced endoplasmic reticulum stress. *Environmental Toxicology* 2018;33(4):436-445.

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

100. Ferguson KK, Colacino JA, Lewis RC, Meeker JD. Personal care product use among adults in NHANES: associations between urinary phthalate metabolites and phenols and use of mouthwash and sunscreen. *J Expo Sci Environ Epidemiol* 2017;27(3):326-332.

101. Harley K, Kogut K, Madrigal D, et al. Reducing Phthalate, Paraben, and Phenol Exposure from Personal Care Products in Adolescent Girls: Findings from the HERMOSA Intervention Study. *Environ Health Perspect* 2016;124(10):1600-1607.

102. Berger KP, Kogut KR, Bradman A, et al. Personal care product use as a predictor of urinary concentrations of certain phthalates, parabens, and phenols in the HERMOSA study. *J Expo Sci Environ Epidemiol* 2018.

103. Tahan G, Santos N, Albuquerque A, Martins I. Determination of parabens in serum by liquid chromatography-tandem mass spectrometry: Correlation with lipstick use. *Regul Toxicol Pharmacol* 2016;79:42-48.

104. Ye X, Wong LY, Jia LT, Needham LL, Calafat AM. Stability of the conjugated species of environmental phenols and parabens in human serum. *Environ Int* 2009;35(8):1160-1163.

105. Barr L, Metaxa sG, Harbach C, Savoy L, Darbre P. Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum. *J Appl Toxicol* 2012;32(3):219-232.

106. Valle-Sistac J, Molins-Delgado D, Diaz M, Ibanez L, Barcelo D, Silvia Diaz-Cruz M. Determination of parabens and benzophenone-type UV filters in human placenta. First description of the existence of benzyl paraben and benzophenone-4. *Environ Int* 2016;88:243-249.

107. Sajid M, Basheer C, Narasimhan K, Choolani M, Lee H. Application of microwave-assisted micro-solid-phase extraction for determination of parabens in human ovarian cancer tissues. *J Chromatogr B: Analyt Technol Biomed Life Sci* 2015;1000(1 Sept 2015):192-198.

108. Fisher M, MacPherson S, Braun JM, et al. Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product Use. *Environ Sci Technol* 2017;51(7):4009-4017.

109. Wang L, Asimakopoulos A, Kannan K. Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue. *Environ Int* 2015;78:45-50.

110. Wang L, Wu Y, Zhang W, Kannan K. Characteristic profiles of urinary p-hydroxybenzoic acid and its esters (parabens) in children and adults from the United States and China. *Environ Sci Technol* 2013;47(4):2069-2076.

111. Artacho-Cordon F, Fernandez MF, Frederiksen H, et al. Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. *Environ Int* 2018;119:203-211.

112. Nassan F, Coull B, Gaskins A, et al. Personal care product use in men and urinary concentrations of select phthalate metabolites and parabens: Results from the Environment And Reproductive Health (EARTH) Study. *Environ Health Perspect* 2017;125(8):087012-087011-087012-087010.

113. Messerlian C, Mustieles V, Minguez-Alarcon L, et al. Preconception and prenatal urinary concentrations of phenols and birth size of singleton infants born to mothers and fathers from the Environment and Reproductive Health (EARTH) study. *Environ Int* 2018;114:60-68.

114. Pollack A, Mumford S, Krall J, et al. Exposure to bisphenol A, chlorophenols, benzophenones, and parabens in relation to reproductive hormones in healthy women: A chemical mixture approach. *Environ Int* 2018;120.

115. Ashrap P, Watkins D, Calafat A, et al. Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends. *Environ Int* 2018;121:990-1002.

116. Aung MT, Ferguson KK, Cantonwine DE, et al. Associations between maternal plasma measurements of inflammatory markers and urinary levels of phenols and parabens during pregnancy: A repeated measures study. *Sci Total Environ* 2019;650(Pt 1):1131-1140.

117. Aker AM, Ferguson KK, Rosario ZY, et al. A repeated measures study of phenol, paraben and Triclocarban urinary biomarkers and circulating maternal hormones during gestation in the Puerto Rico PROTECT cohort. *Environ Health* 2019;18(1):28.

118. Handa O, Kokura S, Adachi S, et al. Methylparaben potentiates UV-induced damage of skin keratinocytes. *Toxicology* 2006;227(1-2):62-72.

119. Mundy RD, Cormack B. Expression of Candida glabrata adhesins after exposure to chemical preservatives. *J Infect Dis* 2009;199(12):1891-1898.

120. Sonnenburg A, Schreiner M, Stahlmann R. Assessment of the sensitizing potency of preservatives with chance of skin contact by the loose-fit coculture-based sensitization assay (LCSA). *Arch Toxicol* 2015;89(12):2339-2344.

121. Epstein S, Ahdoot M, Marcus E, Asbell P. Comparative toxicity of preservatives on immortalized corneal and conjunctival epithelial cells. *J Ocul Pharmacol Ther* 2009;25(2):113-119.

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

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

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

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

126. Minguez-Alarcon L, Chiu Y, Messerlian C, et al. Urinary paraben concentrations and in vitro fertilization outcomes among women from a fertility clinic. *Fertil Steril* 2016;105(3):714-721.

127. Wu C, Huo W, Li Y, et al. Maternal urinary paraben levels and offspring size at birth from a Chinese birth cohort. *Chemosphere* 2017;172:29-36.

128. Aker AM, Ferguson KK, Rosario ZY, et al. The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico. *Environ Res* 2018;169:41-51.

129. Smarr MM, Honda M, Kannan K, Chen Z, Kim S, Louis GMB. Male urinary biomarkers of antimicrobial exposure and bi-directional associations with semen quality parameters. *Reprod Toxicol* 2018;77:103-108.

130. Smarr MM, Kannan K, Sun L, et al. Preconception seminal plasma concentrations of endocrine disrupting chemicals in relation to semen quality parameters among male partners planning for pregnancy. *Environ Res* 2018;167:78-86.

131. Minguez-Alarcon L, Williams PL, Chiu YH, et al. Secular trends in semen parameters among men attending a fertility center between 2000 and 2017: Identifying potential predictors. *Environ Int* 2018;121(Pt 2):1297-1303.

132. Harley KG, Berger KP, Kogut K, et al. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. *Hum Reprod* 2019;34(1):109-117.

133. Bellavia A, Chiu Y, Brown F, et al. Urinary concentrations of parabens mixture and pregnancy glucose levels among women from a fertility clinic. *Environ Res* 2019;168:389-396.

134. Wu C, Xia W, Li Y, et al. Repeated measurements of paraben exposure during pregnancy in relation to fetal and early-childhood growth. *Environmental Sci Technol* 2018;53(1):422-433.

135. Philippat C, Heude B, Botton J, Alfaidy N, Calafat A, Slama R. Prenatal Exposure to Select Phthalates and Phenols and Associations with Fetal and Placental Weight among Male Births in the EDEN Cohort (France). *Environ Health Persepect* 2019;127(1):017002-017001 - 017002-017008.

136. Liu W, Zhou Y, Li J, et al. Parabens exposure in early pregnancy and gestational diabetes mellitus. *Environ Int* 2019;126:468-475.

137. Jiang Y, Zhao H, Xia W, et al. Prenatal exposure to benzophenones, parabens and triclosan and neurocognitive development at 2 years. *Environ Int* 2019;126:413-421.

138. Shoaff JR, Calafat AM, Schantz SL, Korrick SA. Endocrine disrupting chemical exposure and maladaptive behavior during adolescence. *Environ Res* 2018;172:231-241.

139. Warembourg C, Basagana X, Seminati C, et al. Exposure to phthalate metabolites, phenols and organophosphate pesticide metabolites and blood pressure during pregnancy. *Int J Hyg Environ Health* 2019;222(3):446-454.

140. Geer L, Pycke B, Waxenbaum J, Sherer D, Abulafia O, Halden R. Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York. *J Hazard Mater* 2017;323(Pt A):177-183.

141. Philippat C, Botton J, Calafat A, Ye X, Charles M, Slama R. Prenatal exposure to phenols and growth in boys. *Epidemiology* 2014;25(5):625-635.

142. Fernandez M, Arrebola J, Jimenez-Diaz I, et al. Bisphenol A and other phenols in human placenta from children with cryptorchidism or hypospadias. *Reprod Toxicol* 2016;59:89-95.

143. Guo J, Wu C, Lu D, et al. Urinary paraben concentrations and their associations with anthropometric measures of children aged 3 years. *Environ Pollut* 2016;222:307-314.

144. Smith K, Souter I, Dimitriadis I, et al. Urinary paraben concentrations and ovarian aging among women from a fertility center. *Environ Health Perspect* 2013;121(11-12):1299-1305.

145. Meeker J, Yang T, Ye X, Calafat A, Hauser R. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. *Environ Health Perspect* 2011;119(2):252-257.

146. Jurewicz J, Radwan M, Wielgomas B, et al. Human semen quality, sperm DNA damage, and the level of reproductive hormones in relation to urinary concentrations of parabens. *J Occup Environ Med* 2017;59(11):1034-1040.

147. Kolatorova L, Vitku J, Hampl R, et al. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. *Environ Res* 2018;163:115-122.

148. Adoamnei E, Mendiola J, Monino-Garcia M, et al. Urinary concentrations of parabens and reproductive parameters in young men. *Sci Total Environ* 2018;621:201-209.

149. Nishihama Y, Toshima H, Yoshinaga J, et al. Paraben exposure and semen quality of Japanese male partners of subfertile couples. *Environ Health Prev Med* 2017;22(1):5.

150. Koeppe E, Ferguson K, Colacino J, Meeker J. Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007-2008. *Sci Total Environ* 2013;445-446:299-305.

151. Savage J, Matsui E, Wood R, Keet C. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *J Allergy Clin Immunol* 2012;130(2):453-460.

152. Li Y, Xu S, Li Y, et al. Association between urinary parabens and gestational diabetes mellitus across prepregnancy body mass index categories. *Environ Res* 2019;170:151-159.

153. Quiros-Alcala L, Hansel N, McCormack M, Matsui E. Paraben exposures and asthma-related outcomes among children from the US general population. *J Allergy Clin Immunol* 2019;143(3):948-956.

154. Bethea TN, Wesselink AK, Weuve J, et al. Correlates of exposure to phenols, parabens, and triclocarban in the Study of Environment, Lifestyle and Fibroids. *J Expo Sci Environ Epidemiol* 2019.

155. Fisher J, Turner K, Brown D, Sharpe R. Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. *Environ Health Perspect* 1999;107(5):397-405.

156. Garcia T, E S, V K, et al. Effects on the reproductive system of young male rats of subcutaneous exposure to n-butylparaben. *Food Chem Toxicol* 2017;106(Pt A):47-57.

157. Taxvig C, Vinggaard A, Hass U, et al. Do parabens have the ability to interfere with steroidogenesis? *Toxicol Sci* 2008;106(1):206-213.

158. Scientific Committee on Consumer Safety (SCCS). The SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 9th revision, SCCS/1564/15. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf. European Commissioner; Directorate-Health & Food Safety. Last Updated: Accessed: 9/25/2018.

159. Cowan-Ellsberry CE, Robison SH. Refining aggregate exposure: example using parabens. *Regul Toxicol Pharmacol* 2009;55(3):321-329.

160. Brand W, Boon P, Hessel E, Meesters J, Weda M, Schuur A. Exposure to and toxicity of methyl-, ethyl-, and propylparaben. A literature review with a focus on endocrine-disrupting properties. The Netherlands 2018 2018. RIVM Report 2017-0028. https://www.rivm.nl/dsresource?objectid=c9762c40-21f5-4b0d-a045-8f61ee2a7f4c&type=pdf&disposition=inline.

161. Soni M, Carabin I, Burdock G. Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food Chem Toxicol* 2005;43(7):985-1015.

162. Aylward L, Vilone G, Cowan-Ellsberry C, et al. Exposure to selected preservatives in personal care products: case study comparison of exposure models and observational biomonitoring data. *J Expo Sci Environ Epidemiol.* 2018. http://www.ncbi.nlm.nih.gov/pubmed/30518793.

163. Csiszar SA, Ernstoff AS, Fantke P, Jolliet O. Stochastic modeling of near-field exposure to parabens in personal care products. *J Expo Sci Environ Epidemiol* 2017;27(2):152-159.

164. ACD/Labs. 2015. Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2017 ACD/Labs).

165. US National Center for Biotechnology Information. PubChem Compound Database; CID=7184; Butyl 4-hydroxybenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/7184. Last Updated: Accessed: 1/10/2017.

166. US National Center for Biotechnology Information. PubChem Compound Database; CID=8434; Ethylparaben. https://pubchem.ncbi.nlm.nih.gov/compound/8434. Last Updated: 2017. Accessed: 3/25/2017.

167. Csordas L, Medgyaszay M. Crystal-physical explanation of the melting point alteration of some organic compounds. *Proceedings of the Conference of Applied Physical Chemistry* 1971;2:697-703.

168. Ramirez N, Marce R, Borrull F. Development of a thermal desorption-gas chromatography-mas spectrometry method for determining personal care products in air. *J Chromatog A* 2010;1217(26):4430-4438.

169. Sidgwick N, Bayliss N. Parachor of coördinated hydrogen in the o-substituted phenols. *Journal of the Chemical Society* 1930:2027-2034.

170. Barry J, Bram G, Decodts G, et al. Solid-liquid phase-transfer catalysis without added solvent. A simple, efficient, and inexpensive synthesis of aromatic carboxylic esters by alkylation of potassium carboxylates. *Synthesis* 1985;1985(1):40-45.

171. US National Center for Biotechnology Information. PubChem Compound Database; CIR=7175; Propyl 4-hydroxybenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/7175. Last Updated: 2017. Accessed: 3/30/2017.

172. US National Center for Biotechnology Information. PubChem Compound Database; CID=54686127; Calcium bis(4-hydroxybenzoate). https://pubchem.ncbi.nlm.nih.gov/compound/54686127. Last Updated: 2017. Accessed: 1/10/2017.

173. US National Center for Biotechnology Information. PubChem Compound Database; CID=23663689; Potassium butyl 4-oxidobenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/23663689. Last Updated: 2017. Accessed: 1/10/2017.

174. US National Center for Biotechnology Information. PubChem Compound Database; CIR=23696798; Potassium ethyl 4-oxidobenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/23696798. Last Updated: 2017. Accessed: 3/30/2017.

175. US National Center for Biotechnology Information. PubChem Compound Database; CIR=23663677; potassium methyl 4-oxidobenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/23663677. Last Updated: 2017. Accessed: 3/20/2017.

176. US National Center for Biotechnology Information. PubChem Compound Database; CID=23672310; Potassium 4-hydroxybenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/23672310. Last Updated: 2017. Accessed: 10/10/2017.

177. US National Center for Biotechnology Information. PubChem Compound Database; CID=23662516; Potassium propyl 4-oxidobenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/23662516. Last Updated: 2017. Accessed: 1/10/2017.

178. US National Center for Biotechnology Information. PubChem Compound Database; CID=23671890; 36457-20-2. https://pubchem.ncbi.nlm.nih.gov/compound/23671890. Last Updated: 2017. Accessed: 1/10/2017.

179. US National Center for Biotechnology Information. PubChem Compound Database; CID=23662515; Sodium isobutyl 4-oxidobenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/23662515. Last Updated: 2017. Accessed: 1/10/2017.

180. US National Center for Biotechnology Information. PubChem Compound Database; CIR=23663626; 5026-62-0. https://pubchem.ncbi.nlm.nih.gov/compound/23663626. Last Updated: 2017. Accessed: 3/25/2017.

181. US National Center for Biotechnology Information. PubChem Compound Database; CID=16219477; Sodium 4-hydroxybenzoate. https://pubchem.ncbi.nlm.nih.gov/compound/16219477. Last Updated: 2017. Accessed: 1/10/2017.

182. US National Center for Biotechnology Information. PubChem Compound Database; CID=23679044; 35285-69-9. https://pubchem.ncbi.nlm.nih.gov/compound/23679044. Last Updated: 2017. Accessed: 1/10/2017.

183. ChemDraw. Cambridge, MA: Cambridge Soft Corporation; 2002.

184. Tandon P, Baboo R, Singh A, Purwar G, Purwar M. Simple one-pot conversion of organic compounds by hydrogen peroxide activated by ruthenium(III) chloride: organic conversions by hydrogen peroxide in the presence of ruthenium(III). *Appl Organomet Chem* 2005;19(10):1079-1082.

185. US Food and Drug Administration (FDA). 2011. Estimation Programs Interface Suite™ for Microsoft® Windows. Vol 4.0. Washington, DC, USA: FDA.

186. Henchoz Y, Romand S, Schappler J, Rudaz S, Veuthey J, Carrupt P. High-throughput log P determination by MEEKC coupled with UV and MS detections. *Electrophoresis* 2010;31(5):952-964.

187. Registry of Toxic Effects of Chemical Substances (RTECS). Isobutylparaben and Isopropylparaben entries. *RTECS database*. National Library of Medicine Toxicology Data Network (TOXNET). 1993.

188. Homburger F. Carcinogenicity of several compounds. NTIS Report PB No. 183 027. 1968. Pages1-26.

189. Bijlsma U. Solbrol-p-hydroxybenzoic acid methyl ester. *Arch Int Pharmacodyn Ther* 1928;34:173-179.

190. Mason M, Cate C, Baker J. Toxicology and carcinogenesis of various chemicals used in the preparation of vaccines. *Clin Toxicol* 1971;4:185-204.

191. Adler-Hradecky C, Kelentey B. On the toxicity and local analgetic effect of *p*-hydroxybenzoic acid esters. *Arch Int Pharmacodyn Ther* 1960;128:135-142.

192. Park C, Nah W, Lee J, Oh Y, Gye M. Butyl paraben-induced changes in DNA methylation in rat epididymal spermatozoa. *Andrologia* 2012;44(Suppl. 1):187-193, 187.

193. Pop A, Drugan T, Gutleb A, et al. Individual and combined in vitro (anti)androgenic effects of certain food additives and cosmetic preservatives. *Toxicol In Vitro* 2016;32:269-277.

194. National Toxicological Program (NTP). Testing Status of Propyl-4-hydroxybenzoate M88006. https://ntp.niehs.nih.gov/testing/status/agents/ts-m88006.html. Last Updated: 1/17/2019. Accessed: 4/29/2019.