Safety Assessment of Parabens as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	February 23, 2018
Panel Meeting Date:	March 5-6, 2018

The 2018 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 Lillian C. Becker, Scientific Analyst/Writer and Bart Heldreth.

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MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Bart Heldreth, PhD, CIR Executive Director

Date: February 23, 2018

Subject: Parabens

Attached is the updated draft review of 20 parabens as used in cosmetics. These parabens are reported to function in cosmetics primarily as preservatives. In 2017, the Panel agreed to add Sodium Methylparaben to the Priority List due to the large number of reported uses in the FDA's Voluntary Cosmetic Registration Program (VCRP) database. The Expert Panel agreed that it would be appropriate to group this ingredient with 7 parabens reviewed in the CIR safety assessment published in 2008: Methylparaben; Ethylparaben; Propylparaben; Butylparaben; Benzylparaben; Isopropylparaben; and Isobutylparaben. In addition, the Panel included 12 other paraben salts that had not yet been reviewed: Calcium Paraben; Potassium Butylparaben; Potassium Ethylparaben; Potassium Methylparaben; Potassium Paraben; Potassium Propylparaben; Sodium Butylparaben; Sodium Ethylparaben; Sodium Isobutylparaben; Sodium Isopropylparaben; Sodium Paraben; and Sodium Propylparaben. At the June 2017 meeting, the Panel also elected to add 4-Hydroxybenzoic Acid to the group.

However, 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 specific cell marker in the testes of offspring of female rats orally dosed with 10 mg/kg/day Butylparaben during the 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 the 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. The Panel agreed that a subject matter expert should be consulted to review the reproductive toxicity data available for the parabens, and identify additional relevant data that the Panel should consider, if any. [*parabe032018Rep*]

This expert should provide professional opinions on the relevance of the animal-model toxicity endpoints reported in the DART studies available for assessing the safety of the parabens as used in cosmetics. This expert should evaluate the quality, and facilitate the interpretation of, the data on which NOAELs, lowest-observed adverse effect levels (LOAELs), and MOS values may be derived to assess the safety of these cosmetic ingredients. The Panel agreed to table the re-review of the parabens pending the input of such an expert. Just such an expert is scheduled to present on this topic at this CIR Expert Panel Meeting.

Furthermore, someadditional references have been submitted by stakeholders or discovered by CIR. These references are not yet incorporated into the report, and the Panel is asked to consider the merit and relevance of these documents to this safety assessment. [*parabe032018Refs*] Specifically, these are titled:

> *Urinary concentrations of parabens and reproductive parameters in young men*
>
> *Effects on the reproductive system of young male rats of subcutaneous exposure to n-butylparaben*
>
> *Personal Care Product Use in Men and Urinary Concentrations of Select Phthalate Metabolites and Parabens: Results from the Environment and Reproductive Health (EARTH) Study*
>
> *Human Semen Quality, Sperm DNA Damage, and the Level of Reproductive Hormones in Relation to Urinary Concentrations of Parabens*

The Panel should review the information presented at this meeting and either affirm or change the conclusion from the 2008 report on the original seven paraben ingredients. The Panel should also determine if this conclusion can be applied to the newly added ingredients, or if a split conclusion is warranted. Whether the conclusion remains the same (and extends to all of the new ingredients) or is to be changed and/or split, the Panel should issue a Tentative Amended Report, and develop the basis for the Discussion. However, if information (and/or added ingredients) raise new questions for the Panel that are not answered by the available data, then an Insufficient Data Announcement, with a list of data needs, should be issued.

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INTRODUCTION

This is a re-review of the available scientific literature and unpublished data relevant to assessing the safety of parabens as used in cosmetics. According to the web-based *Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*; wINCI), The ingredients in this paraben group are reported to function as preservatives, and two are reported to function as fragrances (Table 1).[1]

In 2017, the Expert Panel (Panel) agreed to add Sodium Methylparaben to the Priority List due to the large number of reported uses in the FDA's Voluntary Cosmetic Registration Program (VCRP) database. The Expert Panel agreed that it would be appropriate to group this ingredient with the 7 parabens reviewed by the Cosmetic Ingredient Review (CIR) in the safety assessment published in 2008: Methylparaben; Ethylparaben; Propylparaben; Butylparaben; Benzylparaben; Isopropylparaben; and Isobutylparaben. In addition, the Panel included 12 other paraben salts that had not yet been reviewed: Calcium Paraben; Potassium Butylparaben; Potassium Ethylparaben; Potassium Methylparaben; Potassium Paraben; Potassium Propylparaben; Sodium Butylparaben; Sodium Ethylparaben; Sodium Isobutylparaben; Sodium Isopropylparaben; Sodium Paraben; and Sodium Propylparaben. At the June 2017 meeting, the Panel also elected to add 4-Hydroxybenzoic Acid to the group. However, 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 specific cell marker in the testes of offspring of female rats orally dosed with 10 mg/kg/day Butylparaben during the 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 the 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. The Panel agreed that a subject matter expert should be consulted to review the reproductive toxicity data available for the parabens, and identify additional relevant data that the Panel should consider, if any. This expert should provide professional opinions on the relevance of the animal-model toxicity endpoints reported in the DART studies available for assessing the safety of the parabens as used in cosmetics. This expert should evaluate the quality, and facilitate the interpretation of, the data on which NOAELs, lowest-observed adverse effect levels (LOAELs), and MOS values may be derived to assess the safety of these cosmetic ingredients. The Panel agreed to table the re-review of the parabens pending the input of such an expert.

Pertinent data were discovered in the European Chemicals Agency (ECHA) database.[2-8] Data were also discovered in reports by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Union's (EU) Scientific Committee on Consumer Products (SCCP).[9-17]

An exhaustive search was conducted for new data on the safety of parabens in preparation of the previous iteration of this report. A few short-term, but no new acute, subchronic or chronic toxicity studies, were discovered.

New epidemiology studies explored the possibility of associations between markers of paraben exposure and adverse health outcomes, including prospective and retrospective studies. Exposures to Methylparaben, Propylparaben and Butylparaben were evaluated in all of these studies. In addition, aggregate exposures to Ethylparaben and Benzylparaben were considered. Taken together, these studies reported numerous comparisons between exposure markers and outcomes, only a fraction of which were statistically significant. The safety assessment report provides relatively brief summaries of all of these studies, focused on the statistically-significant results reported.

Dermal penetration, toxicokinetics, short-term toxicity, DART, endocrine-activity, genotoxicity, and epidemiology studies are also briefly summarized in the body of the report, and in most cases details are provided in tables. However, toxicity studies conducted in animals exposed to individual parabens by subcutaneous injection, and toxicity tests in animals exposed to mixtures of parabens with other compounds (e.g., phthalates), were not included because they lack relevance in assessing the toxicity of these ingredients in cosmetics.

CHEMISTRY

Definition and Structure

The ingredients in this safety assessment are paraben phenolic acids and their salts and carboxylic acids. The basic paraben structure is provided in Figure 1.

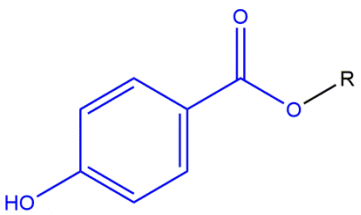


Figure 1a. Paraben phenolic acids: a generic structure wherein R is an alkyl group from 1 to 4 carbons long, or is benzyl.

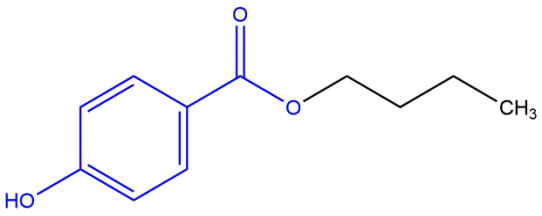


Figure 1b. Paraben phenolic acids: an example, Butylparaben (wherein R from the generic structure in Figure 1a, is an alkyl group 4 carbons long).

The salts of these phenolic acids are being added to this re-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 2).

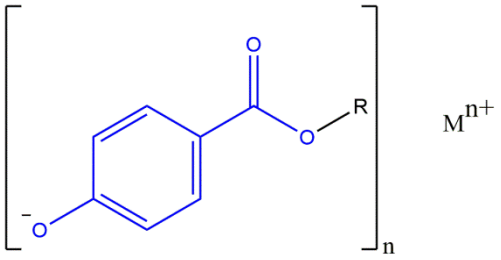


Figure 2a. Paraben phenolic salts: generic structure wherein R is an alkyl group from 1 to 4 carbons long and M is sodium or potassium.

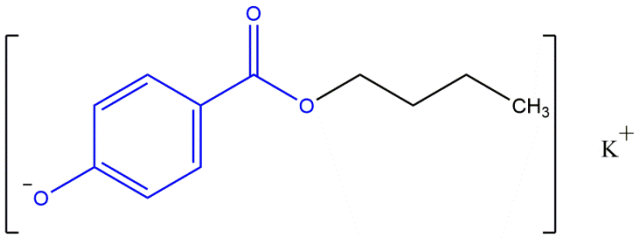


Figure 2b. Paraben phenolic salts: an example, Potassium Butylparaben (wherein R, from the generic structure in Figure 2a, is an alkyl group 4 carbons long and M is potassium).

Also added to this re-review, are the carboxylic acids of parabens (i.e., not esters). The carboxylic proton 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 3).

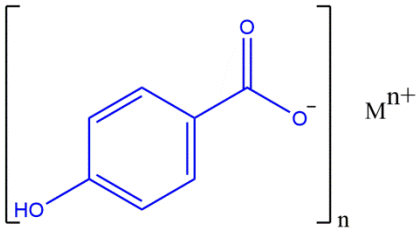


Figure 3a. Paraben carboxylic salts: a generic structure wherein M is sodium, potassium, or calcium.

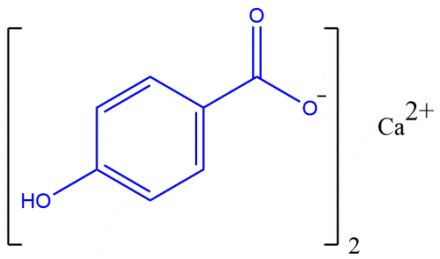


Figure 3b. Paraben carboxylic salts: an example, Calcium Paraben (wherein M, from the generic structure in Figure 3a, is calcium and n is 2).

Physical and Chemical Properties

Physical and chemical properties of the parabens in this safety assessment are presented in Table 3.

Parabens form small colorless crystals or white crystalline powders with practically no odor or taste.[18] 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.

The median particle diameter (D_{50}) of Sodium Methylparaben was reported to be 117.1 ± 17.5 µm, Ethylparaben was 307.5 ± 21.9 µm; Sodium Ethylparaben was 49.5 ± 6.4 µm; and Sodium Propylparaben was 37.8 ± 4.9 µm (Table 4).[2,3,5,6]

Parabens are stable against hydrolysis during autoclaving and resist saponification.[19]

Method of Manufacture

Paraben phenolic acids (and salts) are prepared by esterifying *p*-hydroxybenzoic acid (PHBA) with the corresponding alcohol in the presence of an acid catalyst, such as sulfuric acid, and an excess of the specific alcohol.[18] The acid is then neutralized with caustic soda, and the product is crystallized by cooling, centrifuged, washed, dried under vacuum, milled, and blended. Benzylparaben can also be prepared by reacting benzyl chloride with sodium *p*-hydrobenzoic acid. Paraben carboxylic salts may be prepared by deprotonating PHBA with an appropriate alkaline salt (e.g., sodium hydroxide could be used to prepare Sodium Paraben).[20]

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the 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 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 2017, Methylparaben was reported to be used in 13,797 formulations (10,784 in leave-on formulations, 2889 in rinse-off formulation, and 124 in bath formulations); this is an increase from 8786 formulations reported in 2006 (Table 5 and Table 6).[18,21] Propylparaben had the next highest number of reported uses at 10,642 (8668 in leave-on formulations, 1875 rinse-off formulations, and 99 in bath formulations); this was an increase from 7118 formulations 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.[18,22] 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.

In some cases, reports of uses were received in the VCRP, but concentrations of use data were not provided. For example, Sodium Butylparaben is reported to be used in 5 cosmetic formulations, but no use concentration data were reported. In other cases, no uses were reported in the VCRP, but concentration of use data were received from industry; Sodium Paraben had no reported uses in the VCRP, but a use concentration in the category of other skin care preparations was provided in the industry survey. Therefore, it should be presumed there is at least one use in every category for which a concentration is reported.

The ingredients not in use according to the VCRP and industry survey are listed in Table 7.

Several of the parabens with reported uses are used in products that can be ingested incidentally (e.g., Methylparaben at up to 0.35% in lipstick), used near the eye (e.g., Methylparaben at up to 0.8% in mascara), come in contact with mucous membranes (e.g., Methylparaben at up to 0.5% in bath oils, tablets and salts), or in baby products (e.g., Methylparaben at up to 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) and could possibly be inhaled. These ingredients are reportedly used at concentrations up to 0.41% in spray products (e.g., Methylparaben in the category of other fragrance products). 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.[23-26] 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.[23,25] 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.[23] 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, and could possibly be inhaled. These ingredients are reportedly used in loose powder products (e.g., Ethylparaben in face powders at up to 0.5%). 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.[27-29]

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.[30] There are no established adverse outcome pathways for this weak estrogenic activity.

NICNAS published the following conclusion:

> "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 available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak oestrogenic activity, but there are no established adverse outcome pathways for this effect. Should further information on adverse outcome pathways in mammals associated with weak oestrogenic activity become available, further assessment of these chemicals at Tier III could be required." [30]

The SCCP of the EU has published several opinions on parabens over the last few years (Table 8).[11-17] The current SCCP opinion 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-… Therefore, for these compounds, the human risk cannot be evaluated. The same is true for Benzylparaben…"[15,17]

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-1.[18] The opinion restated the acceptable daily intake (ADI) of 0 to 10 mg/kg day-1 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 No-observed-adverse-effect-level (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]

An evaluation by the JECFA determined that the acceptable daily intake (ADI) of the sum of the Ethylparaben and Methylparaben to 0-10 mg/kg.[31] In view of the adverse effects in male rats, Propylparaben was excluded from the group ADI for the parabens used in food.[32]

TOXICOKINETIC STUDIES

Dermal Penetration

2008

Parabens in cosmetic formulations applied to skin penetrate the stratum corneum in inverse relation to the ester chain length.[18] Carboxylesterases present in keratinocytes hydrolyze parabens in the skin. The extent of the breakdown to PHBA is different between rodent and human skin. In vitro studies also indicate a difference in the extent of hydrolysis to PHBA, 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 concentration (0.1%) of Methylparaben penetrated porcine skin (fresh or after stored frozen) in 4 h.[33] In 24 h, 2.0%- 5.8% and 2.9%-7.6% 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 lipophilicity.[34] Increasing the ethanol concentration or the exposure duration increased the retention of the parabens in the dermis, compared 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. Penetration of parabens in 3 commercial facial cream through rabbit ear skin ranged from 20%-60%, after 8 h in Franz-type diffusion cells, increasing (Propylparaben < Ethylparaben < Methylparaben) with increasing water solubility of the paraben, regardless of the formulation tested.[35] 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%).[36] Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin. The authors state that the results show that parabens may be classified as moderate penetrants. Penetration was inversely proportional to the lipophilicity of the parabens tested (0.057% for Methylparaben to 0.007% for Butylparaben), and increased with repeated applications.[37]

Human

Butylparaben

Dermal penetration was studied in 26 healthy Caucasian male volunteers, 21 to 36 years old, after application of 2% (w/w) Butylparaben in Essex cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate.[38] Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week (control week) were followed by daily application of the cream with the test substances for 1 week. Concentrations of Butylparaben were measured in blood serum samples. Butylparaben serum concentrations 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.[39] 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 4-cyanophenol 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. This suggests that CP enhances skin permeation of Methylparaben primarily by increasing the solubility of Methylparaben in the SC (especially in the nonlipid regions), and Methylparaben increases 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 p-hydroxybenzoic acid, conjugated, and the conjugate excreted in the urine.[40] 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 p-hydroxybenzoic 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.[41] Excretion is in the urine. Small amounts of the ester are excreted unmetabolized or hydrolyzed to the benzyl alcohol and p-hydroxybenzoic 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 p-hydroxybenzoic acid; 20% was not recovered.[42]

2008

Ingested parabens are quickly absorbed from the gastrointestinal tract, hydrolyzed to p-hydroxybenzoic acid, conjugated, and the conjugate excreted in the urine.[18] 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.

In Vitro

CELL-FREE SYSTEMS

Cell-free systems ADME studies are presented in Table 10.

Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for α-fetoprotein (AFP).[43] On the other hand, the 50% inhibitory concentration (IC_{50}) of Benzylparaben was 0.012 µM. Butylparaben was biotransformed to *p*-hydroxybenzoic acid with maximum rate at saturating concentration (V_{max}) of 8.8 nmol/min/mg protein.[44]

Methylparaben and Ethylparaben were stable in human plasma, but Propylparaben, Butylparaben and Benzyl-paraben concentrations decreased by 50% within 24 h.[45] All parabens tested were rapidly hydrolyzed when incubated with human liver microsomes (HLM), 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.[46] In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. 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 (the longer the alcohol moiety, the slower the hydrolysis).[47] 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, 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.

CELL CULTURES

Cell culture ADME studies are presented in Table 10.

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference. [48] Butylparaben was extensively hydrolyzed to *p*-hydroxybenzoic acid as the major metabolite for both sexes and species. The other metabolite observed in the human hepatocytes was 4-hydroxyhippuric acid.

Animal

DERMAL EXPOSURE

Animal dermal ADME studies are presented in Table 10.

In rats exposed to a single dermal dosage of 100 mg/kg bw radiolabeled 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.[49] Most of the dosage (≥46.4%) was not absorbed, and less than 25.8% 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. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of radiolabeled Butylparaben was absorbed 72 h following application to the skin in rats.[48] 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.

ORAL EXPOSURE

Oral ADME studies are presented in Table 10.

In rats exposed to a single oral dosage of 100 mg/kg bw radiolabeled Methylparaben, Propylparaben, or Butylparaben, 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.[49] 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 100 mg/kg bw/day dosage of radiolabeled Butylparaben.[48] 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. In general, tissue levels at 24 h were considerably higher in female rats. Metabolites detected in urine included newly discovered metabolites arising from ring hydroxylation followed by glucuronidation and sulfation.

Human

DERMAL EXPOSURE

Human dermal ADME studies are presented in Table 10.

All 26 healthy male volunteers showed increased excretion of Butylparaben following daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben. [50] 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. Free and conjugated parabens and their major, non-specific metabolites (*p*-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.[51] Minor metabolites discovered had hydroxy groups on the alkyl side chain or oxidative modifications on the aromatic ring.

AGGREGATE EXPOSURE

Aggregate exposure ADME studies are presented in Table 10.

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.[52] 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.

Methylparaben, Butylparaben, and Benzylparaben were detected in all placenta samples collected from healthy mothers.[53] The highest measured concentration was 11.77 ng Methylparaben/g tissue.

TOXICOLOGICAL STUDIES

Acute Dose Toxicity

No new published acute toxicity studies were discovered and no unpublished data were submitted.

1984

Acute toxicity studies in animals indicate that parabens are practically nontoxic by various routes of administration.[40]

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.[41]

1995

Isobutylparaben had a subcutaneous LD_{50} of 2,600 mg/kg in mice.[42]

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.[42]

Dermal

Short-term dermal toxicity studies are presented in Table 11.

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.[54] Macroscopic and microscopic examinations revealed mild-to-moderate skin damage in female rats. No-observed-adverse-effect-levels (NOAEL) for Isobutylparaben and Isopropylparaben were 600 mg/kg bw/day, and 50 mg/kg bw/day, respectively.

Oral

Short-term oral toxicity studies are presented in Table 11.

At 100 and 300 mg/kg bw/day Propylparaben administered orally, 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.[55] 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. Serum markers of lipid-peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically-significantly elevated in rats exposed to 250 mg/kg bw/day Methylparaben.[56] Malondiadehyde 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.[57]

Subchronic Toxicity Studies

No new published subchronic toxicity studies were discovered and no unpublished data were submitted.

1984

Subchronic… oral studies indicate that parabens are practically nontoxic.[40]

Chronic Toxicity Studies

No new published chronic toxicity studies were discovered and no unpublished data were submitted.

1984

…[C]hronic oral studies indicate that parabens are practically nontoxic.[40]
A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses up to 2 g/kg/day.

1995

Mice were orally dosed with 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks.[42] *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.*

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.[18] *Isobutylparaben and Butylparaben were noncarcinogenic when given to mice in a chronic feeding study.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

1984

Methylparaben was nonteratogenic in rabbits, rats, mice and hamsters, and Ethylparaben was nonteratogenic in rats.[40]

2008

Methylparaben was nonteratogenic in rabbits, rats, mice, and hamsters, and Ethylparaben was nonteratogenic in rats.[18] *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 day- 1, even with some maternal toxicity at that dose. The maternal toxicity NOAEL dose was 100 mg/kg day-1.*

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 reported no spermatotoxic effects. Propylparaben did affect sperm counts at all levels from 0.01% to 1.0%. Epididymis and seminal vesicle weight decreases were reported in rats given a 1% oral Butylparaben dose decreased sperm number and motile activity in F1 offspring of rats maternally exposed to 100 mg/kg day-1 were reported. Decreased sperm numbers and activity were reported in F1 offspring of female rats exposed to Butylparaben at 100 or 200 mg/kg day-1, but there were no abnormalities in the reproductive organs.

Methylparaben was studied using [male] rats at levels in the diet up to 10000 ppm (estimated mean dose of 1141.1 mg/kg day^{-1}) with no adverse effects. Butylparaben was studied using rats at levels in the diet up to 10000 ppm (estimated mean dose of 1087.6 mg/kg day^{-1}) in a repeat of the study noted above, but using a larger number of animals and a staging analysis of testicular effects. No adverse reproductive effects were found.

Butylparaben binds to estrogen receptors in isolated rat uteri, with an affinity orders of magnitude less than natural estradiol. The estrogenic effect of parabens has been estimated by their competitive binding to the human estrogen receptors α and β. With DES 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.

Parabens and PHBA have been studied in several uterotrophic assays. PHBA at 5 mg/kg day- 1 (s.c.) was reported to produce an estrogenic response in one uterotrophic assay using mice, but there was no response in another study using rats (s.c. up to 5 mg/kg day-1) and mice (s.c. up to 100 mg/kg day-1) and in a study using rats (s.c. up to 100 mg/kg day-1).

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 20000 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 25000 less; the potency of Propylparaben was 1612 to 20000 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 PHBA 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 p-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 17 β-estradiol and DES (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.

Dermal Exposure

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

Oral Exposure

Oral DART studies are summarized in Table 12.

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 gestation day (GD) 7-GD21.[58] Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring were statistically-significantly reduced at 10 mg/kg bw/day or more. Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day. CYP19 and estrogen receptor (ER)α expression was statistically-significantly increased, and the expression of steroidogenic acute regulatory protein (StAR), cytochrome cholesterol side-chain cleavage enzyme (P450scc), estrogen sulfotransferase (SULT1E1), and androgen receptor (AR) in the testes and methylation rate of the ERα promoter were statistically-significantly reduced, in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD7 to GD21.[59] Weights of the testes, epididymal cauda sperm counts, and daily sperm production in male offspring were statistically significantly-reduced in the 400 and 1000 mg/kg bw/day groups of rats orally exposed to Butylparaben on GD7 to post-natal day (PND) 21.[60] 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.[61] Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

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.[62] 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 effects on serum hormone concentrations, estradiol concentrations were statistically-significantly 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.[63]

F2 pups exhibited statistically-significantly 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.[64] 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.[65]

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.[44] 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 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.

GENOTOXICITY STUDIES

1984

Numerous mutagenicity studies, including the Ames Test, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the parabens are nonmutagenic.[40]

1995

Chinese hamster fibroblast cell lines treated with 0.03% Isobutylparaben had no chromosomal aberrations after 48 h.[42]

At a concentration of 1 mg/plate Isobutylparaben and Isopropylparaben had negative Ames tests in S. 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

Numerous genotoxicity studies, including Ames testing, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the parabens are generally nonmutagenic, although Ethylparaben and Methylparaben were judged to induce significant chromosomal aberrations (11.0% and 15.0% increases, respectively) in an in vitro assay using Chinese Hamster ovary cells.[18]

In Vitro

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.[66] Alterations in cell proliferation were assessed by microscopic analysis of the mitotic index and by flow cytometry. Cells treated with 500 µM Propylparaben were analyzed for cell-cycle distribution. Induction of DNA double-strand breaks (DSBs) was examined by indirect immuno-fluorescence against the phosphorylated form of the variant histone γ-H2AX. Possible oxidative DNA damage was evaluated by immunocytochemical analysis of 8-hydroxydeoxyguanosine (8-OHdG). 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) at 500 µM increase in 8-OhdG staining at all concentrations tested (maximum intensity at 500 µM).

Propylparaben, Butylparaben

Chinese hamster ovary (CHO-K1) cells were grown, and incubated for 1 or 3 h with 0, 0.5, 1, 1.5, 2, or 2.5 µM Propylparaben or 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 Butylparaben (depending on the test), at

37°C in Ham's F-12 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL).[67] Sister chromatid exchange (SCE), chromosome aberration (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 Butylparaben (≥0.4 µM) or Propylparaben (≥1 µM). Butylparaben, in particular, showed comparatively high incidences of fragmentation. Statistically-significantly elevated SCEs/cell were observed in cells incubated with Butylparaben (0.75 µM) or Propylparaben (≥1.5 µM) for 3 h. Statistically-significantly elevated CAs/cell were observed in cells incubated with Butylparaben (0.75 µM) or Propylparaben (≥1 µM) for 3 h.

In Vivo

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

CARCINOGENICITY STUDIES

No new published dermal, oral, or inhalation carcinogenicity studies were discovered and no unpublished data were submitted.

1984

Methylparaben was noncarcinogenic when injected subcutaneously in mice or rats when administered intravaginally in rats and was not co-carcinogenic when injected subcutaneously in mice.[40] 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.[42]

In Vitro

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.[68] 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).

Methylparaben

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.[69] 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.[69] Some cells 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 ($p < 0.001$).

OTHER RELEVANT STUDIES

Endocrine Activity

In vitro and in vivo endocrine activity studies are summarized in Table 13.

Weak activation of mPPARα was seen in murine NIH-3T3-L1 cells at the highest concentrations of Butylparaben tested (100 µM).[70] Butylparaben activated murine peroxisome proliferator-activated receptor (mPPAR)γ 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.

Isobutylparaben antagonized the androgen receptor (AR) in Chinese Hamster ovary cells. The effect was statistically significant at ≥ 25 µM.[71] 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).[72] The EC_{50} for glucocorticoid-like activity in MDA-kb2 cells was 1.75 mM for Butylparaben and 13.01 mM for Propylparaben.[73] 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.[74]

Butylparaben exhibited estrogen agonism at all concentrations tested in T47D-KBluc cells.[75] 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 NOECs from 0.05 - 8 µM for the parabens tested.[76] 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.[77] 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.[78] 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.[79] 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.

Relative uterine weights were elevated in immature Sprague-Dawley rats after treatment with ≥0.16 mg/kg bw/day Benzylparaben on PND21-PND23.[80] LOELs for increased relative uterine weight after treatment of immature female rats with Methylparaben or Ethylparaben on PND21-PND23 were 20 and 4 mg/kg bw/day, respectively.[81] No observed effects levels (NOEL) 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.[82] 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 bw dosage of Butylparaben.[83] Transferase uridyl nick end labeling (TUNEL) assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h.

In 26 healthy Caucasian males, minor differences in inhibin B, luteinizing hormone (LH), estradiol, total thyroxine (T4), free thyroxine (FT4), and thyroid stimulating hormone (TSH) concentrations were observed after daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben, compared to the concentrations measured before the treatment.[38] The differences could not be attributed to the treatment.

DERMAL IRRITATION AND SENSITIZATION STUDIES

No new published animal or human irritation and sensitization studies were discovered and no unpublished data were submitted.

1984

Methylparaben (100% and 10%), Propylparaben (10%), and Ethylparaben (100% and 10%) were, at most, mildly irritating when applied to rabbit skin.[40]

Parabens are practically nonirritating and 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 showed no evidence of significant irritation or sensitization potential for these ingredients.

Parabens are practically nonsensitizing in the [human] population with normal skin. 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. Skin sensitization tests on product formulations containing from 0.1 to 0.8 percent of one or two of the parabens showed no evidence of significant irritation or sensitization potential for these ingredients.

Practically all animal sensitization tests indicate that the parabens are nonsensitizing.

1986

Benzylparaben …was neither an eye nor skin irritant when tested in rabbits.[41]

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.

Parabens are practically nonirritating 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

Methylparaben, Ethylparaben, Propylparaben,
Isopropylparaben, Butylparaben, Isobutylparaben, Benzylparaben

The parabens were tested individually for irritancy and sensitization potential in co-cultured human keratinocyte and peripheral blood mononuclear cells (PBMCs).[84] 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 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} for cell death ≤ 50 µM were considered to be irritating, EC_{50} ranging from 50 - 1000 µM weakly irritating, and substances that did not reach the 50% threshold for cytotoxicity, or for which EC_{50} > 1,000 µM, were considered non-irritating. Substances with EC_{50} for CD86-expression ≤ 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

Photocontact sensitization and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methyl-, Propyl-, and/or Butylparaben gave no evidence for significant photoreactivity.[40]

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 ethanol vehicle.[85] 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 UVB (15 or 30 mJ/cm^2) after replacing the 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 UVA and UVC. After irradiation, the cells were incubated in culture medium without Methylparaben for various durations. Methylparaben statistically-significantly reduced cell viability 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 statistically-significantly elevated ($p < 0.5$) 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

No new published animal or human ocular irritation studies were discovered and no unpublished data were submitted.

1984

Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits.[40]

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.[41]

2008

There were no adverse reactions to 0.1 g of Benzylparaben.[18]

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.[86] 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.[40]

EPIDEMIOLOGICAL STUDIES

Primary Studies

PROSPECTIVE STUDIES

Prospective epidemiological studies are summarized in Table 14.

Preterm birth (PTB) 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).[87] 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. In another prospective study, in vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility.[88] 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.[89]

RETROSPECTIVE STUDIES

Retrospective epidemiological studies are summarized in Table 14.

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).[90] Linear regression analyses indicated an association between urinary Ethylparaben concentrations in 3-year old children and their body weights and heights.[91] The latter parameter was also associated with calculated estimates of aggregate exposures to parabens, including Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben. All regression coefficients calculated for girls and all other coefficients for boys were not statistically significant.

Linear regression analyses of data from the U.S. National Health and Nutrition Examination Survey (NHANES) program indicated an association between reduced serum thyroxine (T4) concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben.[92]

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 anthral follicle count (AFC).[93]

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).[94] The results also indicated an 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).

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.[95] The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

SUMMARY OF NEW DATA

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

In 2016, Sodium Methylparaben was included on the 2017 CIR Priority List due to the number of uses reported in the FDA VCRP database. It is appropriate to group this ingredient with additional paraben phenolic acids and their salts and carboxylic acids, some of which have already been reviewed by the CIR Expert Panel.

According to VCRP survey data received in 2017, Methylparaben was reported to be used in 13,797 formulations; this is an increase from 8786 formulations in 2006. Propylparaben had the next highest number of reported uses at 10,642; this was an increase from 7118 formulations 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 2016 indicate Methylparaben had the highest reported maximum concentration of use; it is used at 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.

In Franz-type diffusion cells, 2.3% - 3.3% of the applied concentration (0.1%) of Methylparaben penetrated porcine skin (fresh or after stored frozen) in 4 h. In 24 h, 2.0% - 5.8% and 2.9% - 7.6% 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 lipophilicity. Increasing the ethanol concentration or the exposure duration increased the retention of the parabens in the dermis, compared epidermis. Binary combinations of the parabens reduced their permeation rates, which was attributed by the authors to high retention in the epidermis and dermis. Penetration of parabens in three commercial facial cream through rabbit ear skin ranged from 20% to 60%, after 8 h in Franz-type diffusion cells, increasing (Propylparaben < Ethylparaben < Methylparaben) with increasing water solubility of the paraben, regardless of the formulation tested. 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%). Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin. The authors state that the results show that parabens may be classified as moderate penetrants. Penetration was inversely proportional to the lipophilicity of the parabens tested (0.057% for Methylparaben to 0.007% for Butylparaben), and increased with repeated applications.

Dermal penetration was studied in 26 healthy Caucasian male volunteers, 21 to 36 years old, after application of 2% (w/w) Butylparaben in Essex 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 (control week) were followed by daily application of the cream with the test substances for 1 week. Concentrations of Butylparaben were measured in blood serum samples. Butylparaben serum concentrations 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 three 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.

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. 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 CP. Receptor fluid (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 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 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. This suggests that CP enhances skin permeation of Methylparaben primarily by increasing the solubility of Methylparaben in

the SC (especially in the nonlipid regions), and Methylparaben increases skin permeation of CP by enhancing both the solubility and diffusivity of CP in the SC.

In in vitro tests, Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for AFP. On the other hand, the IC_{50} of Benzylparaben was 0.012 µM. Butylparaben was biotransformed to *p*-hydroxybenzoic acid with maximum rate at saturating concentration (V_{max}) of 8.8 nmol/min/mg protein.

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. The other metabolite observed in the human hepatocytes was 4-hydroxyhippuric acid. 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. 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 (the longer the alcohol moiety, the slower the hydrolysis). This trend was also observed for 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, 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.

In rats exposed to a single dermal dosage of 100 mg/kg bw radiolabeled Methylparaben, Propylparaben, or Butylparaben, C_{max} ($\geq$ 693 and $\geq$ 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 ($\geq$ 46.4%) was not absorbed, and less than 25.8% was found in the urine. Urinary excretion was the main route of elimination. Radioactivity was eliminated rapidly in the urine with averages $\geq$ 11.9% recovered in the first 48 h. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of radiolabeled Butylparaben was absorbed 72 h following application to the skin in rats. 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 radiolabeled Methylparaben, Propylparaben, or Butylparaben, C_{max} ($\geq$ 11432 and $\geq$ 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. Radioactivity was eliminated rapidly, with averages $\geq$ 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 100 mg/kg bw/day dosage of radiolabeled Butylparaben. 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 dosage excreted within 24 h. In general, tissue levels at 24 h were considerably higher in female rats. Metabolites detected in urine included newly discovered metabolites arising from ring hydroxylation followed by glucuronidation and sulfation.

All 26 healthy 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. Minor metabolites discovered had hydroxy groups on the alkyl side chain or oxidative modifications on the aromatic ring.

One or more of 5 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isobutylparaben) was detected 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 statistically significantly 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.

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, serum urea concentrations, lipid peroxidation and NO generation, and E2 concentrations. Statistically-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, among other effects.

Serum markers of lipid-peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically-significantly elevated in rats exposed to 250 mg/kg bw/day Methylparaben.

Malondiadehyde 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.

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. Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring were statistically-significantly reduced at 10 mg/kg bw/day or more. Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day. CYP19 and ERα expression was statistically-significantly increased, and the expression of StAR, P450scc, SULT1E1, and AR in the testes and methylation rate of the ERα promoter were statistically-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 statistically 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. Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

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. 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 effects on serum hormone concentrations, estradiol concentrations were statistically-significantly 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.

F2 pups exhibited statistically-significantly 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. 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. The authors concluded that the 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.

In prospective studies, PTB 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). 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. In another prospective study, in vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility. 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.

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 children and their body weights and heights. The latter parameter was also associated with calculated estimates of aggregate exposures to parabens, including Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben. All regression coefficients calculated for girls and all other coefficients for boys were not statistically significant.

Linear regression analyses of data from the U.S. 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.

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). The results also indicated an 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).

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. The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

PREVIOUS DISCUSSIONS

1984

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben[40]

It is important to note the concentrations at which the parabens are used in cosmetic products. In only two instances are the parabens reported to be used at concentrations greater than 5 percent. In fact, 99.7 percent of the products that contain parabens have concentrations of less than or equal to 1 percent. This information can be used to evaluate the adequacy of the data contained in this report with respect to the concentrations tested versus the concentrations used in cosmetic products.

A number of acute, subchronic, and chronic toxicity tests have been performed on the parabens using a wide variety of routes of administration. From these data, it is readily apparent that these ingredients exhibit a very low order of toxicity and must certainly be considered safe in this respect for cosmetic use in the usual quantities employed as a preservative. When tested on human skin, each of the parabens began producing evidence of irritation only when concentrations exceeded 5 to 12 percent. Considering the order of magnitude of these concentrations, it may be concluded that the parabens are relatively nonirritating at the concentrations used in cosmetic products.

The Food and Drug Administration's Ophthalmic Drug Panel concluded that Methylparaben and Propylparaben are unsafe as antimicrobial agents in OTC ophthalmic products because they are irritating to the eyes if used at concentrations effective against microorganisms. Supportive data were not available in the references cited in the Ophthalmic Drug Panel's report. Data available to the Cosmetic Ingredient Review indicate that there is no evidence for significant ocular irritation potential. Methylparaben and Ethylparaben, each at 100 percent concentration, and a number of product formulations containing Methyl-, Ethyl-, Propyl-, and/or Butylparaben at concentrations of 0.1 to 0.8 percent produced no more than minimal, transient ocular irritation in rabbits. Instillation of aqueous solutions of 0.1 to 0.3 percent Methylparaben several times daily into the eyes of more than 100 human subjects produced no irritation.

Sensitization to parabens has been reported, especially in cases where paraben-containing medicaments have been applied to damaged skin. However, in a total pool of over 27,000 subjects with chronic dermatitides, only 2.2 percent became sensitized to paraben preparations of 1 to 30 percent concentration. The results of tests obtained using healthy human skin confirm the results obtained in animals, both indicating that the parabens are free from allergenic behavior under these circumstances. Frequently, patients sensitized to parabens on damaged skin can tolerate usage on intact skin. In light of these data, it is recommended that parabens not be used on damaged skin due to the increased risk of sensitization.

1986

Benzylparaben[41]

Section 1 paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the CIR Procedures, the Expert Panel informed the public of its decision that the data on Benzylparaben are insufficient to determine that this ingredient, under the relevant condition of use, is either safe or not safe. The Panel released a "Notice of Insufficient Data Announcement" on October 10, 1984, outlining the data needed to assess the safety of Benzylparaben. The types of data required included:

1. *UV absorption spectrum. If absorption occurs between 280 and 360 nm,*
1. *a photosensitization study is required (in animals only, not in clinical assays).*
2. *Data detailing the possible presence of impurities.*
3. *Subchronic feeding study-go-day in rats.*
4. *Mutagenicity studies and/or in vitro assays for genotoxicity.*
5. *Eye irritation study at concentration of use.*
6. *Metabolism and associated pharmacokinetic studies are not requested at this time. If significant toxicity is shown in the above tests, the Expert Panel may request this additional type of testing.*

Acute animal oral toxicity and animal eye and skin irritation data were received in response to the above requests and are included in this report. The eye test data included in this report cannot be interpreted without an adequate description of the methodology used. The Expert Panel again concurred with the decision made during its earlier review that similar data on methylparaben, ethylparaben, propylparaben, or butylparaben were not necessarily applicable to the safety evaluation of Benzylparaben.

1995

Isobutylparaben and Isopropylparaben[42]

The Expert Panel recognizes that the actions and effects of Isobutylparaben and Isopropylparaben closely resemble those of Butylparaben, Ethylparaben, Methylparaben, and Propylparaben. In the evaluation of those parabens (Elder, 1984), the Panel issued a "safe as used" conclusion. The Panel acknowledges that since publication of that report there have been additional isolated cases of Paraben sensitivity. However, the fact that Parabens may be sensitizing was addressed in the discussion of Parabens in 1984, and the Expert Panel feels that the new case reports do not warrant a reevaluation of that conclusion. Furthermore, the body of evidence concerning Isobutylparaben and Isopropylparaben supports the conclusions drawn in 1984 concerning Parabens.

2008

Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isopropylparaben, Isobutylparaben, and Benzylparaben[18]

As previously considered, available acute, subchronic, and chronic toxicity tests, using a range of exposure routes, demonstrate a low order of parabens' toxicity at concentrations that would be used in cosmetics.

Parabens are rarely irritating or sensitizing to normal human skin at concentration used in cosmetics. Some individuals, however, may develop allergic reactions to parabens. The Expert Panel is aware of the "paraben paradox" in which paraben-sensitive patients who react with allergic contact dermatitis when paraben-containing pharmaceuticals are applied to eczematous or ulcerated skin can tolerate paraben-containing cosmetics applied to normal, unbroken skin. No reaction is induced even when these cosmetics contact the thin, delicate membrane of the eyelid. Clinical patch testing data available over the past 20 years demonstrate no significant change in the overall portion of dermatitis patients that test positive for parabens.

Although parabens do penetrate the stratum corneum and are available for distribution throughout the body, the Expert Panel noted that metabolism of parabens takes place within viable skin. Although the extent of this metabolism is different in different reports, the Expert Panel believes that a conservative estimate of 50% penetration of unmetabolized parabens may be used to compare exposures with adverse effects levels. The metabolism of parabens in the skin is likely to result in as low as 1% of unmetabolized parabens available for absorption into the body.

The Expert Panel considered that the most important new data available for assessing the safety of parabens as used in cosmetics are those data generally in the category of endocrine disruption, but which include male reproductive toxicity and various estrogenic activity studies. The Expert Panel believes that the available data demonstrate that parabens are, at most, weakly estrogenic. For example, the binding efficiency of parabens with estrogen receptors is around 4 orders of magnitude lower than estradiol.

The CIR Expert Panel compared exposures to parabens resulting from use of cosmetic products to a no observed adverse effect level (NOAEL). If that exposure is lower than the level shown to have no effect, then safety may be inferred. The CIR Expert Panel selected a NOAEL of 1000 mg/kg day-1 based on the most statistically powerful and well conducted study of the effects of Butylparabens on the male reproductive system. The Panel did note the several studies in which spermatotoxic effects were noted at lower doses. In the Expert Panel's experience, studies of sperm counts are particularly unreliable and evaluation of reproductive organs is a much more reliable and reproducible indicator. The benchmark study noted above included a careful staging analysis of reproductive organ damage, which was likely to detect even subtle forms of damage.

The Expert Panel acknowledged that one study has reported estrogenic activity in the uterotrophic assay system of the paraben metabolite, PHBA. Three other studies did not detect any estrogenic activity. In considering the benchmark end point of male reproductive effects, the Expert Panel noted that the available animal studies of Methylparaben and Ethylparaben (parabens with the shortest ester side chains) have demonstrated an absence of an effect, so it is considered unlikely that PHBA has any significant estrogenic activity.

The CIR Expert Panel considered exposures to cosmetic products containing a single paraben preservative (use level of 0.4%) separately from products containing multiple parabens (use level of 0.8%). The CIR Expert Panel recognized that industry survey data indicate lower use concentrations in products for infant use, and that use levels in many adult products will be lower, but these values are conservative for purposes of determining if there is any possibility of adverse effect. Adult (60 kg body weight) use of cosmetic products was estimated to be 17.76 g per day and infant (4.5 kg) use of cosmetic products was estimated to be 378 mg per day. Infants were separately considered because they would be a sensitive subpopulation for any agent capable of causing male reproductive effects.

Based on the available data demonstrating the metabolism of parabens in the human body and the absence of any tissue accumulation over time, the Expert Panel considered that infant exposure to parabens via breast-feeding was unlikely and that the only exposure of infants to parabens from cosmetic products would be from direct product use.

For adults, the relevant calculations are:

Systemic dose (single paraben) = 17.76 g/day of product
x 0.4% use concentration ÷ 60 kg person x 50% absorption
x 1000 mg/kg = 0.59 mg/kg day^{-1}

Systemic dose (multiple parabens) = 17.76 g/ day of product

x 0.8% use concentration ÷ 60 - kg person
x 50% absorption x 1000 mg/kg = 1.18mg/kg day^{-1}

For infants, the relevant calculations are:

Systemic dose (single paraben) = 378 mg/day of product
x 0.4% use concentration ÷ 4.5 kg infant x 50% absorption
= 0.168mg/kg day^{-1}

Systemic dose (multiple parabens) = 378 mg/day of product
x 0.8% use concentration ÷ 4.5 kg infant x 50% absorption
= 0.336mg/kg day^{-1}

Based on these systemic doses and the NOAEL for Butylparaben of 1000 mg/kg day^{-1}, a margin of safety (MOS) may be determined by dividing the NOAEL by the systemic dose to yield the MOS values shown in Table 15. The Expert Panel considers that these MOS determinations are conservative and likely represent an overestimate of the possibility of an adverse effect (e.g., use concentrations may be lower, penetration may be less). As presented, the MOS over the level demonstrated to produce no adverse male reproductive toxicity is around 3 orders of magnitude or greater. The CIR Expert Panel considers this MOS adequate to assure the safety of cosmetic products in which these preservatives are used.

DISCUSSION

[*To be developed*]

CONCLUSION

[*To be developed*]

TABLES

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

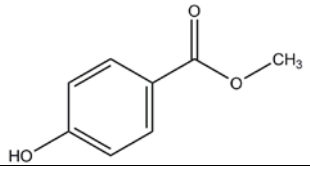
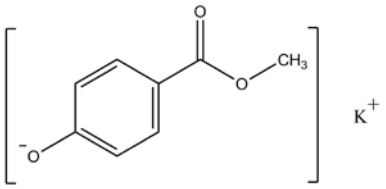
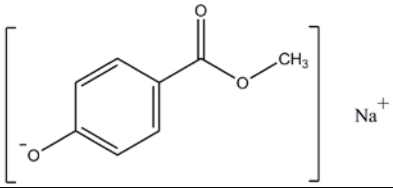
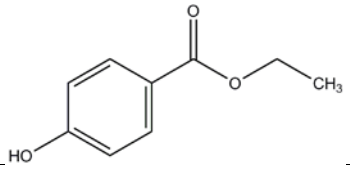
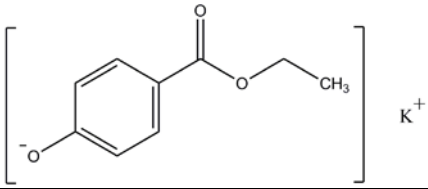
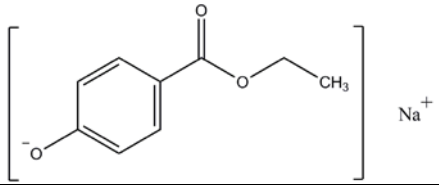
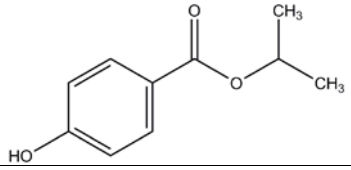
Ingredient CAS No.	Definition & Structure	Function
Parabens and Paraben Salts		
Methylparaben 99-76-3	Methylparaben is the ester of methyl alcohol and p-hydroxybenzoic acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Methylparaben 26112-07-2	Potassium Methylparaben is the 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 *p*-hydroxybenzoic 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 *p*-hydroxybenzoic acid. It conforms to the formula: 	Preservative

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

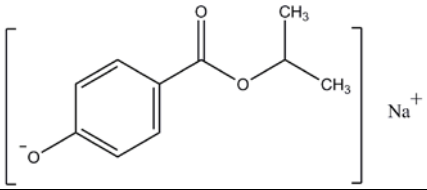
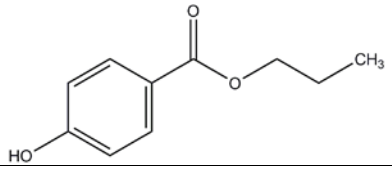
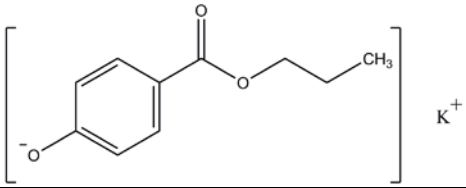
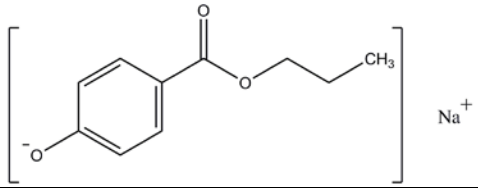
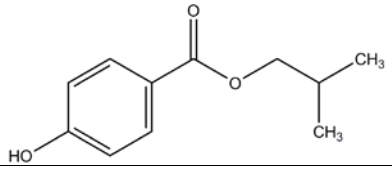
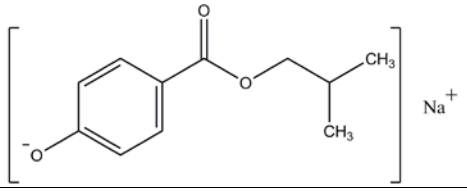
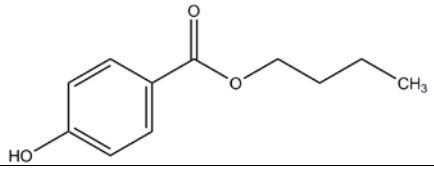
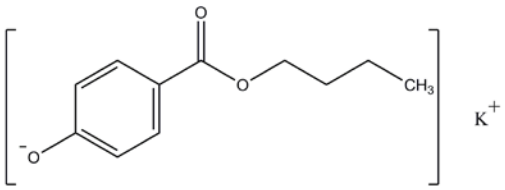
Ingredient CAS No.	Definition & Structure	Function
Sodium Isopropylparaben	Sodium Isopropylparaben is the sodium salt of Isopropylparaben: 	Preservative
Propylparaben 94-13-3	Propylparaben is the ester of n-propyl alcohol and *p*-hydroxybenzoic acid. It conforms to the formula: 	Fragrance ingredient, 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 *p*-hydroxybenzoic 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 *p*-hydroxybenzoic acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Butylparaben 38566-94-8	Potassium Butylparaben is the potassium salt of Butylparaben that conforms to the formula: 	Preservative

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

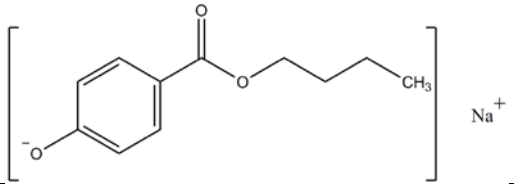
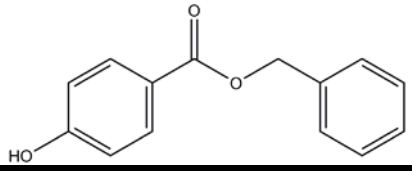
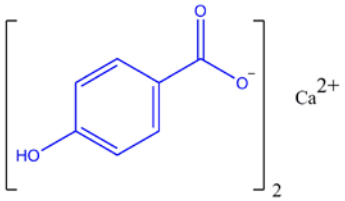
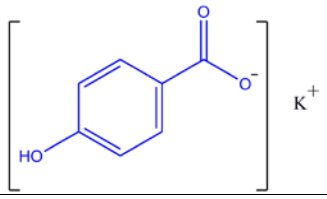
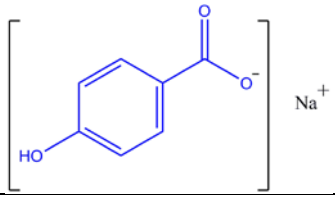
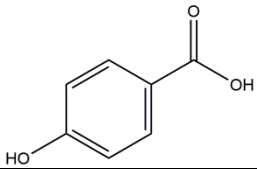
Ingredient CAS No.	Definition & Structure	Function
Sodium Butylparaben 36457-20-2	Sodium Butylparaben is the sodium salt of Butylparaben that conforms to the formula: 	Preservative
Benzylparaben 94-18-8	Benzylparaben is the ester of benzyl alcohol and *p*-hydroxybenzoic acid. It conforms to the formula: 	Preservative
Paraben Carboxylic Salts and Free Acid (non-esters)		
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
4-Hydroxybenzoic Acid 99-96-7	4-Hydroxybenzoic Acid is the aromatic acid that a conforms to the formula: 	Fragrance ingredient; preservative

Table 2. Previous safety assessments of parabens in this safety assessment.

Parabens	Conclusion	Reference
Methylparaben, Ethylparaben, Propylparaben, and Butylparaben	Safe as cosmetic ingredients in the present practices of use	1984[40]
Benzylparaben	Available data are insufficient to support the safety	1986[41]
Isobutylparaben and Isopropylparaben	Safe as cosmetic ingredients in the present practices of use	1995[42]
Methylparaben, Ethylparaben,	Safe in the present practices	2008[18]

Propylparaben, Butylparaben, Benzylparaben, Isopropylparaben, and Isobutylparaben	and concentrations

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Sodium Methylparaben		
Physical Form	Crystalline solid	[2]
Color	White	[2]
Molecular Weight g/mol	174.131	[96]
Density @ 20°C	1.42	[2]
Melting Point °C	313	[2]
Water Solubility g/L @ 20°C & pH 11.4	> 10.0	[2]
log P_{ow}	-0.63	[2]
Disassociation constants pKa @ 23°C	8.4	[2]
Calcium Paraben		
Molecular Weight g/mol	314.306	[97]
Potassium Butylparaben		
Molecular Weight g/mol	232.32	[98]
Potassium Ethylparaben		
Molecular Weight g/mol	204.266	[99]
Potassium Methylparaben		
Molecular Weight g/mol	190.239	[100]
Potassium Paraben		
Molecular Weight g/mol	176.212	[101]
Potassium Propylparaben		
Molecular Weight g/mol	218.293	[102]
Sodium Butylparaben		
Molecular Weight g/mol	216.212	[103]
Sodium Ethylparaben		
Physical Form	Solid, powder	[5]
Color	White	[5]
Molecular Weight g/mol	188.157	[30]
Density g/cm^3 @ 20°C	1.34	[5]
Melting Point °C	268	[5]
Water Solubility g/L @ 23°C & pH 10.4	> 1000	[5]
log K_{ow}	-0.14	[5]
Sodium Isobutylparaben		
Molecular Weight g/mol	216.212	[104]
Sodium Paraben		
Molecular Weight g/mol	160.104	[105]
Sodium Propylparaben		
Physical Form	Solid, powder	[6]
Color	White	[6]
Molecular Weight g/mol	202.185	[106]
Density @ 20°C	1.24	[6]
@ 25°C	1.24	[6]
Vapor pressure mmHg @ 20°C	< 0.001	[6]
Melting Point °C	302	[6]
Boiling Point °C	310 (decomp)	[6]
Water Solubility g/L @ 23°C	> 100	[6]
log P_{ow}	0.27	[6]

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Methylparaben		
Physical Form	Powder	19
	Liquid	19
Color	White or colorless	19
Odor	Characteristic	19
Molecular Weight g/mol	152.16	18
Density g/cm^3 @ 137.2°C	1.1208	107
@ 20°C	1.209±0.06 est.	108
Vapor pressure mmHg @ 25°C	2.37x10^{-4}	19
Melting Point °C	131	18
	125-128	18
Boiling Point °C	270-280	18
	265	109
	140-141	110
Water Solubility g/L @ 25°C	2.50x10^3	19
	Slightly soluble	18
Other Solubility		
Alcohol	Very soluble	18
Benzene	Slightly soluble	18
Ether	Very soluble	18
Glycerin	Slightly soluble	18
log K_{ow}	1.93	35
Disassociation constants (pKa, pKb)		
pK_a	8.17	18
Ethylparaben		
Physical Form	Crystals or powder	111
Color	Colorless or white	111
Molecular Weight g/mol	166.18	18
Density @ 20°C	1.291	3
Vapor pressure mmHg @ 25°C	9.29x10^{-5}	111
Melting Point °C	116-118	18
	115-118	18
Boiling Point °C	297-298	18
Water Solubility g/L @ 25°C	0.885	111
Other Solubility		
Alcohol	Very soluble	18
Ether	Very soluble	18
Glycerin	Slightly soluble	18
log K_{ow}	2.47	3,111
	2.27	35
Disassociation constants (pKa, pKb)		
pK_a	8.22	18
	8.34	111

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Propylparaben		
Physical Form	Crystal or powder	112
Color	Colorless or white	112
Odor	Odorless or faint	112
Molecular Weight g/mol	180.21	18
Density	1.0630	18
	1.28	112
Vapor pressure mmHg @ 25°C	$5.55x10^{-4}$ est.	112
Melting Point °C	96.2-98	18
	95-98	18
Boiling Point °C	294	109
	271	112
Water Solubility g/L	0.0500	112
	Insoluble	18
Other Solubility		
Alcohol	Soluble	18
Ether	Soluble	18
log K_{ow}	2.34	4
	2.81	35
Disassociation constants (pKa, pKb)		
pK_a	8.35	18
Isopropylparaben		
Molecular Weight g/mol	180.22	18
Melting Point °C	96-97	113
Boiling Point °C	294	109
Butylparaben		
Physical Form	Crystals or powder	114
Color	White	114
Odor	Odorless	114
Molecular Weight g/mol	194.23	114
Vapor pressure mmHg @ 25°C	$1.86x10^{-4}$	114
Melting Point °C	68-69	18
	68-72	18
Boiling Point °C	309.2±15.0	108
Water Solubility g/L @ 20°C	$0.0027x10^{2}$	114
	Insoluble	18
Other Solubility g/L		
Alcohol	Soluble	18
Ether	Soluble	18
Glycerin	Slightly soluble	18
Disassociation constants (pKa, pKb)		
pK_a	8.37	18
	8.47	114
Isobutylparaben		
Physical Form	Solid, powder	7
Color	White	7
Molecular Weight g/mol	194.25	18
Density g/cm^3 @ 20°C	1.105±0.06	108
Vapor pressure mmHg @ 25°C	0.000381	7
Melting Point °C	72.95 est.	7
Boiling Point °C	302.3±15.0	108
Water Solubility g/L @ 25°C	2.24	7
log P_{ow}	3.04	7

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Benzylparaben		
Physical Form	Solid, crystalline	[8]
Color	White	[8]
Odor	Odorless	[8]
Molecular Weight g/mol	228.25	[18]
Molecular Volume m^3/kmol		
Density g/cm^3 @ 20°C	1.224±0.06 est.	[108]
Vapor Density mmHg	0 est.	[8]
Melting Point °C	110-112	[18]
Boiling Point °C	389.8±17.0 est.	[108]
Water Solubility g/L @ 25°C	1.08	[8]
	10	[18]
Other Solubility g/L		
Propylene glycol	130	[18]
log P_{ow}	3.97	[8]
Disassociation constants (pKa, pKb)		
pK_a	8.18±0.15 est.	[108]
Isobutylparaben		
Physical Form	Solid, crystalline	[115]
Molecular Weight g/mol	214.5	[115]
Density g/cm^3 @ 25°C	1.46	[115]
Disassociation constants (pKa, pKb)		
pK_a	4.57±0.10	[108]
4-Hydroxybenzoic Acid		
Molecular Weight g/mol	138.12	[116]
Density g/ml @ 25°C	1.46	[117]
Melting Point °C	214.5	[118]
Boiling Point °C	336.2 est.	[116]
log K_{ow}	1.39 est.	[119]
Disassociation constants (pKa, pKb)		[120]
pK_a	4.57±0.10 est	

Decomp=decomposes on melting

Table 4. The particle size range of parabens in this safety assessment.

Ingredient	D_{10} (µm)	D_{50} (µm)	D_{90} (µm)	Reference
Sodium Methylparaben	7.9±3	117.1±17.5	693.5±96.8	[2]
Ethylparaben	50±4.3	307.5±21.9	770.6	[3]
Sodium Ethylparaben	6.5±0.3	49.5±6.4	147.1±28.3	[5]
Sodium Propylparaben	6.7±0.3	37.8±4.9	164.5±36.7	[6]

Table 5. Current and historical frequency and concentration of use of parabens according to duration and exposure.[18,21,22]

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2017	**2006**	**2016**	**2003**	**2017**	**2005****	**2016**	**2003**
	Methylparaben				**Ethylparaben**			
Totals*	**13,797**	**8786**	**0.000001-0.9**	**0.0003-1**	**4635**	**2679**	**0.00000032-0.65**	**0.00002-0.98**
Duration of Use								
Leave-On	*10,784*	*6468*	*0.0000043-0.8*	*0.0008-1*	*3528*	*2066*	*0.00000032-0.65*	*0.00002-0.6*
Rinse-Off	*2889*	*2105*	*0.000001-0.9*	*0.001-0.46*	*1059*	*562*	*0.0000008-0.5*	*0.0001-0.98*
Diluted for (Bath) Use	*124*	*213*	*0.21-0.5*	*0.0003-0.5*	*48*	*51*	*0.005-0.1*	*0.00004-0.15*
Exposure Type								
Eye Area	2187	1610	0.000002-0.8	0.07-0.6	716	543	0.000002-0.65	0.00002-0.49
Incidental Ingestion	330	301	0.000032-0.35	0.07-1	98	72	0.000008-0.3	0.0002-0.2
Incidental Inhalation-Spray	142; 3313[a]; 2263[c]	111; 1382[a]; 968[c]	0.0000043-0.41; 0.0024-0.5[a]; 0.25-0.6[c]	0.1-0.35; 0.07-0.5[a]; 0.15-0.44[c]	20; 933[a]; 904[c]	23; 431[a]; 330[c]	0.000031-0.22; 0.00059-0.2[a]; 0.06-0.15[c]	0.02-0.2; 0.0001-0.6[a]; 0.0004-0.4[c]
Incidental Inhalation-Powder	496; 23[b]; 2263[c]	376; 33[b]; 968[c]	0.004-0.4; 0.001-0.6[b]; 0.25-0.6[c]	0.1-0.5; 0.2-0.4[b]; 0.15-0.44[c]	101; 12[b]; 904[c]	122; 12[b]; 330[c]	0.0057-0.5; 0.0002-0.48[b]; 0.06-0.15[c]	0.04-0.5; 0.0004-0.4[c]
Dermal Contact	11,080	6898	0.000001-0.6	0.0003-0.7	3728	2147	0.000002-0.65	0.00004-0.98
Deodorant (underarm)	30[a]	35[a]	0.000075-0.00012[d]; 0.15-0.4[e]	0.0008-0.3[a]	12[a]	10[a]	0.00005[d]; 0.5[e]	0.002-0.1[a]
Hair - Non-Coloring	1658	1137	0.0002-0.9	0.1-0.4	461	229	0.0000008-0.3	0.001-0.6
Hair-Coloring	272	197	0.0000016-0.4	0.05-0.35	115	92	0.000004-0.2	0.2
Nail	77	37	0.0000012-0.41	0.002-0.4	45	10	0.00000032-0.2	0.01-0.2
Mucous Membrane	1091	751	0.000001-0.5	0.0003-1	406	170	0.000008-0.3	0.00004-0.2
Baby Products	40	60	0.13-0.4	0.2-0.4	15	15	0.032	NR

	2017	**2006**	**2016**	**2003**	**2017**	**2006**	**2016**	**2003**
	Propylparaben				**Isopropylparaben**			
Totals*	**10,642**	**7118**	**0.00000014-0.7**	**0.00002-0.7**	**323**	**48**	**0.000005-0.32**	**0.00001-0.3**
Duration of Use								
Leave-On	*8668*	*5585*	*0.00000014-0.7*	*0.00002-0.7*	*271*	*39*	*0.00004-0.32*	*0.00001-0.3*
Rinse-Off	*1875*	*1422*	*0.00000026-0.3*	*0.01-0.5*	*50*	*8*	*0.000005-0.22*	*0.03-0.2*
Diluted for (Bath) Use	*99*	*140*	*0.0001-0.3*	*0.04-0.3*	*2*	*1*	*NR*	*0.005*
Exposure Type								
Eye Area	1874	1477	0.00000014-0.7	0.02-0.5	54	10	0.19	0.06-0.2
Incidental Ingestion	631	527	0.000004-0.3	0.03-0.62	31	1	0.12	0.2
Incidental Inhalation-Spray	64; 2517[a]; 1630[c]	62; 996[a]; 706[c]	0.00000014-0.31; 0.0003-0.25[a]; 0.02-0.25[c]	0.1-0.3; 0.001-0.5[a]; 0.03-0.4[c]	19; 87[a]; 21[c]	2; 6[a]; 6[c]	0.00004; 0.00004[a]	0.0005-0.3[a]; 0.1-0.2[c]
Incidental Inhalation-Powder	407; 24[b]; 1630[c]	308; 31[b]; 706[c]	0.0018-0.3; 0.0001-0.3[b]; 0.02-0.25[c]	0.1-0.7; 0.2[b]; 0.03-0.4[c]	7; 21[c]	5; 6[c]	NR	0.00001-0.00002; 0.1-0.2[c]
Dermal Contact	8615	5598	0.00000014-0.4	0.00002-0.7	241	39	0.031-0.32	0.00001-0.3
Deodorant (underarm)	24[a]	29	0.000025-0.000058[d]; 0.025-0.15[e]	0.002-0.2[a]	NR	NR	NR	NR
Hair - Non-Coloring	847	623	0.0000055-0.4	0.03-0.5	25	6	0.000005-0.22	0.001
Hair-Coloring	177	150	0.00000026-0.25	0.04-0.5	NR	NR	NR	NR
Nail	67	27	0.0000003-0.2	0.002-0.4	6	NR	0.00012	0.1
Mucous Membrane	1178	832	0.000004-0.3	0.02-0.62	59	2	0.12	0.005-0.2
Baby Products	39	56	0.15	0.05-0.2	NR	NR	NR	NR

Table 5. Current and historical frequency and concentration of use of parabens according to duration and exposure.[18,21,22]

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2017	**2006**	**2016**	**2003**	**2017**	**2006**	**2016**	**2003**
	Butylparaben				**Isobutylparaben**			
Totals*	**4685**	**3001**	**0.00000006-0.5**	**0.00002-0.54**	**2291**	**642**	**0.00000006-0.3**	**0.000007-0.5**
Duration of Use								
Leave-On	*3754*	*2409*	*0.0000000-0.5*	*0.00002-0.4*	*1729*	*435*	*0.00000006-0.3*	*0.000007-0.5*
Rinse-Off	*890*	*551*	*0.0000004-0.33*	*0.00004-0.54*	*528*	*178*	*0.0000004-0.23*	*0.0001-0.4*
Diluted for (Bath) Use	*41*	*41*	*0.00002-0.1*	*0.00004-0.07*	*34*	*29*	*0.000012-0.005*	*0.00002-0.2*
Exposure Type								
Eye Area	999	812	0.000002-0.5	0.00002-0.3	263	59	0.00000006-0.14	0.000007-0.5
Incidental Ingestion	297	219	0.0000026-0.2	0.0008-0.1	73	11	0.000004-0.09	0.0001-0.4
Incidental Inhalation-Spray	36; 773[a]; 733[c]	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]	29; 447[a]; 499[c]	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	172; 7[b]; 733[c]	88; 21[b]; 320[c]	0.0057-0.3; 0.0001-0.24[b]	0.07-0.14; 0.05[b]; 0.0004-0.4[c]	28; 2[b]; 499[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	3840	2406	0.0000004-0.4	0.00004-0.54	1883	525	0.0000006-0.3	0.00001-0.5
Deodorant (underarm)	10[a]	10[a]	0.000025[d]	0.002[a]	7[a]	3[a]	NR	0.002[a]
Hair - Non-Coloring	323	246	0.00000011-0.22	0.0004-0.25	166	83	0.0000004-0.17	0.01-0.3
Hair-Coloring	48	28	0.0000005-0.05	0.03	42	1	0.000036-0.00008	NR
Nail	48	21	0.00000006-0.07	0.003-0.2	41	3	NR	0.006
Mucous Membrane	601	312	0.0000026-0.2	0.00004-0.11	297	63	0.000004-0.09	0.00002-0.4
Baby Products	12	28	NR	0.05	5	7	NR	NR

	2017	**2006**	**2016**	**2003**
	Benzylparaben			
Totals*	**NR**	**1**	**NR**	**NR**
Duration of Use				
Leave-On	*NR*	*1*	*NR*	*NR*
Rinse-Off	*NR*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR
Dermal Contact	NR	1	NR	NR
Deodorant (underarm)	NR	1[a]	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	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.

** Suspected to be a typo in the publication and may actually be 2006.

NR – no reported use

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

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

[c] 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

[d] Spray products

[e] Not spray products

Table 6. Frequency of use according to duration and exposure of parabens.[21,22]

Use type	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)
	Sodium Methylparaben		**Sodium Butylparaben**		**Sodium Ethylparaben**		**Sodium Isobutylparaben**	
Total/range	**434**	**0.000005-0.4**	**5**	**NR**	**35**	**0.000012-0.062**	**1**	**NR**
*Duration of use**								
Leave-on	*232*	*0.00001-0.4*	*4*	*NR*	*32*	*0.000012-0.062*	*1*	*NR*
Rinse-off	*192*	*0.000005-0.4*	*1*	*NR*	*3*	*0.0036*	*NR*	*NR*
Diluted for (bath) use	*10*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure type								
Eye area	50	0.000012-0.4	1	NR	12	0.0036	NR	NR
Incidental ingestion	1	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	2; 50[a]; 82[b]	0.00002; 0.00022-0.3[b]	3[a]	NR	7[a]; 6[b]	NR	1[a]	NR
Incidental inhalation-powders	82[b]	0.00013; 0.00016-0.3[c]	NR	NR	6[b]	0.0036[c]	NR	NR
Dermal contact	278	0.000005-0.4	5	NR	31	0.0036-0.062	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	69	0.00002-0.4	NR	NR	NR	0.0036	NR	NR
Hair-coloring	74	0.3-0.4	NR	NR	NR	NR	NR	NR
Nail	NR	0.000046	NR	NR	NR	0.000012	NR	NR
Mucous Membrane	29	0.25	NR	NR	2	NR	NR	NR
Baby	NR	NR	NR	NR	NR	NR	NR	NR

	Sodium Paraben		**Sodium Propylparaben**	
Total/range	**NR**	**0.008**	**141**	**0.000015-0.28**
Duration of use				
Leave-on	*NR*	*0.008*	*106*	*0.000017-0.28*
Rinse-off	*NR*	*NR*	*31*	*0.000015-0.1*
Diluted for (bath) use	*NR*	*NR*	*4*	*NR*
Exposure type				
Eye area	NR	NR	21	0.004-0.28
Incidental ingestion	NR	NR	NR	NR
Incidental Inhalation-sprays	NR	NR	17[a]; 42[b]	NR
Incidental inhalation-powders	NR	NR	42[b]	0.0051[c]
Dermal contact	NR	0.008	129	0.0004-0.28
Deodorant (underarm)	NR	NR	NR	NR
Hair-noncoloring	NR	NR	3	0.000015
Hair-coloring	NR	NR	1	0.0051
Nail	NR	NR	NR	0.000017
Mucous Membrane	NR	NR	10	0.1
Baby	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 uses in 2017 or historic according to the VCRP and the Council survey.[18,21,22]

Calcium Paraben	Potassium Butylparaben
Potassium Ethylparaben	Potassium Methylparaben
Potassium Paraben	Potassium Propylparaben
Sodium Isopropylparaben	

Table 8. SCCP 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.	11
2005	Methyl and ethyl paraben 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.	12
2006	The conclusion of opinion SCCP/0873/05 remains unchanged.	13
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.	14
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.	15
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.	16
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 Propyl- and Butylparaben in humans.	17

Table 9. Dermal penetration and penetration enhancement studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
Dermal Penetration In Vitro							
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 and skin samples (~3.3 cm^2 discs, intact or tape-stripped 20 times; diffusion area 2 cm^2) maintained at 32°C; 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; Freezing skin samples reduces hydrolysis of Methylparaben slightly	33
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 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	34
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 froze, 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 86 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	35

Table 9. Dermal penetration and penetration enhancement studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
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 mounted on Franz diffusion cells	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-MS/MS and replaced by fresh receptor medium	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 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	[36]
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	[37]

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	43
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 *p*-hydroxybenzoic 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 *p*-hydroxybenzoic 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	44
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 for hepatocytes from males and females, respectively, corresponding to Cl_{int}=92 ± 5 and 111 ± 22 mL/min/kg); Butylparaben was extensively hydrolyzed to *p*-hydroxybenzoic 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%)	48

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
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 showed the highest hydrolytic activity towards Butylparaben, with activity decreasing with decreasing side-chain length – carboxylase 1 exhibited a similar activity pattern; Rat small-intestinal microsomes exhibited higher activity toward longer-side-chain parabens – carboxylase 2 showed a similar activity pattern; 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	46
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	45

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
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; p-hydroxybenzoic 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 all rat tissue fractions tested, hydrolysis rates for the parabens increased as the ester chain length increased, in contrast to human tissue fractions; 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	[47]

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
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; 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 After sacrifice, the brain, heart, kidneys, liver, lungs, spleen, stomach and forestomach, small and large intestine, pancreas, and skeletal muscle sample were collected and weighed, and analyzed for radioactivity	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 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 *p*-hydroxybenzoic acid	[49]

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
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-^{14}C(U) – 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 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; protective appliance was removes, dose-site skin was excised and washed with a series of water-wetted gauzes and appliance, dose-site skin, rinsate, and gauzes were stored frozen before analysis	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	[48]

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
ANIMAL						
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, 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 After sacrifice, the brain, heart, kidneys, liver, lungs, spleen, stomach and forestomach, small and large intestine, pancreas, and skeletal muscle sample were collected and weighed, and analyzed for radioactivity	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 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 *p*-hydroxybenzoic acid	[49]

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
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-^{14}C(U) – 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	[48]
HUMAN						
Dermal						
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean=26 years old), n=26	2% (w/w) Butylparaben in Essex 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; 24-h urine samples were collected and analyzed for total and unconjugated Butylparaben by LC-MS/MS	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 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	[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
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, *p*-hydroxybenzoic 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, *p*-hydroxybenzoic 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	51
			HUMAN			
			Aggregate			
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Human	Female breast cancer patients undergoing radical mastectomy, n=40	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-MS/MS) in breast tissue samples excised from four serial locations (quadrants) across the breast, from axilla to sternum	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 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	52

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
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	[53]

AFP=α-Fetoprotein; Cl_{int}=intrinsic clearance; DMSO=Dimethyl sulfoxide; ESI=Electrospray ionization; 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. Short-Term Toxicity Studies.

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
Short-Term							
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; 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)	[54]

Table 11. Short-Term Toxicity Studies.

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
Short-Term							
Animal							
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; 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	[55]

Table 11. Short-Term Toxicity Studies.

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
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 ($p<0.01$)	[56]
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 ($p < 0.05$) by 11.34%, 27.03%, and 41.02% at 13.33, 20 and 40 mg/kg bw/day, respectively; GSH levels were reduced by ($p < 0.05$) by 22.22%, 44.53% and 55.74% at 13.33, 20 and 40 mg/kg bw/day, respectively; Statistically-significant ($p < 0.05$), dose-dependent reductions in SOD, CAT, GPx, GR, and GST levels were noted	[57]

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 12. Oral developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Procedure	Results	Reference
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	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; 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 only at 100 mg/kg; Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day	[58]
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	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	[59]
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	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; 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; Butylparaben reduced epididymal cauda sperm counts and daily sperm production in a dose-dependent manner; this difference was statistically significant in offspring in the 400 and 1000 mg/kg bw/day groups	[60]

Table 12. Oral developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Procedure	Results	Reference
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	[61]
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)	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	[62]

Table 12. Oral developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Procedure	Results	Reference
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-1B, 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 ($p<0.01$)	[63]
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	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	[64]
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	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	[65]

Table 12. Oral developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben	Rat (Sprague-Dawley)	7-week-old males, 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	[121]
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 8 weeks; 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 from Butylparaben-treated rats and corresponding controls	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%, 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	[44]

AR=Androgen receptor; CYP19=Aromatase; E2=17β-estradiol; EE=17α-ethynylestradiol; ERα=Estrogen receptor α; FSH=Follicle-stimulating hormone; GD=Gestation day; IL-1B=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 of 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 13. 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	[70]
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	[71]
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	[122]

Table 13. 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	[72]
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	[73]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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%	[74]
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	[75]
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Human	MCF-7 human breast adenocarcinoma cells	Range of concentrations tested was not specified, ethanol vehicle	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; 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	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 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 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	[76]
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) Propylparaben inhibited the Propylparaben-induced effects on acini	[77]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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	[78]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	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 1 h before the cells were treated with Butylparaben or DMSO in the presence of the antagonist	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; 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)	[79]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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%; 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	[80]
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 be/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	[81]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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	[82]
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 digoxigenin-dUTP nick-end-labeling (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 microscopy revealed condensed chromatin and shrinkage of cytoplasm and nucleus of apoptotic spermatocytes.	[83]

Table 13. 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 Essex 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	Minor differences in serum inhibin B, LH, estradiol, 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	[38]

AR=Androgen receptor; CHO=Chinese hamster ovary; 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; ER=Estrogen receptor; FBS=Fetal bovine serum; FCS=Fetal calf serum; FSH=Follicle stimulating hormone; FT4=Free thyroxine; GPER=G-protein coupled estrogen receptor 1; GR=Glucocorticoid receptor; hADSC=Human adipose-derived stem cells; HER2=Human epidermal growth factor receptor; 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; 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 14. 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 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 - 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 Limitations: - 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	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 Low Birth Weight and Maternal Urine Concentrations Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben Low Birth Weight and Cord Blood Concentrations Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben Preterm Birth and Maternal Urine Concentrations Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben Preterm Birth and Cord Blood Concentrations Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	**OR** 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 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)**	[87]

Table 14. 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	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	88

Table 14. 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	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 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.	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 Ethylparaben Propylparaben Butylparaben <u>Body Weight at 36 Months</u> Methylparaben Ethylparaben Propylparaben Butylparaben	**<u>β Coefficient</u>** 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) 116 (-53.3-285) 111 (-71.2-294) 193 (-3.88-389) 113 (-101-327) 159 (-49.4-368) 179 (-45.3-404)	89

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
				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		
				REROSPECTIVE STUDIES		
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 Limitations - 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	Methylparaben	**OR (unadjusted)**	90
				<0.4 ng/g	1.00	
				0.44-1.91 ng/g	1.00 (0.32-3.09)	
				1.96-11.69 ng/g	**3.18 (0.88-11.48)**	
				Concentration as continuous variable	1.17 (0.94-1.46)	
				Ethylparaben		
				<LOD	1.00	
				0.07-0.89 ng/g	0.29 (0.08=1.06)	
				0.91-5.49 ng/g	1.51 (0.44-5.15)	
				Concentration as continuous variable	1.07 (0.74-1.55)	
				Propylparaben		
				<LOD	1.00	
				0.06-1.15 ng/g	1.23 (0.30-5.04)	
				1.16-5.52 ng/g	**4.72 (1.08-20.65)**	
				Concentration as continuous variable	**1.90 (1.12-3.22)**	
				Butylparaben		
				<0.08 ng/g	1.00	
				0.16-0.74 ng/g	2.29 (0.65-8.05)	
				0.79-1.60 ng/g	2.31 (0.72-7.46)	
				Concentration as continuous variable	2.27 (0.8-6.42)	
				Methylparaben	**OR (adjusted)**	
				<0.4 ng/g	1.00	
				0.44-1.91 ng/g	1.04 (0.33-3.26)	
				1.96-11.69 ng/g	3.24 (0.83-12.69)	
				Concentration as continuous variable	1.17 (0.93-1.48)	
				Ethylparaben		
				<LOD	1.00	
				0.07-0.89 ng/g	0.26 (0.07-1.00)	

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			mechanisms occurring at different critical stages in gestation	0.91-5.49 ng/g	1.25 (0.34-4.60)	
				Concentration as continuous variable	1.00 (0.68-1.47)	
				Propylparaben		
				<LOD	1.00	
				0.06-1.15 ng/g	1.39 (0.33-5.91)	
				1.16-5.52 ng/g	6.42 (1.16-35.47)	
				Concentration as continuous variable	2.16 (1.16-4.01)	
				Butylparaben		
				<0.08 ng/g	1.00	
				0.16-0.74 ng/g	2.26 (0.62-8.21)	
				0.79-1.60 ng/g	2.11 (0.62-7.16)	
				Concentration as continuous variable	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	Weight z Score (Boys)	**β Coefficient**	91
				Methylparaben	0.08 (-0.06-0.23)	
				Ethylparaben	**0.16 (0.03-0.28)**	
				Propylparaben	0.00 (-0.16-0.17)	
				Butylparaben	0.12 (-0.09-0.32)	
				Benzylparaben	-0.04 (-0.18-0.10)	
				∑Parabens	0.17 (-0.04-0.39)	
				Height z Score (Boys)		
				Methylparaben	0.11 (-0.02-0.26)	
				Ethylparaben	**0.15 (0.03-0.27)**	
				Propylparaben	0.05 (-0.11-0.21)	
				Butylparaben	0.14 (-0.06-0.34)	
				Benzylparaben	0.08 (-0.06-0.21	
				∑Parabens	**0.23 (0.03-0.43)**	

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			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 Limitations: - Spot urine samples may cause exposure misclassification - Specific diet information was not sufficiently obtained and evaluated	All β coefficients calculated for girls and all other β coefficients for boys were not statistically significant		
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 subsample of U.S. 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 U.S. 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	Adults, Total T4 (µg/dL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, ln-Free T3 (pg/mL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, ln-Free T4 (ng/mL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, T4 (µg/dL) Methylparaben Ethylparaben Propylparaben Butylparaben	β Coefficient -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)***	[92]

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			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) Limitations: - 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	All other β coefficients calculated were not statistically significant		
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	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)	[93]

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			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 <u>Limitations</u>: - 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 - 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 Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 sub-sample of the U.S. 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	<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	 **<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)**	94

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			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 Limitations: -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	Tertile 1 Tertile 2 p_{trend}=0.9 Nonatopic Asthma (males and females) Methylparaben Tertile 1 Tertile 2 Tertile 3 **p_{trend}=0.04** Nonatopic Wheeze (males and females) Methylparaben 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	1 (Reference) **1.55 (1.02-2.33)** **OR (adjusted)** 1.0 (Reference) 0.43 (0.47-3.73) **0.25 (0.07-0.90)** 1 0.51 (0.18-1.46) **0.23 (0.05-0.99)**	
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	Comet Tail % Butylparaben <0.2 µg/L 0.2-0.6 µ/L >0.6 µg/L **P_{trend}=0.03**	**β Coefficient (adjusted)** 0 6.81 (-1.80-15.4) 8.23 (-0.41-16.9)	95

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			(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 <u>Limitations</u>: - 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 - Cross-sectional design restricts the ability to draw conclusions about causal relationships - Relatively small sample size provided low statistical power	No other comparisons were statistically significant in this study		

*** 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; BMI=Body mass index; CI=Confidence interval; E2=Estradiol; EDI=Estimated daily intake; EEQ=Estrogen equivalency; FSH=Follicle stimulating hormone; HPLC-MS/MS=High-performance liquid chromatography-mass spectrometry/mass spectrometry; ICSI=Intracytoplasmic sperm injection; 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; MDL=Method detection limit; MGH=Massachusetts General Hospital; MPC=Mean percent change; NA=Not applicable; NHANES=National Health and Nutrition Examination Survey; OR=Odds ratio; OV=Ovarian volume; $P_{parabens}$=Sum molar concentrations of the parabens; PIR=Poverty income ratio; PTB=Preterm birth; SART= Society for Assisted Reproductive Technology; SG=Specific gravity; UPLC-MS/MS=Ultra-high-performance liquid chromatography-tandem mass spectrometry; WHO=World Health Organization

Table 15. Margins of safety for parabens in cosmetics as a function of exposed population and single versus multiple paraben usage.[18]

Exposed population	Paraben exposure	MOS
Infant	Single paraben	5952
Infant	Multiple parabens	2976
Adult	Single paraben	1690
Adult	Multiple parabens	840

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Historical Minutes of Parabens

METHYLPARABEN

April 1983

The following conclusion of the report was unanimously approved:

"From the available information, the Panel concludes that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben are safe as cosmetic ingredients in the present practices of use."

Dr. Hoffmann suggested that the organic/inorganic impurities be specified in the Physical Properties section of this as well as all future CIR reports.

Subject to minor revisions, the document will be announced as a Tentative Report for a 90-day comment period.

BENZYLPARABEN

October 1984

Dr. Schroeter recommended an Insufficient Data Announcement be issued. Clinical data would not be requested, as those data could be extrapolated from the report on the Methylparaben group of ingredients.

The Panel unanimously accepted and approved the following statement in connection with Benzylparaben:

The Expert Panel requests:
1. UV absorption spectrum. If _absorption occurs between 280 and 360 nm, a photosensitization study is required. (In animals only, not human).
2. Data detailing the possible presence of impurities.
3. Subchronic feeding study - 90-day in rats.
4. Mutagenicity and teratogenicity studies.
5. Eye irritation study at concentration of use.
6. Metabolism and associated pharmacokinetic studies are not requested at this time. If significant toxicity is shown in the above tests, the Expert Panel may request this additional type of testing."

The Insufficient Announcement will shortly be issued for a 90-day public comment period.

February 1985

A Notice of Insufficient Data Announcement was issued on this ingredient on October 10, 1984.

The two Teams met separately in closed session to evaluate the additional data submitted by industry during the public comment period. Dr. Bergfeld stated that the eye irritation data lacked details, and that acute oral and dermal tests were submitted although not requested. Dr. Hoffmann

recommended deleting the request for teratogenicity studies from the insufficient data report. All Panel members concurred.

The following Discussion Section and Conclusion were unanimously accepted and approved:

"DISCUSSION

"Section 1 paragraph (p) of the CIR Procedures states that 'A lack of information about an ingredient shall not sufficient to justify a determination of safety.• In accordance with Section 30(j)(2)(A) of the CIR Procedures, the Expert Panel informed the public of its decision that the data on Benzylparaben are insufficient to determine that this ingredient, under the relevant condition of use, is either safe or not safe. The Panel released a Notice of Insufficient Data Announcement on October 10, 1984 outlining the data needed to assess the safety of Benzylparaben. The types of data required included:

1. UV absorption spectrum. If absorption occurs between 280 and 360 nm, a photosensitization study is required. (In animals only, not human).
2. Data detailing the possible presence of impurities.
3. Subchronic feeding study - 90-day in rats.
4. Mutagenicity studies.
5. Eye irritation study at concentration of use.
6. Metabolism and associated pharmacokinetic studies are not requested at this time. If significant toxicity is shown in the above tests, the Expert Panel may request this additional type of testing.

Acute animal oral toxicity, animal eye and skin irritation data were received in response to the above requests, and are included in this report.

The eye test data included in this report cannot be interpreted without an adequate description of the methodology used. The Expert Panel again concurred with the decision made during its earlier review that similar data on Methylparaben, Ethylparaben, Propylparaben or Butylparaben were not necessarily applicable to the safety evaluation of Benzylparaben."

"CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Benzylparaben as used in cosmetics ..."

The document will be issued as a Tentative Report for a 90-day public comment period.

ISOBUTYLPARABEN AND ISOPROPYLPARABEN

August, 1993

INFORMAL DATA REQUESTS. The Schroeter and Belsito Teams issued informal data requests on the following ingredients: Dibutyl Adipate, Isobutylparaben/Isopropylparaben, Nonoxynols, and Phloroglucinol.

November, 1993

Dr. Belsito said that his Team concluded that Isopropylparaben and Isobutylparaben are safe as used. He also noted that his Team had originally suggested that the report on these ingredients should be an addendum to the original CIR report on methyl, ethyl, propyl, and butyl parabens.

Similarly, Dr. Schroeter said that his Team agreed that Isobutylparaben and Isopropylparaben are safe as used, and that the report on these ingredients should be an extension of the original document on parabens.

Dr. Belsito questioned the accuracy of a statement in the report indicating that parabens appear to be rapidly absorbed through intact skin. He said that his impression is that parabens are poorly absorbed and that this is why high sensitization rates are observed in intradermal studies.

Dr. Andersen said that the statement on dermal absorption in the original parabens report will be checked for accuracy.

The Panel agreed that whether or not the statement on dermal absorption is true or false will not affect the conclusion, safe as used.

Dr. Bergfeld noted that the issue of whether or not there is dermal absorption of parabens must be clarified.

The Panel concluded that Isobutylparaben and Isopropylparaben are safe as used in cosmetics, and voted in favor of issuing a Tentative Final Report with this conclusion.

February/March, 1994

The Panel voted in favor of issuing a Final Report on Isobutylparaben and Isopropylparaben.

METHYLPARABEN, ETHYLPARABEN, PROPYLPARABEN, BUTYLPARABEN, AND BENZYLPARABEN

December 2005

Dr. Bergfeld mentioned that Dr. George Daston (with Procter and Gamble) had given a presentation on the possible estrogenic effects of the parabens on the preceding day. This slide presentation, which includes data supporting the safety of parabens, is inserted at the end of the minutes.

Dr. Daston presented an overview of parabens data developed by both COLIPA and CTFA. He addressed the metabolism of paraben ingredients to p-hydroxybenzoic acid and the corresponding alcohol, the absence of any significant effect of p-hydroxybenzoic acid, and the margin of safety calculations that were developed, predicated on both adult and infant exposure to cosmetic products containing parabens preservatives.

Dr. Marks noted that a CIR Final Report with the following conclusion was published in 1984: From the available information, the Panel concludes that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben are safe as cosmetic ingredients in the present practices of use.

Dr. Marks also noted that a CIR Final Report with the following conclusion on Benzylparaben was published in 1986: The CIR Expert Panel concludes that the available data are insufficient to support the safety of Benzylparaben as used in cosmetics.

Dr. Marks stated that the Panel has reopened the two safety assessments, particularly in light of the concern about these parabens as endocrine active chemicals. However, he noted that this concern has been allayed by the existence of margin of safety calculations for adult and baby exposures. Dr. Marks added that his Team determined that Benzylparaben, because of how it is metabolized, can now be considered safe.

With the preceding comments in mind, Dr. Marks said that his Team agreed that a Tentative Amended Final Report with a safe as used conclusion should be issued.

Dr. Andersen expressed his appreciation for the comments (from Shiseido) on the two keratinocyte studies, which contributed to the Panel's perception of the value of these studies.

The Panel voted unanimously in favor of issuing a safe as used conclusion. The conclusion is stated as follows: Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben are safe as cosmetic ingredients in the practices of use and concentration as described in this safety assessment.

It is important to note that this conclusion is an amended conclusion for Benzylparaben, and that the Panel's conclusion in the published CIR Final Report on the remaining parabens remains unchanged.

June 2006

Dr. Belsito stated that a Tentative Amended Final Report with the following conclusion was issued at the December 12-13, 2005 Panel meeting: The CIR Expert Panel concluded that Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben are safe as cosmetic ingredients in the practices of use and use concentrations described in this safety assessment.

Dr. Belsito added that the document is an amended report because, previously, the Panel found the available data on Benzylparaben to be insufficient. He noted, however, that the available data on this ingredient that are now included in the Tentative Amended Final Report were found to be sufficient.

Dr. Belsito stated that since the issuance of the Tentative Amended Final Report, technical comments were received from CTFA and additional unpublished reproductive toxicity data on Methylparaben have been added. A section reviewing the American Contact Dermatitis Group patch testing experience with Parabens has also been added. This information shows that the level of sensitization among dermatitis patients has remained constant over the last several decades, and, generally, is < 1% of dermatitis patients (not 1% of the population).

Dr. Belsito said that his Team had looked again at studies on gene expression profiles in breast cancer cells exposed to Parabens and estrogens, because of reports of weak estrogen receptor activity in these cells. He said that his Team had also looked specifically at the issues of male reproductive toxicity in going over the margin of safety calculations that the Panel had previously performed in December of last year.

Dr. Belsito noted that a no-observed-adverse effect level of 1000 mg/kg/day (for Butylparaben - the Paraben of greatest concern here) for male reproductive toxicity in the Charles River study was reported. Using these results, the margin of safety calculations were ~11,900 (for infants exposed to a single Paraben) and ~6,000 (for infants exposed to multiple Parabens). For the latter value, the worst case scenario of 0.08% Parabens in a product was assumed. Dr. Belsito made the observation that this value (~6,000) needs to be corrected due to a calculation error.

For adults, the margins of safety were ~1700 (for exposure to a single Paraben) and ~840 (for exposure to multiple Parabens).

Dr. Andersen stated that the correct margin of safety values are: 5,952 (for infants exposed to a single Paraben) and 2,976 (for infants exposed to multiple Parabens). He added that the margin of safety values for both infant calculations are over three orders of magnitude, and that the margin of safety values for both adult calculations are around three orders of magnitude.

Also referring to the calculations on page 103 of the safety assessment, Dr. Belsito noted that the actual infant exposure to multiple Parabens should be 0.168 mg/kg/day.

Dr. Andersen said that all of the corrections relating to these calculations will be made.

Dr. Bergfeld stressed the need to make sure that all of the calculations have been done correctly.

The Panel voted unanimously in favor of issuing a Final Report with the following conclusion: The CIR Expert Panel concluded that Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben,

Butylparaben, Isobutylparaben, and Benzylparaben are safe as cosmetic ingredients in the practices of use and use concentrations described in this safety assessment.

MARCH 2012 - NEW DATA/SCCS OPINION

Dr. Belsito's Team

DR. BELSITO: Anything more with formaldehyde? Okay. So, parabens. We got asked by Helyna and the PCPC to come back and look at these again because the SCCS has just updated their opinion specifically regarding propyl and butyl paraben and lowering the acceptable amount for one or any mixture of the two to.19 and this was based actually on there is no new data. Okay, we have looked at all the same data they have looked at. The major difference, and I thought I wrote down a page number, the major difference has to do in calculation of the margin of safety. We both did calculations of margin of safety and, in fact, in our calculation -- this is page -- numbers didn't come out very well in my book. It looks --

DR. LIEBLER: Panel book 73.

DR. BELSITO: Yes, maybe, I don't know. It's the opinion on parabens of the SCCS.

DR. LIEBLER: Oh, the SCCS comments?

DR. BELSITO: Yes.

DR. LIEBLER: That's 4.6.

DR. BELSITO: Yes, 4.6.

DR. LIEBLER: Panel book 106.

DR. BELSITO: Yes. So, if you look at their calculations, which are at the bottom of that page, just before number 5 opinion, okay, dermal absorption, they used 3.7 percent; we actually used 50 percent in our calculation. Intended concentration of the finished product, we both used.4 percent; body weight was the same, cumulative exposure to preservatives was the same. The major difference was they took a NOEL of 2 milligram/kilogram per bodyweight per day. We took a NOAEL of 1,000 milligram/kilogram per day. So, we ended up with a great margin of safety; they ended up with a margin of safety of 46.6. To get it to 100, they reduced the concentration to.19.

So, I'm a dermatologist. Do we go with a NOEL or a NOAEL in terms of doing or margin of safety and this all has to do with endocrine disruption and repro toxicity, which is not my area of expertise. So, I turn it over to Paul then and Curt at this point. I think I've explained where the differences have occurred.

DR. LIEBLER: So, I looked at this and I was trying to find the reference that the SCCS document cited. I'm referring to the 1,000 milligram/kilogram exposure, the NOEL.

DR. BELSITO: Well, we used that.

DR. LIEBLER: Oh, we used that.

DR. BELSITO: We used 1,000.

DR. LIEBLER: Right, so, they referred to that as an inadequate study. They criticized the study and the test.

DR. EISENMANN: Right, and there was a reason why the study that was done that way. It was because there was an original study done in Japan that found the facts, and they were trying to repeat the study exactly the same --

DR. LIEBLER: Oh, as an attempt to repeat the Oishii studies?

DR. EISENMANN: Yes.

DR. BELSITO: Yes.

DR. LIEBLER: Okay, so, I was tracing my way through the literature on this, and it was clear that the CIR document comes up used as 1,000 and in the SCCS document, they cite that as the Holderman,

et al., study, but I was confused because of the CIR document, there's no literature citation for anything by Holderman, et al.

DR. EISENMANN: They might have been cited (inaudible) instead.

DR. LIEBLER: Maybe that was it. So, it was confusing because it wasn't clear in the CIR document where the citation came from, and that page where the CIR presents the MOS calculation, it says why the 1,000 was selected, but there's no citation for it. So, that part was just confusing to me, and I don't know if that means we need to do anything because I can see the reason for the difference. Obviously, it's whether you use that Fisher study to make per kilogram or you use the "Holderman study," 1,000 per kilogram.

DR. BELSITO: Without sensitization or irritation. I wash my hands, says Pontius Pilate.

DR. ANDERSEN: Well, the paragraph on Panel Book page 73, and I couldn't find the actual reference quickly either. That was the Paul Snyder Memorial paragraph --

DR. SNYDER: Okay.

DR. ANDERSEN: That essentially said look, guys, all this sperm stuff is not a particularly good endpoint. So, Europe, go sit on it.

DR. SNYDER: I mean, the sufficient study that they're using for the basis was a single subcutaneous injection and only looked at the minimal epithelium (inaudible) or sperm production, and so, we had a lengthy discussion about that at the panel meeting and talked about that the other study that was done by the (inaudible) actually did testicular staging and much more robust study. And at that time, we thought the robustness of the study and the negative results at the 1,000 milligram were significant enough where we used for our analysis. I think the only other issue is that I think we need to address both that specification of that study and then the dermal absorption being so great because we did not have or at least we didn't reference those janjua, J-A-N, janjua.

DR. BELSITO: But it doesn't matter. We assumed dermal absorption was 50 percent.

DR. SNYDER: Okay.

DR. BELSITO: So, we overestimated even compared to the Europeans. The Europeans gave it 3.4 percent.

DR. LIEBLER: And I think that 50 percent is a reasonable estimate given that the reported data on absorption of these compounds, the metabolism is all over the map.

DR. BELSITO: Right. But, in reality, parabens are probably poorly-absorbed in human skin because in contact dermatitis, there's what's called the paraben paradox, and that's where parabens, if you tape strip the stratum corneum, you can induce sensitization quite easily, but, in reality, the incidents of sensitization to parabens as used in cosmetics is the lowest of any of the preservatives listed inside there. So, in guinea pig maximization test, that was predicted to be a huge allergen, and it just hasn't developed that right.

So, I mean, I guess the question is: Do we need to do anything? I mean, I think PCPC just wanted us to be aware of what's happened in Europe and make a decision whether we want to change our mind or not. Is that correct?

DR. BRESLAWEC: Yes.

DR. LIEBLER: That doesn't seem to me that there's a basis for doing that.

DR. BELSITO: So, that's it. We looked at it and we don't even have to make a comment, do we?

DR. ANDERSEN: Well, there's piece two, which is Denmark has banned use of parabens for children under three.

DR. BELSITO: Three months.

DR. ANDERSEN: No, three years.

DR. BELSITO: Three years of age. Three years.

DR. ANDERSEN: Yes. And my reading of that second SCCS document said we can find no basis for the Danish position, but it does seem like there's not a lot of data on exposure to any population

under six months of age. So, they at least opened a small door, but they didn't take a step through it. They just made the comment.

DR. LIEBLER: And most of that discussion was simply speculation about the lack of development of biotransformation enzymes that might affect handling the compound.

DR. ANDERSEN: Yes, and focusing on the Danish apparent adoption or the precautionary (inaudible) since we don't know the answer to some of those questions unless err on that side. So, I didn't count that as new data either.

DR. LIEBLER: Well, that changes our outcome.

DR. ANDERSEN: For infants, we already had an almost 6,000 margin of safety.

DR. BELSITO: Yes.

DR. ANDERSEN: By our approach.

DR. SNYDER: It would be interesting to look at -- there are three papers here that I circled about this different absorption distributing factors due to impurity of the young children.

DR. KATZ: What page?

DR. SNYDER: Page 7 of the second SCC document (inaudible) document on skin production.

DR. LIEBLER: It's Panel Book, Paul.

DR. SNYDER: In Panel Book. Oh, Panel Book --

DR. BELSITO: It's (inaudible) Panel Book.

DR. SNYDER: It's the second one that's --

DR. BELSITO: It's the introduction for the scientific rationale for the Danes (inaudible).

DR. LIEBLER: Okay, (inaudible) children. I just -- it was nothing we ever discussed, but it might be -- is it relevant looking at as a panel perspective? I was never aware they were different.

Paul, you were saying page 6 of that report?

DR. SNYDER: Page 7.

DR. BELSITO: Page 7.

DR. LIEBLER: Page 7.

DR. SNYDER: The first bullet point.

DR. BELSITO: 3.1 introduction.

DR. BRESLAWEC: Are you talking about the Holderman studies?

DR. BELSITO: No, we're talking about the second part of the SCC opinion on restriction in children.

DR. BRESLAWEC: All right.

DR. BELSITO: 3.1.

DR. ANDERSEN: Makri, Renwick, and Schwenk are the three separate citations.

DR. BELSITO: Yes.

DR. SNYDER: For different absorption rates for young children.

DR. BELSITO: No, not absorption. No, no, they're talking about metabolism.

DR. KLAASSEN: I think so, too.

DR. BELSITO: There is good data to show that except that in premature infants, absorption through infant skin is not significantly different than absorption across adult skin. Now, of course, there were differences in the fact that in a diaper, you have occluded skin. There are differences because of the larger body surface area and weight, but no, what they're talking about here is not absorption, it's metabolism. Elimination kinetics.

DR. ANDERSEN: There is pretty good evidence in both in laboratory and humans that babies don't metabolize as well as adults as far as their livers are concerned, and that's a pretty well-known phenomena.

DR. SNYDER: I just raised it because there were two or three references there that --

DR. BELSITO: Right. That we've never seen.

DR. SNYDER: We've never seen before.

DR. ANDERSEN: Well, and down further, the Boberg citations. Go down three more bulletins, are new

to us.

DR. SNYDER: Yes. Yes. So, it might be just useful to enhance our knowledge base about some of those primaries.

DR. ANDERSEN: Well, since the council very practically used the word "reexamine" and didn't ask us to reopen it, we could take the time out and reexamine those three papers.

DR. BELSITO: Well, five papers.

DR. ANDERSEN: Five.

DR. BELSITO: The Boberg, as well.

DR. ANDERSEN: Yes.

DR. SNYDER: Well, in that light, also, there's a hypothetic. On page 27 on that same document, the Prusakiewicz.

DR. LIEBLER: Prusakiewicz.

DR. SNYDER: Prusakiewicz 2007 is not in our report as is the Shaw and (inaudible) is not in our report. And so, there are some others.

DR. ANDERSEN: Arguably, fleshing out the stuff that has not been seen before --

DR. SNYDER: Well, I mean, again, as you said, and I'm not proposing reopening, but certainly looking at if there's new available data we have not looked at before, it doesn't necessarily mean that we're going to reopen. We can just take a look at it.

DR. ANDERSEN: Yes. So, you're not --

DR. BELSITO: So, but there are seven papers you want to look at. Just the papers? I mean, how do you want to deal with this, Paul? So, you're asking for the three papers that deal with metabolism in kids, the two papers that are new to the paraben, the disruption by Boberg, and then the Prusakiewicz or however you pronounce it and the --

DR. SNYDER: Shaw.

DR. BELSITO: -- Shaw and (inaudible).

DR. SNYDER: Yes, the write-up -- can just maybe look at those, write a little brief synopsis, and we could then --

DR. BELSITO: Well, there are seven papers. Why didn't the writer just send us the seven papers? Why write a brief synopsis? I mean, aside from our review of the seven papers whether we need to pursue anything further.

DR. ANDERSEN: Yes, except what I was planning on doing was asking Ivan to do that and his perspective might end up being useful.

DR. BELSITO: Okay, where's Ivan?

SPEAKER: He's not here.

DR. ANDERSEN: He was right here. (inaudible) I mean, I think what --

DR. BELSITO: You leave the room, you get an assignment. (Laughter)

DR. ANDERSEN: The first issue is a more global issue. It's not necessarily related to parabens. I mean, it is and it isn't, but it's also related to a review assessment if there are differences in metabolism that we're not aware of or something.

DR. BELSITO: Yes.

DR. KLAASSEN: Okay, let me tell you. So, in regards to the first three, I mean, I'm sure that's what those papers are about. And we can actually come up with 20 or 30 papers at least to show what's known about drug metabolism in children compared to adults, but it's not specific to the parabens, of course.

Now, these two articles that are kind of specific to parabens, the Boberg papers, one is update on uptake distribution, metabolism, and excretion of endocrine disrupting the activity of parabens could be useful and then a second one is a possible endocrine disrupting effects of parabens. So, we probably aren't going to learn a lot from that, but I think it's probably wise to go through and look at these lateral ones at least that are -- and maybe for people that aren't aware of what's known

about drug metabolism in children to become a little aware of that.

DR. BELSITO: And, so, maybe what we could ask Ivan to do since he's not here is not only take a look at those three papers, but do a little bit of a literature search on what's known about metabolism in skin of young children and bring that to the panel and then the writer of this report can just get the two papers that Paul is requesting so that we can look at them without doing anything to the paraben report. So, basically holding it, doing a little paper which would benefit all of us in terms of the chemicals we look at for the use in baby products and just updating us on the two papers we didn't see on endocrine disruption.

DR. ANDERSEN: Okay, and just to close the loop, the other group is going to suggest that this might create a spinoff not related to parabens, but maybe there is a useful discussion like we did with aerosols, talking about dermal penetration in infants. Just the point that Don made, this is a special population and if we know something, maybe we ought to tell people.

DR. KLAASSEN: Dermal penetration and metabolism.

DR. BELSITO: Right.

DR. KLAASSEN: I would suggest --

DR. ANDERSEN: Yes, yes.

DR. KLAASSEN: I mean, these other metabolism papers that are referenced here basically deliver.

SPEAKER: Right.

DR. ANDERSEN: But it's a packaged deal.

DR. KLAASSEN: Yes, yes.

DR. ANDERSEN: So, just don't be surprised if you hear that separate suggestion or another summary document, if you will.

DR. KLAASSEN: Well, we need to be educated.

SPEAKER: That's fine.

DR. BELSITO: Anything more on parabens? Okay, re-reviews.

Dr. Marks' Team

DR. MARKS: Okay, team, are we ready? And for our recorders, this really sounds loud. This is good for you all? Let us know if not. Yes, I hear loudness and echoing. I agree with Jay. I'm not sure why that was. Maybe it was a different tone of voice.

Okay, we're going to start with the parabens, and team members, let me know if you need a break. We need to get through all these this afternoon as you know. So there's a memo from our director, Dr. Andersen, dated February 10 that the council asked the panel to reexamine our report on parabens. And this was based on two changes: One in March of last year there was a revised opinion on the parabens issued by the ECSC or SCCS in which the concentrations for the parabens were changed, and then also a declaration by the Danish that parabens should not be used in children. And that SCCS had set the safe concentration of methylene ethyl at 0.4 percent for one, total of 0.8 percent for any mixture. And propyl and butyl parabens were lower at 0.19 percent. And, of course, these concentrations are less than the concentration of use that was in our final safety assessment.

So the first question should be, do we need to reopen parabens to address these issues? Or should we note that and make it as -- I'll ask Alan to help us -- whether we would just leave the minutes of this meeting and tomorrow morning address the issues, or whether we need to have some sort of formal comment in the literature? In the past we did that in terms of re-reviews. So does this need to be opened to re- review or not? I'll ask Tom, Rons.

DR. SHANK: I think we should reopen it, not necessarily for the concentrations issue, but for the information from the Danish report that children under the age of one have a greater absorption of these compounds through the skin and don't have the same activity of the carboxy esterase that adults have. It's less, and we based our safety on skin penetration and metabolism by the

esterase. And I think we need to look at that more carefully, so that would require opening it.

DR. SLAGA: I agree, and one of the things I think we have to in the future be careful is addressing children like this anyway on a large number of ingredients that potentially would penetrate easier or more so in a very young person. I'm not quite sure why they're saying three years of age, though. I don't understand that. If someone -- huh?

DR. BERGFELD: It's six months.

DR. SLAGA: It's six months, not three years?

DR. ANDERSEN: The Danish decision was under three.

DR. MARKS: Under three.

DR. SLAGA: Under three?

DR. BERGFELD: But the studies were at six months.

DR. MARKS: Alan has a comment that it appears the studies were really relevant to children under six months and for products used under the nappy area, which is the diaper area. I interpret nappy also as meaning diaper, Alan.

DR. ANDERSEN: Yes.

DR. MARKS: So, Ron, you would reopen. So we're clear, you feel our conclusions, the use concentration in the report that we have for methyl is 1 percent, for ethyl is essentially the same. That's over double that the SCCS has. And for propyl it was.7 and.54 in the report and it's.19. But you're not concerned about the concentrations of those? You wouldn't reopen to change the concentration?

DR. SHANK: Right. I'm not concerned with it. If we're going to reopen it, then that will come up again anyway if there are any new data.

DR. MARKS: Right. And then, Ron, would you repeat, particularly in terms of the children, your concerns. There were two reasons. You said one was the absorption; the other was the metabolism?

DR. SHANK: Yes, the Danish cite somewhere that children under the age of one have a lower activity of carboxy esterase in the skin, and we relied on this enzyme to hydrolyze the parabens before systemic distribution. And they suggest that when there is nappy dermatitis, skin absorption rates are higher. So I think we need to look at that.

DR. MARKS: Okay.

DR. BERGFELD: Can I make a comment? I'd like to make a comment on that. It was mentioned by Tom that if we're really going to reopen it and look at baby skin and its absorption and the various enzyme differences between child and adult or infant and adult, I think that it might be deserving a little broader look at it for all of the cosmetic ingredients and perhaps ultimately a boilerplate.

DR. SHANK: I think that's a great suggestion. I have one question for Dr. Bergfeld and Dr. Marks. Parabens are antimicrobials. They're added as preservatives. Wouldn't an antimicrobial be actually beneficial on nappy dermatitic skin?

DR. MARKS: Diaper dermatitis, yes, we'll use that. That's easier.

DR. SHANK: Diaper dermatitis. You're going to tie my tongue one way or the other.

DR. MARKS: Perhaps because I think most of the dermatitis is irritant contact, so the antimicrobial effect of the parabens is more for the ingredient you're putting on it than actually for the skin, if that's the way you're directing it. Now we're in the margin of safety. Does it talk about the metabolism and carboxy you were talking about in metabolism, on page 72 or 73, Ron? Does it specifically say in our discussion that we're concerned about that enzyme being -- it was a carboxy which?

DR. SHANK: Carboxy esterase.

DR. MARKS: Esterase, okay.

DR. SHANK: We just say metabolism. We don't say the enzyme itself.

DR. MARKS: Yeah, you aren't specific, but the Danish are more specific saying that this esterase is decreased in infant skin, particularly less.

DR. ANDERSEN: Before we get off this, I guess I -- it would be nice to look in -- and I'm not sure the Panel Books are going to make this easy because Panel Book numbers seem to have disappeared -- but if you look at the second Scientific Committee on Consumer Safety document, it's the last one in the book, and look at page 7 in particular. This is the Scientific Committee on Consumer Safety's evaluation of the Danish mindset. And they review what they see as the Danish position. Number one: Different absorption and distribution factors ineffective in activation and elimination kinetics, and there are three references cited. Clearly those three references could be used for an ongoing discussion, but they were all in our original safety assessment.

And it goes on to say "infants have a higher body surface area in the body mass ratio" -- So what else is new? You guys have been saying that since I've been on the panel -- "and potentially enhanced target organ sensitivity in the young organism" and there is a 2000 citation for that. "Impaired development of an organ may be irreversible and, therefore, more severe," but that citation was in our original safety assessment as well.

Then they go on to talk about "parabens affecting reproductive or endocrine endpoints in rats and mice, and both boys and girls may be at risk." And then it goes into the estrogenicity of parabens and those are more recent citations, but that seems to be an expression of the precautionary principle -- maybe we'd better keep it low just in case.

And then they talk about "parabens having no adequate reproductive and developmental studies." I thought the panel was pretty comfortable that there was a sensitive endpoint that could be used, and you had a nice margin of safety for that. And then they reiterate the high body surface area and raise the question of potential higher exposure because kids spend a lot of time out in the sun. That one kind of threw me a little bit, but that's a Danish EPA citation.

With the exception of the Boberg 2009 and 2010 citations that are referenced, there isn't anything new here. So I just want to make sure that that's okay, but that's my reading of it.

DR. MARKS: We certainly have a very large margin of safeties if you look in Panel Book page 73, table 33 there for infants. So again, I guess, certainly we can reopen just to address this but they're very large margins of safety.

DR. ANDERSEN: And I guess the other piece to it, though -- and I'm going to say this with some trepidation -- the Scientific Committee on Consumer Safety as I read it appears to be saying there's no basis for the Danish ban. But they did go on to say when we relook at it, folks, there just aren't enough data for children under six months of age. And I'm not that we can disagree with that because I don't think there are any data on children less than six months of age.

DR. BERGFELD: There's rarely any data on children under six months on anything.

DR. SLAGA: On anything.

DR. MARKS: So Ron Hill, you were going to say something I thought, and then Tom, and then let's go back to the -- I will be making the motion tomorrow whether or not we reopen or not. At this point at least it appears we're going to move to reopen it, but Ron Hill, Tom.

DR. HILL: One thing I was going to add is if it does get reopened, it looked like the uses of benzylparaben had dropped to a very small number. I thought if it was reopened, we should get the best possible new survey of concentration data and use --

DR. MARKS: Yeah, that would come out.

DR. HILL: -- because for me that was the one that was of the biggest concern in terms of unknowns. I mean, I read the rationale of all the European studies beginning to end, and I concur with all of their logic. But I also agree with everything Alan just said.

DR. MARKS: Tom?

DR. SLAGA: This could be a discussion item that we can handle. I mean, I --

DR. BERGFELD: Infants were separate because --

DR. SLAGA: Yeah, we already say that.

DR. BERGFELD: We already said it in the discussion.
DR. MARKS: Pardon?
DR. SLAGA: Infants were separately considered because they would be a sensitive subpopulation for any agent capable of causing male reproductive effects.
DR. MARKS: Right, and this was actually when we had the outside -- as I recall -- expert discuss endocrine disruptors, so we are very so to speak sensitive about that potential issue relevant to parabens.
So Ron Shank, in light of looking at now that memo that Alan pointed out and looking at our going back to the margin of safety calculations and specifically relevant to infants, do you think we need to reopen?
DR. SHANK: I can't find in the Danish report yet where these -- I thought they actually had experimental evidence that the carboxy esterase activity in infant skin was lower. But I can't find it, so --
DR. MARKS: It's kind of interesting, Alan, if I were to -- the reason the Danish mention the sun exposure is because of the presence of parabens in sunscreens. I'm not sure of their practices in infants, but I'm not sure whether they leave the nappy area open when they're out getting sun exposure or not. It certainly is probably more barrier compromised, but again, looking at the margins of safeties, they're in the thousands calculating for infants.
DR. BERGFELD: I think this is rather a political problem rather than a scientific one. And whether you reopen or not is immaterial to me actually, but the reality is I think with a re-review statement we don't need to reopen. However, if one thinks you have to specifically address the baby skin under six months of age, then I think we have to pull other kinds of scientific documentation on skin absorption in infant skin.
DR. MARKS: So we can certainly address this in the re-review statement, say that it was considered -- that would be published, be public knowledge, that we re-reviewed it and did not re-open and addressed those two issues that were in the memo.
Jay, you were going to --
DR. ANSELL: I would just agree with Wilma that if we want to start working on boilerplate, our experience with the aerosol suggests that it would best be done outside of a specific chemical.
DR. MARKS: Yes.
DR. ANSELL: And addressed much more broadly.
DR. MARKS: Okay, so Tom --
DR. SLAGA: I agree with Wilma, too.
DR. MARKS: So handle it as a re-review statement, not reopen? Ron, what do you feel? Does that sound okay?
DR. SHANK: Yeah, that's all right.
DR. MARKS: Okay.
DR. ANDERSEN: I think, Jim, the question of exactly what would this be, we have some flexibility on. The council used the word "reexamine." So they've asked you to reexamine it. If you want to look at more data, for example the couple of new Danish citations and more detail on what data are exactly available for infant skin, then you could ask CIR to prepare a re-review package. This isn't technically a re-review package. This is kind of pre-re-review. So if you wanted to look at those data, you would ask us to prepare a re-review package. Then you would have the opportunity to look at all of those data and say yes, we want to reopen it or no we don't. The council is very elegantly I think here given us a pre-step so that we have that flexibility of gathering additional information. It would allow any interested party to throw other data on the table for consideration by the panel in a re-review package that would occur later this year. I don't want to promise June, but later this year. So I think we have that flexibility because this is a non-usual request. They didn't say re-review it. They said reexamine it.
DR. MARKS: So I think that's quite reasonable. I mean, we have for today or tomorrow two re-review

summaries, but they were pretty straightforward. This is slightly different, so we could just say we're going to see the re- review summary before it actually becomes the final summary so to speak. Does that -- is that what you're envisioning?

DR. ANDERSEN: We would put together a package that would -- for example, the Boberg 2009 and the Boberg 2010 citations that couldn't have been in the CIR report because they weren't published yet -- get those and include summaries of that information so that you have it all to look at and can make a formal decision on reopen or not reopen.

DR. HILL: And if we go that route, I'd just make the request that we have an exhaustive look for whatever is known about human biotransformation of isobutylparaben, and also I mentioned already the use data for benzyl.

DR. ANDERSEN: And I had a question that, I don't know if Jay will have the answer, but I'd like to know what the answer is. I was thrown by the SCCS initial opinion for the parabens in general, not related to the Danish, in which they refer to pentylparaben which by my count is not a cosmetic ingredient. So that threw me a little bit whether it was a typo and they really meant phenyl, but they included phenylparabens. It was a strange thing in the SCCS report that I couldn't explain.

DR. ANSELL: I'm with you there.

DR. MARKS: David, do you want to come up to the mike? Yes, please.

DR. STEINBERG: On the question of benzylparaben, from around 1982-83 I think is when my data goes back through 2010, the total world production of benzylparaben was 0 kilos. The first production that took place was in 2011. In most people's history, they made 200 kilos. It was made in Europe. I believe it was exported to China. We have not used benzylparaben in the United States.

I think the pentyl was a mistake. I think they meant heptyl, which is used or was at one time used in beer and not in cosmetics.

DR. MARKS: Okay, so if --

DR. ANDERSEN: David, would you identify yourself?

DR. STEINBERG: I'm David Steinberg, Steinberg & Associates.

DR. MARKS: Thank you.

DR. HILL: Did you say pentyl or phenyl because they definitely mention phenyl?

DR. ANDERSEN: No question, but they also had pentyl.

DR. HILL: Okay.

DR. ANDERSEN: And that seems to not exist.

DR. MARKS: And Alan, you don't have a -- and again in this re-review I'm going to put in parentheses "package" -- we don't have a good reason why the SCCS decreased their concentrations to.19 percent for propyl and butyl.

DR. ANDERSEN: Well, their explanation is that while there are no new data, they have reevaluated the existing dermal penetration and metabolism data and believe that the number should be lowered for the two higher molecular weight or higher chain length, I guess would be a better way to say it, parabens. So it's again no new data, and we would endeavor to include the gist of that explanation in the package that we give you for the upcoming meeting.

DR. MARKS: Okay, so -- yes? Please identify yourself.

DR. LORETZ: Linda Loretz at the council. Yeah, they calculated that. The SCCS in a, I think it's an earlier opinion where they came to the.19 in the lower concentrations, it was based on that they used a different reproductive study from the one that was used by the panel, and then they calculated --

DR. MARKS: So that's going to be in the package, too?

DR. LORETZ: It would be in the previous opinion, the details of that.

DR. MARKS: All right. Let's get back; did we see that reproductive study that you talked about? They used a different one?

DR. LORETZ: Yeah, right, but you based it on a different study that they didn't use, so yes.

DR. MARKS: Okay. So tomorrow I'm going to move that we not reopen the safety assessment of the parabens; however, what we expect is that there will be a robust re-review package presented so that we can address these issues with the idea that a re-review summary would be produced explaining the reasons why we are not reopening. Did I capture that correctly?

DR. ANDERSEN: Sounds good.

DR. BERGFELD: Are you going to make the suggestion also that perhaps baby skin be looked at and a boilerplate for baby skin under age six months be established?

DR. ANDERSEN: I think we probably already got that message when we made the note --

DR. BERGFELD: Well, I was thinking that, Jim, when you present maybe you'd throw it on the table?

DR. MARKS: I guess the question is, is the age cutoff arbitrary and with this particularly I'm not exactly sure when the barrier -- so I guess certainly we can explore infant skin and perhaps a boilerplate, but we get into the issue of diaper dermatitis, too.

DR. ANDERSEN: I think Jay's admonition to separate such an effort --

DR. MARKS: Yes.

DR. ANDERSEN: -- from parabens would be a good idea.

DR. ANSELL: Yeah, because in particular the Danish discussion would bring us into the drug cosmetic issue since they're really talking about nappy or diaper dermatitis skin protectants, which would fall outside of the cleaning cosmetic application. So I think it would be much, much cleaner to just raise that issue as a topic if the panel decides outside of the discussion of a unique chemical.

DR. MARKS: Oh, I agree. I think so. Rachel, you had a comment. And you always point out to us when a product's being used in a baby, and do we feel comfortable.

MS. WEINTRAUB: Right, and that's exactly what I was thinking. I think it would be very helpful to us in other applications for other ingredients as well because I think it's an issue that I especially -- and I know others do -- look at in particular. And having all of the scientific evidence in one place that we could use and apply I think would be very helpful moving forward.

And just in terms of the scope, I think we need to sort of rely on the CIR staff's expertise to begin this process, to put together the boilerplate, and then we'll see based on the research that they obtain what the age cutoff should be and whether we should focus on younger children or older. And maybe perhaps we need to include that because maybe there are issues for much, much younger children from 0 to 3 months and older. So I think we should leave that open to further research at this point.

DR. MARKS: Wilma, when do you want me to bring this up tomorrow? Do you want me to bring it up or is this sufficient for discussion here, although both teams need to hear it?

DR. BERGFELD: No, I think it needs to come on the table, but I think that maybe you would deal with whether you reopen or not and get that settled, and then move on to making a suggestion that the staff proceed with looking into this. That's what I would do.

DR. ANDERSEN: That would work.

DR. MARKS: That actually fits in nicely because it's either right before the re-review summaries or it could be mentioned at the end, Wilma, however you would like. So what we want to have is a boilerplate for infant safety.

Okay, anything else with parabens? Move on to methyldibromo glutaronitrile.

Full Panel

DR. BERGFELD: No further comments. Thank you. We'll move on then and we'll take up the parabens, and that is going to be reported by Dr. Marks.

DR. MARKS: The CIR Expert Panel received a memo from Alan dated February 10, 2012, to consider two new issues that have arisen with parabens. One was that the European Commission's

Scientific Committee on Consumer Safety, the SCCS, reiterated that methyl- and ethylparaben are safe up to 0.4 percent for one and a total of 0.8 percent for any mixture. However, they considered that propyl- and butylparaben safety was decreased to -- percent for any one or any mixture so that there was that change in the limit for propyl- and butylparaben concentration. The second issue that was outlined in Alan's memo concerned a Danish clause or safeguard that banned the use of paraben in cosmetic products intended for children under the diaper area, also referred to as the nappy area. At any rate, the issue was in light of these rulings in Europe, should we reopen or not reopen this safety assessment which was published in 2008. Our team felt that we did not need to reopen but that the way we suggest handling it is that there would be a re-review package that the panel would see prior to it being sent off for publication that would address both of these issues.

DR. BERGFELD: Don?

DR. BELSITO: I'm not sure that we were being asked to reopen or re-review. I thought that this was more an FYI and do you want to respond to it. We didn't think we necessarily needed to respond to it. It's whether you take NOEL or whose NOEL you take for reproductive toxicity and that's where the difference in the calculations come. In fact, we had assumed 50 percent absorption and the Europeans assumed 3.7 percent absorption so that we were overly conservative in the amount of parabens absorbed, it just has to do with the NOEL. So if you have confidence in your NOEL then the margin of safety as in our re-review would stand. If you don't have confidence in the NOEL then maybe we need to look at it. I thought we had confidence in the NOEL. Paul expressed an interest in just seeing the two papers that have been published since, just a peek at them. We thought that since the Danes have brought up this issue of not so much absorption because all of the data would suggest that except for premature infants the absorption across infant skin is now significant different from adults, but Curt in particular pointed out that there may be differences in metabolism in infant skin and we thought it would be good to put together an independent paper looking at what is known about absorption, penetration and metabolism in the skin of children as we go forward and deal with issues of products being used on kids. That's what we wanted to do with this, not necessarily open the paraben report, but to create a specific report on infant skin.

DR. MARKS: We concur. We did not feel we need to reopen. I think it's whether or not you react to these two specific things. Then we also discussed the issue of safety and infant skin and I think largely concur with what your team suggested doing. You suggested doing a paper. We suggested actually having a boilerplate that would end up like the aerosols and we've have a boilerplate which we could refer to which would outline the safety issues of applying cosmetics to infant skin.

DR. BELSITO: But it would be I think hard to create a boilerplate until we had data to look at. This isn't a matter of a company saying this is the size of the particles that come out of a pump and I'm saying those aren't respirable and as long as there could be issues if they are absorbed from the tracheobronchial area, but if there is no systemic toxicity then it's not an issue. Here it would be put together a document where we know what's known about absorption across infant skin, penetration, what we know about metabolism, and is it or is it not significant different, the only thing we have to worry about is that infants have a bigger surface area to weight ration. So I think we need data before we create anything.

DR. MARKS: Obviously you couldn't create a boilerplate without having the data and with the aerosols we had a lot of data. In fact, we had that one outside expert come in and discuss aerosols to us. If such a person exists for infant skin, I bet that person does exist in the industry which looks at that issue and perhaps we should have an expert come in and discuss the biology and physiology of infant skin. Ron Shank brought up the issue that carboxylesterases are lower in infant skin and perhaps you would metabolize cosmetic ingredients differently in infant skin than in adult.

DR. BELSITO: I would see this like a hair dye epidemiology statement or the ethylene glycol repro thing we put together.

DR. MARKS: We certainly concur. It's the question of how do you proceed forward.

DR. BERGFELD: It appears to me that we were asked to reexamine and not to re-review. The opinion, at least the grassroots opinion, is to re-review and we've looked at it, but we're not going to do a re-review document. Coming out of this it's even more important that we look baby skin with all the dimensions that have been discussed and I think we would charge the CIR office to begin that process for us.

DR. MARKS: Could I ask, Rachel, from a consumer's point of view if you're aware of these two new rulings in Europe? Do you think us having this discussion this morning and deciding not to reopen and ending with that? Or do we need some sort of formal document? I guess maybe Halyna too. I'm comfortable with doing nothing and just leaving it as we've decided today not to reopen, say we noted that that we reviewed it but I wonder whether in the interests of the public if somebody says the panel is aware of this but they didn't react so to speak.

MS. WEINTRAUB: I think the panel is reacting and I think the response is exactly what you're doing, that you are taking a closer look at the issue of baby skin. I think it's unclear what the form is right now, I think that's okay, but I think what you are doing is directing the CIR to look at this issue closely, to perhaps have experts come to do an in-depth analysis on this issue, so that you have a much better understanding moving forward for every ingredient and its impact on baby skin. So I think there is a reaction by the panel and I think it's a good one.

DR. BERGFELD: I wonder if I could call in Linda Katz regarding the issue and what the FDA thinks about baby skin.

DR. KATZ: We would agree with the panel to go ahead and take a closer look, and at this point we also agree with the panel's decision that the rest of the data has been looked at and there is no need to go further with the exception of the baby skin area. Then we would look forward to the results or the opinions of the panel once that issue has been reviewed.

DR. BERGFELD: Thank you. Halyna, do you care to comment?

DR. BRESLAWEC: We brought this issue to the panel because we felt it was important to formally bring it to the panel and ask for a reexamination to see if the panel's decision on the safety of parabens still stands. I'm comfortable with the kinds of discussions that were held in the team meetings that reexamined the basis for our safety decision and the panel's safety decision and really liked the fact that we're focusing on an area of infant and child skin metabolism that will have an impact on all of the ingredients that the panel reviews.

DR. BERGFELD: Alan?

DR. ANDERSEN: I think we declare victory. We've got a new project in front of us. When we can gather information, potentially identify an expert to come and talk with us, then we'll put that back on the agenda and take a look at it as a stand-alone topic not unlinked from parabens because that's how it came up, but it's really much broader than the question of parabens. As for the paraben safety assessment itself, it stands.

DR. LIEBLER: I'd like to note in my reading of the SCCS reaction to the Danish regulatory decision that there was a lot of discussion of the potential impact of insufficiencies in xenobiotic metabolism in infants but a lot of it was sort of hand-waving speculation, not to dump on that particular opinion. It's clear that this is an area where there is a lot of information floating around, it's not very well connected or synthesized particularly in the context of cosmetic ingredients so that this is where we can make a real contribution I think by developing either a paper or a document and/or boilerplate of some type.

DR. BERGFELD: Thank you. Is there any other comment? We move on. I think a very worthwhile project, by the way, to look at baby skin because they don't test baby skin for pharmaceuticals or cosmetics so it is very worthwhile. We'll move on to the re-review summaries. Dr. Marks will be

reporting on these and making recommendations.

DR. MARKS: Both of these summaries were well done and we had no recommendations for any editorial changes.

DR. BERGFELD: Second?

DR. BELSITO: Second.

DR. BERGFELD: Is there any other comment? Seeing none, all those in favor indicate by raising your hand. Thank you. Unanimous.

[Discussion of Parabens is mixed with discussion of Triclosan]

SEPTEMBER 2012

Dr. Belsito's Team

New Data

DR. BELSITO: Okay. Anything else? So now we're back to Buff, the new data, looking at triclosans and parabens. So I guess -- I don't know how you want to do this. The paraben issue has to do with -- well, there are a couple of issues with parabens -- is the increased risk of respiratory and food sensitization with preservatives, and then the levels of paraben in human breast tissue in women undergoing mastectomies for breast cancer and that they enabled this suspension growth of MCF immortalized nontransformed human breast epithelial cells. So the implication is the new data on parabens or do they increase the risk of sensitization and are they a breast cancer risk?

And then we've got a comment from BASF on the aeroallergen and food sensitization issue. I think they've put this in very good perspective; I think it was fairly unbiasly written. I guess the other thing that I would point out, particularly in terms of triclosan but also parabens, is that while they're looking at asthma and food allergy, what they're really missing is how many of these individuals had atopic eczema. Because people with atopic eczema are going to be putting more things on their skin, number one, which are likely to contain parabens because we tell them to stay away from formaldehyde derivatives; and number two, they're staph carriers so they tend to use more antibacterial products, including triclosan. And so we don't know the percentage of these individuals with atopic eczema, which is I think perhaps the most important confounding variable because we know individuals with atopic eczema have high levels of IgE to food and aeroallergens. So quite honestly, I did not think this paper demonstrated anything and, in fact, it was interesting that the -- was it the allergic asthma or non-allergic asthma? There was one form that was negatively correlated with levels.

DR. SNYDER: Methylparabens.

DR. BELSITO: Yeah. And then they also point out that they didn't confound for smoking, but one would hope it would be very low in this population group, but one never knows. So that was my thought.

And then the triclosan with the muscle issue. I mean they're giving it IP. They're giving it in huge doses. I mean I just didn't think it was relevant. And, quite honestly, I thought that we noted these. Do we -- I mean how do we handle this? I think it's important that the public know that we looked at it. And then the question is I personally don't feel that I need to open these reports based upon the information I'm seeing. But how do we -- I mean this is -- it's a hot potato issue. It's been all over the news. EWG is going crazy with it. So do we reopen to close or where do we go? I mean what's -- should we be scientifically correct or politically correct I guess is my dilemma.

DR. ANDERSEN: My strong desire would be to be scientifically correct and then let the political part play out as it will. Now I've got to see if I can remember which meeting we last talked about parabens. I think it was last December when Denmark had raised a series of questions about the use of parabens in baby products, and the Panel -- the Council had asked the Panel to look at those

data, not to reopen or not, just look at those data. You did and you said that there was no need to change the Panel's opinion regarding the use of parabens, that the margin of safety adequately dealt with the issue at hand. I see this as the same thing. You don't have to make a decision to open or not reopen. I think you can simply say that the available data -- and again, in the triclosan report you have repeated dose toxicity study after study after study in which there was no identification of any muscle-related endpoint of concern. So while this is an interesting exercise at high exposure levels, in the available data that you did look at, this endpoint was not of concern. I think that's a scientifically-based view of how important is this information and there's no need to further consider this. As did the researchers, you can always throw in the thing at the end that says "more data would be useful." That's always true. I don't know that it gets you anything to say that. I think you need to make that scientific judgment that these data are not significant as regarding the question of triclosan safety.

DR. BELSITO: And how does that get reflected back to the public, just as part of our minutes?

DR. ANDERSEN: Part of the post-meeting announcement for the parabens discussion, we went through it all in the announcement so that every member of the public can see it. It was part of the meeting minutes so it has been captured as a Panel decision. It's on the Website -- not always easy to find on the Website, but it's there -- and I think that's the right way of handling it. It doesn't need to be a question of opening or reopening every time there's one new study.

DR. BELSITO: And do we send a separate letter back to Alexander Scranton or do we simply say hey, Alex, take a look at our meeting announcement?

DR. ANDERSEN: No, I think a separate email back to Dr. Scranton would be appropriate to say here's what we did with the issues that were raised I think.

DR. SNYDER: With a positive stand, thank you for bringing this to our attention and we fixed it, et cetera, et cetera, et cetera.

DR. BELSITO: We actually put it in the minutes? I mean I think it was Jim and I that sent Alan the article. She was just thanking us for doing due diligence.

DR. ANDERSEN: And I wouldn't want to not do this in the future. You're going to get a series of studies to look at on phthalates in December -- I'm sorry, but you are -- and it's just the renewed data coming out and the question of what's the impact on your view of the safety of phthalates is going to have to be considered. We just need to keep doing this. Certainly the sensitivity leads us to that conclusion, but I'd do the same thing if it were methyldibromo glutarnitrile if there was a significant piece of new data. You just gotta look at it and decide. I hate to nickel and dime you. I'd much rather be doing full-blown safety assessments, but I don't see how we can afford to ignore these kinds of studies.

DR. BELSITO: No, you can't, not when they're getting huge press. And we all know what the 6:00 news is like. You know that your sunscreen maybe causing cancer or underarm deodorant causing breast cancer. I mean here are the facts.

DR. LIEBLER: I fully support Alan, but I don't know that the decision was based on the fact that it attracted press attention. I think that would be a very difficult threshold to watch the news every morning and see. This was published in the proceedings of the National Academy. We looked at it and relative to the doses of the root of exposure and the effects observed, we don't think it's relevant in terms of assessing its use in cosmetic products. And other papers, as they may come up, that are published in legitimate peer reviewed literature that may have an impact should be reviewed. And I think even if we had found it relevant -- well, if we had found it relevant, we should reopen and add it to the literature within the reports.

DR. ANDERSEN: Exactly.

DR. SNYDER: My only comment, Alan, was regarding procedures. And so when an individual article is brought to our attention, do you do any expanded review of the literature, see if there's anything else that has kind of popped up? Or do we just take this as a standalone, ignoring that there may

be some other reports affirming or contradicting? So procedure wise, what is our -- what do our procedures say that we do when these are presented? I understand what happens when we reopen or consider for reopening. We do an extensive literature search and try to data mine and see if there's anything else out there. But in this instance, do we do any additional data mining?

DR. ANDERSEN: Yes in all instances. So the question here gets separated into triclosan and parabens. The lab, who's really focusing on milking this assay system for all it was worth and most of the other background material that's available is on the assay system, not on triclosan. So there wasn't anything else, no more threads to pull, in that direction. Now will there be further assays? Well, maybe. We'll have to wait and see. On parabens the issue of food sensitization is itself an outlier and the authors themselves specifically say that the estrogenic thread isn't the one that's relevant here. It is microbial in origin; if you start killing bugs, you're going to increase sensitization. I get that as a theory. I also agree completely with Don that the selection bias here could have been extreme, and we don't have enough information about it to make any conclusions from this, nor did the authors. They were very clear that this was a piece of information that was a hypothesis and nothing more. But we did pursue the other new parabens data, which was estrogenic in nature. So, yeah, we've got to pull those out and take a look at those. And those will keep coming. There's nothing that's going to stop Darbre's Laboratory in England from doing these studies. They're going to keep coming out, and you're going to have to pay attention to them.

DR. BELSITO: Anything else? So this is just going to be summarized as part of the meeting announcements, that we looked at these, and that we found the following issues and elected not to reopen the reports. Is that what I'm hearing, Alan?

DR. ANDERSEN: Yup. The conclusion stands.

Dr. Marks' Team

DR. MARKS: Oh, good, a half an hour. So -- well, that's because we didn't have the presentations this morning. So, do you think we'll get done Triclosan and the parabens before lunch? That's what we're up to now.

So, what we've gotten are additional studies, papers with these two ingredients, and the obvious question is, does this trigger a reopening? So, that's in the Buff Book under "new data" section.

So, let's do -- let's start out with Triclosan. So, there was a report of urinary levels of Triclosan associated with aeroallergen and food sensitization. That report also talks about parabens, but let's not muddle the two ingredients, let's do one at a time and be clearer since they're separate reports.

And then also there was this report of impaired muscle contractivity and we have some comments from industry and obviously we heard this morning about the issues with getting that paper where there was concern about RYR and calcium channel signaling impaired by the muscle contractivity, both in vivo and in vitro of non-human experimental tissue.

And so, Rons? Ron Shank? Ron Hill? And Tom? Any concerns with either one of these that would trigger enough to reopen Triclosan?

DR. SHANK: I don't think we need to reopen the Triclosan document. I think in the review that we'll have -- shows that the panel has considered these reports and will continue to consider all the new reports that become available.

But the CIR panel report on Triclosan contains a lot of information on repeat oral exposures, which did not indicate any kind of allergenicity response, IG, immunotoxicity, muscle toxicity, and these are interesting reports, but not really pertinent to the use of this compound in cosmetics.

DR. SLAGA: I had a similar conclusion related to this, that it's really hard to relate this to cosmetics and, sure, the combined exposure can create some kind of a different thing, but related to cosmetics, I thought we had sufficient data in the past report.

DR. MARKS: Ron Hill?
DR. HILL: I basically agree. This is used in mouthwashes sometimes, is it not? Toothpaste? Yeah, but toothpaste, most of the time we're talking fluoride toothpaste, so we don't consider that, right? That's not a drug because --
DR. SHANK: Toothpaste.
DR. HILL: Toothpaste? Yeah, but toothpaste, most of the time we're talking fluoride toothpaste, so we don't consider that, right? That's not a drug because --
DR. BRESLAWEC: That is a drug.
DR. HILL: But not mouthwash?
DR. BRESLAWEC: The relevant use here is deodorant.
DR. HILL: Is what? Is deodorant?
DR. BRESLAWEC: The largest use for Triclosan is deodorant.
DR. HILL: Yeah. But there is some use in mouth rinses?
DR. BRESLAWEC: Those are considered drugs as they are anti-gingivitis.
DR. HILL: They give a gingivitis indication and therefore fall out of our scope. Okay.
DR. MARKS: Rachel?
MS. WEINTRAUB: Yeah, so, I spent a lot of time looking through this material and I think one of the comments I think that Dr. Shank made was that, well, if you look at cosmetics use and the interaction of people with cosmetics, that's one thing, but if -- but the problem is that no one's looking at total exposure. And each sort of -- there are different entities, not necessarily one entirely parallel to ours, but I think that's a huge problem here.
I mean, I think this study shows, especially what I found concerning, was sex differences and aeroallergen sensitization. So, what is this explanation? Could there be some link to cosmetics? Some link to the use in deodorant?
I found this data to be of concern and thought that this should be reopened to consider this and see -- and for us to review the impact of this specifically on cosmetics as used in deodorant.
DR. MARKS: Halyna.
DR. BRESLAWEC: If I remember correctly, when CIR last considered the Triclosan report, at the end of the report, Dr. Katz, who was representing FDA at that point, asked the panel to consider the dosage that came out of cosmetic use together with other uses and that the panel determination on Triclosan safety was to have reflected that. That's my recollection. I would like, you know, to check the record on that because I do think that that was something that was a very, very thorough review that the panel did last time.
DR. MARKS: Okay, but --
DR. BRESLAWEC: We have, again, please note for the record the comments that we have provided on the individual studies. There are, we believe, some very serious issues with the study in terms of the relevance to human use and particularly cosmetic use, but, again, my main point here is I think the panel looked at that the last time it did its very thorough review of Triclosan, and I would like the record to be checked to see if that recollection is correct.
DR. MARKS: So, what I recall the prototype of do you consider just cosmetic use or do you consider all uses was with the phthalates in nail polish, and so there was concern of phthalate exposure from many different sources and we limited our consideration, again, to cosmetics because I think once we open up to all exposures it becomes a very difficult to handle, but I would like -- perhaps, Alan, obviously, you comment, but also the two Rons and Tom. I would be more in favor, as Dr. Shank indicated, we're looking at this as a cosmetic use, not in the total use of the universe.
But Alan, do you want to comment?
DR. ANDERSON: Yeah, I think Halyna's recollection is exactly correct, that for Triclosan at the end of the discussion, the panel was focusing on the use in cosmetics and the question was posited whether all of the exposures, and there were a great of information in the safety assessment on Triclosan

in a wide range of product types, and the panels conclusion was, well, none of them, even if you added them all up, reached a threshold of toxicologic concern. And the way you phrased it was available study data, wide variety of studies, then the end points are listed. "Triclosan may be used safely in a wide variety of products in the present practices of use and concentration even if all product types were to contain Triclosan were used concurrently on a daily basis."

So, that was intended, and the discussion record will show that it was beyond just the use in cosmetics.

DR. MARKS: Okay. So, Rachel, that has been addressed before.

DR. SHANK: We have chronic oral exposures with Triclosan and very good skin penetration data, which shows that it is poorly absorbed. Much of it remains in the epidermis and little enters the circulation as Triclosan. Therefore these new studies are very interesting, but are not relevant to cosmetic use.

DR. BRESLAWEC: Many of them are IP studies.

DR. MARKS: Repeat that, you mean these studies are interperitoneal?

DR. BRESLAWEC: The two studies here are interperitoneal, yeah, so you have that issue too.

DR. MARKS: So that, again --

MS. WEINTRAUB: So, why would that not be relevant to cosmetic use? Could you just explain scientifically?

DR. SHANK: In cosmetic use, there is very little transfer from the surface of the skin into the circulation, but in these studies, there was direct injection into the peritoneal cavity, so there was a bonus effect, rapid absorption across the serosa of the intestine, so the blood levels would go very, very high. Never would that be reached by cosmetic use. There would be a slow diffusion at best..

And then some of the other studies were actually adding the Triclosan to media, these were (inaudible) fat amidyls or something like that, where these animals live in a solution of this. Interesting scientific studies, but not relevant -- the results are not relevant to cosmetic use because the amount entering the blood at any one time would be very small.

So, the concentration would never reach anything like these experimental studies that we've just received.

DR. MARKS: Any other -- Rachel, does that help answer the concerns you had?

MS. WEINTRAUB: Yeah.

DR. MARKS: And I thank you, Halyna, for expanding that the panel had in the past addressed for all exposure to it. I had not recalled that.

Now, how should this -- so, this will go in -- the minutes is not reopened? Or will this go in as a re-review in the Journal -- itself -- of Toxicology, not reopened and the reasons why, under a discussion section?

DR. ANDERSON: We still have to talk about parabens, but saying parabens brings to mind the last time we did this, which was in December of last year for parabens. The European Commission had considered the Danish proposal for parabens that they not be used in baby products, and the panel looked at the available information and simply reconfirmed that the margins of safety that it found for the use of parabens were appropriate and no change in the CIR conclusion was needed.

I think that is appropriate here, that further data have been evaluated and no change in the conclusion is appropriate.

Now, if you thought that these data were sufficiently significant, you could have said, I'd like you to reopen this, but if you don't think they cross that threshold, and my reading is you don't, then you would say so in the post meeting announcement. All this would be captured in the minutes as well, so the record would be established.

Now, where CIR would also be obligated to send a response back to Dr. Scranton to Women's Voices for the Earth, that explains what we did as well, because they are on record as encouraging us to

look at these new data and see what their impact is, so we owe her a response and we would do that.

So, I think there will be no lack of public display of where we came down on this..

DR. MARKS: Okay, so this would be handled differently than a formal re-review. It's looking at the data, deciding that we would not reopen it and no change in conclusion. That would be captured in the minutes and in the letter that you will send. Okay.

Any other comments? I mean --

DR. BERGFELD: May I ask a question? Have we ever done these in the Journal where we've said, not reviewed and updated with literature and not changed our conclusion? I thought we had.

DR. MARKS: That's a formal --

DR. ANDERSON: We've done it when --

DR. BERGFELD: For the re-reviews, but this is not --

DR. ANDERSON: I'm trying to figure out a way to describe it succinctly. The first time we looked at parabens a second time was after all of the estrogenic effect data had been published in the late '90s. So, we had reviewed them in the early '90s. Those data weren't even on the radar screen.

Then they appeared and there was sufficient data that warranted an open discussion of those data. So, we reopened it in order to provide that. Not that we -- and the panel clearly said, we're not going to change the conclusion, but these data are sufficiently important to provide an assessment of it.

Subsequent to that, last December, you looked at the EU situation and the Danish proposal and said, this doesn't reach a threshold of having -- in fact, there were no new data, it was simply a reassessment of the existing data, and you said, no need to reopen this.

DR. MARKS: Right.

DR. ANDERSON: So, there is a threshold phenomenon here that we're calibrating and I'm -- I don't know that that's final, and I hate to say it's, you know, we know it when we see it, but it's a question that each time new data are available, what are the significance of those new data, has to be part of the discussion, and if the significance is such that everybody should see a full discussion of that, you should reopen it. I mean, you really should.

But I think the explanation, as Dr. Shank has provided it, that vis-à-vis use in cosmetics, these data are not particularly informative means you cannot reopen it.

DR. HILL: Well, I'm assuming in the -- I'm not assuming anything. In making the response to the Women's Voices group, grant you BSF has an extremely vested interest, but I thought that the letter that Dr. Finken -- I assume it's Dr. Finken -- supplied, it's a sort of a very thoughtful analysis of the Savage papers, it is a very thoughtful analysis, and one of the things they point out near the end was the correlation is between urinary concentrations and allergic sensitization, the IgE stuff and basically that people who are hypersensitive in the first place are advised to practice much stricter hygiene, therefore using much more of this and somewhat more likely to -- so, it's a cause and effect confusion that hasn't been sorted out.

I'm not an immunologist, so that -- once we got much deeper than that I had to stop, but having seen the paper and then this, that was my reaction, it captured my gut reactions pretty well.

DR. MARKS: Ron Shank, when -- in this one paper, and this is just for my own edification, when you talked about Triclosan not being absorbed and not having a systemic effect, is the level of urinary concentration presumably what they're finding in the urine is actually being excreted, perhaps, not being washed off into the urine? Are the levels so low that we aren't -- because there's something -- obviously, either, there's only two explanations -- two or three -- finding it in urine. One, that the assay wasn't correct, two, it was washed off the skin in the urine, three, it was contaminated, or four, it was absorbed and now we're seeing it in the urine. So, just to clarify that if --

DR. BERGFELD: Found in foods?

DR. MARKS: In foods?

DR. BERGFELD: It might be ingested.

DR. MARKS: Ingested. So, and then it was also -- no, that's parabens. So, again, just in case that would come up, somebody would say, well, how is it in the urine if it's not absorbed? It's because other sources?

DR. SLAGA: Yep.

DR. MARKS: Okay, that's fine. I just wanted to confirm that.

Okay, so we --

DR. BERGFELD: I'd like to propose, when you are giving a statement on this, that we considered on these important, worrisome, ingredients, especially those that the FDA has asked us to review, that we not just have it in the minutes, but we have something else -- develop something else that says what we have done and why, so they're a quick reference for anyone that wants to see on these (inaudible), we've been asked to re-review and we decided not to, we can come up with a discussion paragraph and what the references were that we used, and have that be called something and retained.

I would suspect, maybe even on the website, that that would be a good place.

DR. MARKS: I would say, Wilma, we do do that for the hair dye because we update the epidemiologic study, but there are so many hair dye ingredients that that's periodically seen in a report. I don't know how we do it, as you suggested, other than saying, this is a formal re-review and it will go out as a re-review with a conclusion not to reopen and no change in the conclusion and have that paragraph -- that would go in the public literature, so to speak.

But Alan, do you what to -- your proposal was to capture it in the minutes and be very clear and if somebody wanted to go back, I guess we could ask -- where is -- whether or not that would be searchable. Are the minutes searchable?

DR. ANDERSON: Almost certainly not. I mean, I suppose a web search could uncover that information. But we're certainly not making it easy for anyone to find. It's -- while we were clear in December what our conclusion was about the Danish view of life regarding parabens, we didn't go out of our way to make that readily available or hallmarked or at all visible. We didn't try to bury it, but we didn't highlight it.

What we're talking about here is potentially a circumstance where it's important enough to highlight and we don't have a good mechanism for that. Just as you were talking, Wilma, I was thinking about what the Academy does and there's got to be that intermediate thing that gets issued that isn't a publication but is commentary, is something --

DR. BERGFELD: Update.

DR. BRESLAWEC: Press release.

DR. ANDERSON: Well, press release is certainly targeted at visibility.

DR. SHANK: How about a letter to the editor?

DR. ANDERSON: Also appropriate. Interesting, Ron, thank you. Since it concerns a published study, I don't know if PNAS takes letters to the editor, but certainly the -- what the heck is it -- the Academy of Allergy, Asthma, and Immunology I'll bet you takes letters to the editor. That's not a bad idea.

DR. BERGFELD: How about all of the above? I really think that the CIR has been looking for ways to promote itself and to have an impact on many different disciplines with all these safety results because they're a little bit boring when you get to safety if they're all safe, but one that's controversial is certainly a hit in hook, and so I would think highlighting that you actually tackled a difficult subject and had an opinion on it would be most important.

DR. MARKS: Couldn't it be a letter where we publish our reports already? Would the editor accept a letter to the editor? I like that, Ron Hill, in the Journal -- or was it Ron Shank, yeah -- n the Journal of Toxicology?

DR. ANDERSON: It certainly can't hurt to ask. My only concern in that regard is, were I the Journal of Allergy, Asthma and Immunology, I'm not sure I'd like you writing a letter to some other journal commenting on something that appeared in my journal.

DR. SLAGA: Yeah, it would have to be --

DR. ANDERSON: We need to --

DR. MARKS: I guess there though --

DR. ANDERSON: -- scope that out, but --

DR. MARKS: Then we'd need two letters because we're addressing both the allergy issue and also the muscle issue, so now we have two different -- so, that would either generate two different articles or letters or we'd just combine it in one. And then what you could do, perhaps, if the Journal didn't like it is obviously once the letter is formulated you could send it to the respective editors in the other journals.

DR. ANDERSON: Well, the other logic would be a letter to the editor of the International Journal of Toxicology that says, "CIR previously published a safety assessment of Triclosan. Since that was published, two new reports have appeared and here's our analysis of those two new reports." That then packages it in the venue of where we publish. I think that is worth exploring.

DR. BERGFELD: And it's a reference. It's a documented reference.

DR. ANDERSON: Yeah.

DR. MARKS: Which is searchable.

DR. BERGFELD: Yeah.

DR. ANDERSON: Yeah.

DR. MARKS: Good. So --

DR. ANDERSON: Now, that would require a write up, which we would bring back to you, essentially what the letter to the editor would look like, and we come back to you in December, assuming we can get it done, and have you review that.

DR. MARKS: And then I don't know if our discussion included for the allergy, Alan, you had made note in your memo to me that the results were not linked to IgE serum levels. To your point, Rachel, that you made, it's problematic that it's sex differentiated, why did it occur in men but not in women, so that's more problematic in the study is that an issue with this epidemiologic study, and in the last comment you made, Alan, was that this was a cross-sectional study, which is not readily applicable to this issue either.

Okay, so not reopened for Triclosan and no change in the conclusion, and you explore the idea of getting this searchable via a letter to the editor. So, there won't be a --

DR. ANDERSON: And press release.

DR. MARKS: Oh, yeah. That's --

DR. BERGFELD: And the website.

DR. ANDERSON: And the website. So, you know, again, we may have lost some contact with some of the special features of the website and we're working to improve that, but an example of something we did once before was when the panel re-reviewed paraphenylenediamine as a hair dye and said, there's no real new data, it's continues to be safe. However, we really don't like the idea of putting this in tattoo ink or in henna, in particular, and that's a very dangerous practice and is considered unsafe.

That went up on the website as a special alert. Now, that was on the hazard side, but this would be on the flip side that this is to be highlighted. Again, right now our mechanism for doing that probably isn't as good as we would like, but that's impetus to fix it.

DR. MARKS: Okay, we're going to delay the discussion of parabens until after lunch. We're going to break for lunch now and we'll re-adjourn at 1:05..

(Recess)

DR. MARKS: Okay. Rachel's here. Good. Let's start.

So, we finished Triclosan and now we're on to the parabens, and, again, we were sent this second -- part two of this one article is the association urinary level of parabens with aeroallergen and food sensitization, and so the same question -- let me see, were there any other articles that concerned about parabens? Oh, we also have parabens -- Tom, I'll ask you to comment about parabens found in human breast epithelial cells and in parabens concentrations of breast tissue at serial locations across the breast from maxilla to sternum.

DR. BRESLAWEC: Excuse me. Dr. Marks, did we have any studies presented on that in there? Okay, sorry.

DR. MARKS: So, where did I get these from?

DR. BRESLAWEC: I don't know.

DR. HILL: Wave 2.

DR. MARKS: Since they're printed out, they have to be Wave 2. So, the one is by Darby in the Journal of Applied Toxicology, June 2012. That's the one of human -- did you see these, Tom, by any chance? Oh, you didn't? Okay. Well then I'll give you a minute as we discuss the sensitivity, but I'll give you a minute to look at these two.

MS. WEINTRAUB: There's a number of them.

DR. MARKS: Yes. Well, they were the two I printed out.

MS. WEINTRAUB: In Wave 2 there were a number of different abstracts.

DR. MARKS: Thank you. So, the two Rons, were you concerned about the potential link between urinary levels of parabens and food sensitivity or aero sensitivity? It's the same study, same issues that we discuss with Triclosan, so I assume they're similarly applicable. Is that correct? Not enough to reopen?

DR. SHANK: As far as I'm concerned, that's correct. The argument that we use for Triclosan also applies to the parabens.

DR. MARKS: Good, and Lillian, you're sitting in for the director, is that correct?

MS. GILL: Yes.

DR. SLAGA: I totally agree with Ron, related to that article, that I have no problems --

DR. MARKS: Okay. Should we delay the other discussions, Tom, until you've had a while, or Ron -- did you see these abstracts and the articles?

DR. SHANK: I did.

DR. MARKS: Okay, good. Did that raise any concerns in your mind, again, with reopening?

DR. SHANK: No, again, these are interesting observations, but there are no data relating causally parabens to breast cancer. So, how one extrapolates from finding parabens in breast tissue to parabens causing the carcinogenicity is too -- right now it's just too large a gap. And, again, I would say the panel should continue to review these articles and studies as they become available, but right now I don't see a need to reopen the paraben document to consider any kind of a change in the conclusion.

DR. SLAGA: Looking at the abstracts -- I haven't read the whole paper yet, but I agree, it's not -- you can't relate it to cosmetics. There's no causative relationship here. You know, they can be coming from other sources just like we had with the Triclosan, but I don't think this is needed to open it because we really don't have any data related to cosmetics.

DR. SHANK: I think you'd find parabens in a lot of fatty tissues.

DR. SLAGA: Yup, and in your sweat glands you'd find parabens, in BHT, BHA all of those type of things accumulate.

DR. MARKS: And Tom, then, in the original document there was no evidence of parabens having a carcinogenic effect or mutagenic or whatever -- genotoxic -- that whether they're in the tissue or not, you're not really concerned that that could be related as this one was in breast cancer?

DR. SLAGA: Especially at the levels that were used. I think, you know, there were a few that had mixed mutagenicity type of activity, but it wasn't consistent and the concentrations were -- that are used are much below that.

DR. MARKS: Rachel, any other comments? And anyone else have comments?

MS. WEINTRAUB: I mean, I think at a minimum what needs to be documented is that the panel looked at these, considered them, and concluded, based on the information, that it was applicable or not. You know, and I think that's what's minimally important here.

You know, I think, issues of causation -- and there was some other letters -- I don't think it was actually on parabens, I think it was on Retinol A, but there is some interesting information about causation, how to establish causation, I guess, and I think it gets into sort of deep views about how to view this type of information within scientific analysis.

But at a minimum, I think it's very important that the panel establish that it did review these studies and the reasons why it was found persuasive or not in the context of cosmetics.

DR. MARKS: So, I think this is -- Lillian, were you here the end of the morning where we discussed how we would perhaps capture this? So, I talked to Kevin and he felt that our minutes would not be searchable for these ingredients, so what we landed on this morning was that there would be a letter to the editor, so it would be in a peer reviewed journal, which would be quite searchable, that there would be a press release, and then it would be readily available on our website.

MS. GILL: Yes.

DR. MARKS: So, I think, Rachel, that's how we would address and it would have a -- again, we wouldn't reopen, there's no change in conclusions for parabens, but we would have a robust discussion for both of these concerns, in this case, one the allergic concern, the other one the potential cancer concern.

Any other comments about parabens? If not, then tomorrow I will make a motion to not reopen either one of those, if there need be a motion, and of course, that would indicate there's no change in conclusion and then capture the CIR's review of these two ingredients, the Triclosan and the parabens, and the nuances of why we didn't reopen and why we still feel they're safe.

Full Panel

DR. BERGFELD: Any other additive comments? We're going to vote to re-open this group of ingredients. Seeing none, I'll call the question. All those in favor of re- opening? Unanimous. Alright, we're moving on to the last -- I would call it ingredient issue, and that's the triclosan and parabens. Dr. Marks.

DR. MARKS: Well, there were health concerns with both of these cosmetic ingredients for the triclosan, particularly the report relevant to increased sensitivity from this compound, and also the issue of impaired muscle contractivity. We felt that neither one of these reports rose to the level that were of concern, and therefore would not change our previous conclusions of safe, so we move not to re- open triclosan. However, we felt there could be a letter to the editor, a press release, and a website announcement explaining our rationale of not opening the triclosans.

I'll start with that one and then we can move on to the parabens, because there's some other toxicologic concerns with the parabens, although we didn't feel we should re-open that one, either.

DR. BERGFELD: Don?

DR. BELSITO: No, we're fine with that. I think I have a little issue with your phraseology. I think we felt that the data that were presented were not relevant to the use of these products in cosmetics. They were somewhat contradictory in terms of the asthma. There were issues with the fact that while they looked at asthma versus atopic asthma, their definition was patient self-definition of wheezing, which is a huge issue.

What they didn't look at that I thought was an important issue is atopic dermatitis, because we encourage

people who are atopic staph carriers to use antibacterials, so they are likely to use more antibacterial soaps because of that. We don't know that data at all.

In terms of the triclosan on muscle effects, it was given intra-paraneally in much higher doses than people would ever experience in a cosmetic. So, we thought that the data was interesting. There were serious flaws in the one paper that dealt with sensitization, and the paper that dealt with muscle relaxation, which is not relevant to the use in cosmetics.

We would agree that some type of announcement -- that this be looked at -- very seriously be made.

DR. MARKS: To further substantiate that, Don, we also -- there was no link to IgE in the paper with sensitivity or endologic alterations.

There was an unexplained difference in gender that it occurs, sensitivity, in men and not in women, and this was a cross-sectional study which created problems with interpretation, also. So, we concur. We expect that will all be in the letter to the editor and summarized the reasons why we felt there was not -- this report should not be opened and the conclusion should stand.

DR. BERGFELD: So, do you want to make that a motion since that is a vote to re-open or not?

DR. MARKS: I move -- should we do these together or separately? I move not to re-open --

DR. BERGFELD: Separately.

DR. MARKS: -- triclosan.

DR. BELSITO: Second.

DR. BERGFELD: Any further discussion? Seeing none, all those in favor of not to re-open? Unanimous. Now, the parabens.

DR. MARKS: The parabens was included in that same paper with the triclosan concern, where there were allergens to food sensitization. For all the reasons that we discussed were inappropriate for triclosan, it's similar for the parabens. And then, we had some other articles and, Tom Slaga, I'll let you comment about those.

DR. SLAGA: Yeah, the articles are by the same author. Localization of parabens in areas where the accumulation of these parabens. But the concentrations, the levels were so low even though it correlated where cancer would be, if you will, it really -- concentrations were extremely low. And also, they did a study using an immortalized cell line that was not transformed. But if they put estrogens in it, it would become transformed in a soft auger-type assay. And when they put the parabens in, different ones, the levels that they put in were at 10 to the minus 4 to 10 to the minus 5, extremely high levels which would be way beyond what we would find in cosmetics.

DR. BERGFELD: Any further discussion? Is there a motion to not re-open the parabens?

DR. MARKS: I move that we not re-open the parabens.

DR. BELSITO: Second.

DR. BERGFELD: Second. Any other discussion? None? I'll call the question. All those in favor? Unanimous, not to re-open.

Alan?

DR. ANDERSEN: Did that also include the issue to receive the same level of public presentation or not?

DR. BELSITO: Yes.

DR. BERGFELD: Yes, I think generally speaking both of these fall under that umbrella activity.

[Discussion of Parabens]

JUNE 2017

Dr. Belsito's Team

DR. BELSITO: So, now parabens. So seven ingredients that were previously reviewed, there are four total reports, the last was in 2008, and then being asked to add on 13 ingredients which we have not looked at. So sodiumethyl, this came up because sodium methyl paraben was included in the CIR 2017 priority list based on number of uses. And so even though it has been less than 15

years for many of the other parabens, it's like we need to state it or support it, so let's create this regroup the parabens. So we've done that, and we're now being asked for the data sufficient so support this whole new paraben family. Did I summarize that pretty correctly, essentially? So I guess the first question goes to Dan about the carboxcylic salts or parabens. Do they belong here.

DR. LIEBLER: Yes. I have no problem with including them, because the carboxylate salts, as soon as they hit any kind of biological environment, moisture, any moisture is gonna cause them to be protenated, largely protenated just like the rest of the weak acids, you know, the methylethyl probyl parabens, and so they will be equivalent.

DR. BELSITO: Okay. We have no information on how they're manufactured. Do we need them? Is there anything that you see that could be a concern?

DR . LIEBLER: No.

DR. BELSITO: So you're okay with the lack of method of manufacturing and impurities for the carboxylic

DR. LIEBLER: Right. And actually, these are the phenolate salts, and those will very rapidly protenate in the biological milieu.

DR. BELSITO: What about manufacture? Is there

DR. LIEBLER: Oh, the carboxylate. I'm sorry. The carboxylate salts well the same thing is true. So the table includes the paraben and carboxylate salts, non esters, and then the phenolate salts of the esters. But I have no objection to including them all.

DR. BELSITO: Okay. And does the fact we do not have manufacturing methods for any of the carboxylic materials bother you?

DR. LIEBLER: I think it would be good to have it. The methods of producing these kinds of salts are really straightforward. You essentially just add the corresponding base, the paraben plus calcium hydroxide, the paraben plus potassium hydroxide, et cetera, and that could certainly be gotten from a supplier, I assume, and added to the document.

DR. BELSITO: Right. So we would like the method of manufacture? If we don't get it, would this hold you up? I mean, are we willing

DR. LIEBLER: Not really.

DR. BELSITO: if we clear everything else up, would you go safe and

DR. LIEBLER: Yeah.

DR. BELSITO: Okay. I guess the major issue that I had here in this document, was that, you know, if you look on PDF page 43 under "Dermal Penetration, the sort of working with this group has always been that the penetration was inversely related to the ester chain length, so that methyl paraben penetrated less readily than probyl paraben.

DR. LIEBLER: Say that again?

DR. BELSITO: It says the penetration of the stratum corneum is inversely related to the ester chain length.

DR. LIEBLER: Which page are you on, Don?

DR. KLASSSEN: 43.

DR. BELSITO: Page 43.

DR. LIEBLER: Okay. Sorry.

DR. KLAASSEN: Under Toxicokinetics.

DR. LIEBLER: I haven't looked at that reference. Six. It's probably true, although I doubt that there would be a whole lot of difference between most of these. The butyls is the largest, I think.

DR. BELSITO: Well, except in the NEE data we have, it's exactly the opposite.

DR. SNYDER: Page 6 is a (inaudible) report.

DR. BELSITO: What?

DR. SNYDER: Reference number 6 is (inaudible) report.

DR. BELSITO: I know.

DR. LIEBLER: So that's not a primary reference. So that won't really tell you where that data comes from.
DR. ANSELL: Yes. You'd have to be looking at the 2008 report.
DR. LIEBLER: So we would need to look carefully at that report to make sure that there wasn't something misinterpreted, or what type of study supports that assertion from the 2008 report.
DR. BELSITO: Okay. Because here on page 53 in diffusion cells, it was just the opposite.
DR. LIEBLER: You mean 43?
DR. BELSITO: Fifty three. Now, it's saying the penetration decreases with increasing chain length. So in the Franz diffusion cell, methyl paraben was greater than ethyl, greater than probyl, greater than butyl.
DR. LIEBLER: It's 43 in my docket.
DR. BELSITO: No, it's
DR. LIEBLER: Fifty three is EPI in my
DR. BELSITO: Oh, yeah, summary of new data. Sorry. Yeah. The original is probably 43. So, you know, we're contradicting ourselves here within the document. Yeah, so it's right below where we say it's inversely proportional. Now, it
DR. LIEBLER: So we need to resolve that discrepancy. We need to look at the other report.
DR. BOYLE: Okay.
DR. BELSITO: And then
DR. LIEBLER: But as a chemist, I could explain it either way. So (laughter). Just wanted to give you some confidence.
MS. FIUME: Very easy. You can explain it even better. The smaller the numbers, the greater the penetration.
Kind of like being a lawyer. And since we're close to this, on page 44 under the 1984 report, it says that, "Parabens are quickly absorbed from the blood? By definition that makes no sense. You can only you absorb into the blood. You don't absorb from the blood. I don't know what that's talking about.
DR. LIEBLER: I wonder if they're referring to partitioning from blood to tissue.
DR. ANSELL: Could be.
DR. SNYDER: Where's this
DR. KLASSSEN: That's on page 44 of the report under the 1984 the first sentence, "Parabens are quickly absorbed from the blood."
DR. BOYLE: Yeah, these are basically
VOICE: Quotes.
DR. BOYLE: excerpted as the come in those original reports.
DR. BELSITO: Neither of us were on the panel. We can't take the blame.
DR. ANSELL: Well, those people that were on that report at that time, well, explain them.
DR. BELSITO: So what do we make of the breast cancer studies? I think this is what the (inaudible) issue is now.
DR. KLAASSEN: Right.
DR. BELSITO: Endocrine disruption affects some breast cancer. Paul? Jan? Curt, help me out. So that's page 49 of the PDF.
DR. LIEBLER: So these are in vitro studies in cells. Some of the end points are relevant to cancer, but they're not necessarily predictive of carcinogenicity. So, you know, for example being, "Methyl paraben exhibit increased expression of aldehyde hydrate (inaudible) 1, (marker of human mammary stem cells.)" Well, it's true that, you know, something that could do that could be I mean, that's a characteristic of stabilizing stem cells could be a characteristic of a carcinogen, but it doesn't mean that it's carcinogenic. I was scrolling down to the EPI, and it is substantial epi for breast cancer.
DR. BOYER: (inaudible), right? In the epidemiological study section?

DR. SNYDER: But not for cancer anymore. For endocrine activity, right?
DR. BELSITO: Yeah. Lots. So where are you, Dan?
DR. LIEBLER: Well, I looked through the EPI studies
(inaudible) breast cancer. So anything specific to breast cancer.
And then under the other relevant studies on PDF 50, Endocrine Activity, everything is cell model stuff. Some of it is with NCF 7 cells because these are breast cancer cell
(inaudible). In other words, these are NCF 12A and NCF 10
(inaudible) all breast cancer (inaudible). And they observe paraben driven effects in the micro molar range. On molecular end points like ALB H 1 expression. The effects on mammospheres, which are cellular structures, multi cellular structures that have some organ like properties, but don't necessarily recapitulate (inaudible) an organ.
I don't think any of those would be considered to be predictive of carcinogenic potential unless you were predisposed to think that any effect is a carcinogenic effect. This section actually goes from back and forth between different cell types. I'm trying to remember what BT 474 is. I think those are other I think that's another breast cancer cell (inaudible).
DR. BOYER: I think so.
DR. LIEBLER: I think that's right. And it stimulated proliferation at half micro molar concentration. Again, a pretty nonspecific effect.
DR. BELSITO: Unless you have breast cancer.
DR. LIEBLER: But there are a lot of things that can stimulate proliferation of breast cancer cells in vitro that aren't carcinogenic. I mean, it's, you know, it's just an observation.
DR. BELSITO: Yes, I understand that, but we're not talking okay. So we're not saying that parabens cause breast cancer. Let me just throw this out. But a woman who is applying a nipple cream that is preserved with parabens, and has an introductal carcinoma, does this increase her risk of metastacies? Is this safe under those situations? I guess that's the question I'm asking.
DR. LIEBLER: Those are very clear for phenotype, and the thing is that none of these cell models is a model for addressing the question about the relationship between exposure and that phenotype? If you had, you know, some epidemiologic association, you know, with, for example, a particular subtype of breast cancer, you know, ER positive or triple negative, or something like that, (inaudible) breast cancers, then you'd go to an appropriate model system and ask the specific mechanistic questions. If these are just breast cancer cell lives and, in fact, in the paragraph about the BT 474s, for example, the effect was enhancing
DR. BELSITO: Where are you?
DR. LIEBLER: Oh. On PDF 50, the second paragraph. It's about isobutyl paraben.
DR. BELSITO: Okay.
DR. LIEBLER: So this is actually kind of a mixture of cell models and the narrative kind of goes in out of breast cancer cells lives and other cell lives. So it starts out, "Isobutyl paraben antagonize the estrogen receptor in Chinese hampster ovary cells. The effect was statistically significant at great than 25 micro (inaudible)." In other words, a very high concentration.
"Butyl paraben increased the number of BT 474 cells entering S phase concentration half micro molar. The effect was enhanced in the presence of ligand heregulum which is a stimulator of the EGF receptor, or it's a possible stimulator of the EGF receptor."
And then glucocoticoid like activity was 1.5 milli molar for butyl paraben, and 13 milli molar for propyl paraben. These are very high concentrations.
I mean, this is just kind of one off cell, throw in a chemical, make measurement some end points, and this is the type of thing I rail against all the time on this panel when we get data like this because it really doesn't mean anything.
DR. BELSITO: Okay.

DR. LIEBLER: Just throwing in chemical into particular cell lives, and you're observing something, and you put it in a low impact journal.
DR. BELSITO: Okay. So you'll write the defense in the discussion?
DR. LIEBLER: Sure.
DR. BELSITO: And will craft the defense
DR. LIEBLER: I will, sir.
DR. BELSITO: why we're not concerned about the effects on
DR. LIEBLER: Yeah.
DR. BELSITO: breast cancer. The other thing that I found that was sort of just not logical to me was this in Haines study, and the association with (inaudible) and some food sensitizations where the effect was seen only for ethyl paraben, but not for any of the other parabens.
Can anyone come up with an explanation other than it doesn't make sense?
DR. LIEBLER: It makes little sense.
DR. BELSITO: Yeah. I mean, why would ethyl paraben create a respiratory issue when methyl, and propyl, and butyl don't? So this was looking at data, and looking at urine parabens, right? That's where they got urinary concentrations. I'm looking for an association between
(inaudible) allergen and food sensitization or both.
DR. BOYER: This is another study like many
(inaudible) studies where they're really looking for associations between many different things, and they looked at 35, 40, 50 possible associations, and just by chance you'd expect at least some of them to show up as statistically significant.
So it could very well be that that explains why sometimes (inaudible) to tox out like this. It's just chance.
DR. ANSELL: Yeah. For these really data rich chemicals, you really need to rely more heavily on a weight of evidence approach. You know, if you look at a 95 percent percentile significance, and you measure 20 parameters, one of them is going to show a statistical relationship, and I think in the parabens if I'm not mistaken, we often see that. We'll see a statistical significance on the use with a paraben that isn't even used in those products. You just have to aggregate it, (inaudible) together to try to clarify the picture.
DR. BELSITO: Okay. Explain this (inaudible).
MS. LORETZ: And kind of along the same lines, one suggestion we had was two add for hydroxybenzoic acid to the report. It does have an inky name. It's not used by itself so much, but it is common metabolite, and it kind of gets at that question why would be (indiscernible 4:40:59:). I've used it. It wouldn't just be (inaudible)). There is a common metabolite.
So we think that's kind of is important to it makes more sense of the data then.
DR. LIEBLER: Okay.
DR. BELSITO: So do we want to add
DR. LIEBLER: That's fine with me.
DR. BELSITO: Okay.
DR. LIEBLER: I saw the recommendation. Seems reasonable. Other uses?
MS. LORETZ: No.
DR. BELSITO: No. It's not a cosmetic chemical.
DR. LIEBLER: Oh, it's not a not
MS. LORETZ: No, (inaudible).
DR. LIEBLER: Hasn't anything in it. Okay. But there are no uses. But there are data.
MS. LORETZ: Yes.
DR. LIEBLER: Okay.
DR. BELSITO: So the do we need to address the new data also on the thyroid effects? I guess this goes to Paul or Dan.
DR. LIEBLER: This is on page 50 at the end of the endocrine activity section?

DR. BELSITO: Yeah.
DR. ANSELL: It's in the 26th healthy paragraph?
DR. BELSITO: Uh huh.
DR. ANSELL: Well, it ends up there. It says the differences could not be attributed to the treatment. Can someone elaborate a little bit on that?
DR. BOYER: In the way this study was done, for the first week, the subjects were treated with the ointment, with the lotion without the parabens in them, in it, and the
(inaudible) hormone levels were measured in the blood samples. And during the second week, during that daily treatment, a full body application of the ointment with the parabens, again they generated that sort of data, and statistically that could we tell the difference. And there's such a variation from day to day, and hormone levels, and so on, even from hour to hour that there was no way to attribute any differences specifically to the exposures.
DR. KLAASSEN: Okay. So this is really talking about the minor differences.
DR. BOYER: Right. I think the were we were talking about differences. They weren't particularly statistically significant, and they were just simply pointing out that there were these minor differences, but they couldn't explain them.
BELSITO: Okay.
DR. KLAASSEN: I guess I think maybe that needs to be reworded a little bit. I don't know. It almost you know, while it says, "minor differences," I guess that's the tricky word in the whole paragraph is that minor differences I mean to me when they say the word, "differences," it is statistically different.
DR. BOYER: And in this case, they used the word that's their word, "minor," and it to them means that they weren't statistically significant, but they were pointing out they were indicating that their data showed some differences.
DR. KLAASSEN: I think maybe we need to put something in there, "minor differences, however, not statistically significant." Could be if they used the word, "differences," I'd want to use the word, "differences." You might say there was a trend or something, but, yeah, go ahead. You know, in a parentheses, "not statistically significant That would make that paragraph much
DR. BELSITO: Are we sure that they were not statistically significant?
DR. BOYER: I'm positive, yes.
DR. BELSITO: Okay. Okay. So getting back to the addition of the carboxcylic salts, we have absolutely no data on them. You're comfortable with read across from everything else?
DR. LIEBLER: Yes.
DR. BELSITO: Okay. And you're going to draft the
DR. LIEBLER: Couple of sentences on the in vitro well on the endocrine effects of the parabens. It's mostly cell model down at least what's cited here
DR. BELSITO: Right.
DR. LIEBLER: except for the thyroid, thyroxin stuff we just talked about.
DR. BELSITO: Right.
DR. LIEBLER: But for all the cell model stuff, I can draft a two or three sentence section for the discussion and send it to Lillian.
DR. BELSITO: Okay. Then on page 84, or did I just tab it there? Anyway, in the report where you had this whole margin of exposure calculation, it's on page 105 of this report. I guess I flagged it on page 84. So based upon the new data, do we need to recalculate this margin of exposure table?
DR. KLAASSEN: Well, it was based on the (inaudible) for single, and (inaudible) for multiple, right?
DR. BELSITO: Right.
DR. KLAASSEN: If that still holds, it's still valid.
DR. BOYER: Well, it's also based on a NOAEL of 1,000 milligrams per kilograms per day.
DR. BELSITO: Right.

DR. KLAASSEN: And the Hoberman paper that was considered back in 2008
DR. BELSITO: Right. So does our need data change our NOAEL for any of the endocrine end points, or repro end points, or breast cancer end points, or any end points.
DR. BOYER: And the Women's Voices for the Earth comments in particular, they pointed out specifically a study by Bolberg in 2016, which has been incorporated into the safety assessment report. It's an old study done with rats, and they are reporting that for end points like distances and so on, there is an effect of 100 milligrams per kilogram per day. And they're also they also reported that there are some effects on a male that the parameters down to 10 milligrams per kilogram per day. And they also reported that there are some effects on a male reproductive parameters down to 10 milligrams per kilogram per day.
And, in fact, the SCCS opinion that did a similar calculation before the CAR did their calculation, they more or less dismissed the Hoberman study. They didn't use the 1,000 milligrams per kilogram per day. They used a older study that was published by OEC that indicated again based on some effects, did not necessarily consider the adverse effects on male reproductive organs, that the NOAEL should be something like 2 milligrams per day, grams per day. So that's what they used in their calculation is close to 1,000 milligrams per kilogram per day.
So the question really is if you take into consideration the Bolberg 2016 paper, does that provide enough motivation to shift the NOAEL using these calculations from 1,000 down to 10,000 down to 10 milligrams per kilograms per day, or even down to 2 milligrams per kilograms per day?
DR. BELSITO: That was my question.
DR. BERGFELD: It's a big change.
DR. KLAASSEN: Where what page is that study described on?
DR. BOYER: It's actually (inaudible). I think it's page 54.
DR. BELSITO: Yeah. That's where I put my note, I think. Page 84,
DR. BOYER: Page 84, yes.
DR. BELSITO: It's Table 12.
DR. BOYER: If you look at the last column under that entry, and the second paragraph if you look at the last column on that entry, the second paragraph, that pretty much summarizes it. Identifies the end points that were deemed to be statistically different at the 10 milligram per kilogram per day dosage rate.
DR. BELSITO: But, in fact, there was not a NOAEL at 10. Effects were seen at all doses, so it's a LOAEL.
DR. BOYER: That's true, yes.
DR. BELSITO: So the last time that we reviewed this, we were concerned and we calculated the margins of exposure and came out with levels of 1,000 or greater for adults and children. And so my question to you is based upon this new data, do we need to recalculate that and look at this before we sign off on the parabens?
DR. LIEBLER: Unless there's a flaw in the study, I don't think it's anything we can ignore.
DR. BELSITO: I'm sorry. Unless there's a flaw, there's nothing what, we can ignore?
DR. LIEBLER: Unless there's a flaw in the study, I don't think we can ignore this.
DR. BELSITO: So then we have to do the recalculation?
DR. BOYER: What study specifically are we looking at here?
DR. LIEBLER: Table 12, the first entry. Butyl paraben (inaudible).
MS. BECKER: Reference 59.
DR. KLAASSEN: Table 12.
DR. BELSITO: Here we go. Okay. Search for CYP19A1 is probably the quickest way to get to it.
DR. LIEBLER: He's got it.
DR. SNYDER: And then again, there's lots of data there. The only thing that was altered at 10 was the sperm counts, and sperm counts are not considered to be a very sensitive are considered to

not be a very strong parameter for effects, epididymal sperm counts, and so there were effects, but they were all in 100 or greater. Even that's less than 1,000, I guess, so

DR. LIEBLER: I'd like to see that paper, and look at that reference. They say epididymal sperm counts were statistically significantly reduced at all dosages.

DR. SNYDER: Right. So we even include (inaudible).

DR. BOYER: But I guess the issue is whether or not these end points that are identified in the second paragraph, whether or not those are whether those represent effects as opposed to adverse effects. So are we defining no effect level versus a no observed adverse effect level? And that is actually a discussion that you'll see in the literature

MS. LORETZ: Just to mention too, there's more studies than just the Hoberman study that didn't show effects, although, of course, there are slightly different particles, or in some places quite different particles. So there's the weight of the evidence here on some of these results.

DR. BELSITO: For negative studies.

MS. LORETZ: Yeah.

DR. KLASSSEN: How many negative studies does it take to reverse a positive study?

DR. BELSITO: I mean, Curt's point is right on. I mean, usually you use weight of evidence when you have no data on a specific material, and you're using a read across material, or you have a little bit of data that's negative, but you want some supporting material, you don't use weight of evidence to say, oh, that positive study is negative because I have three other studies that are negative.

DR. KLAASSEN: Right.

DR. BERGFELD: But usually mammalian outweighs AMES.

DR. BELSITO: This isn't genotox. This is reproductive tox.

DR. BERGFELD: Oh.

DR. BELSITO: And I just throw it out. I mean, because the last time we justified our lack of concern about any risk factors based upon marginal exposures that were calculated for adults and children, and I don't think we cannot not do that again, particularly in light of new this new data, and then the question is how do we it? I mean so, basically, even if we went to a LOAEL for this study, we're going from 1,000 to 10,000. So we're reducing all of those numbers in the margin of exposure by a factor of 100, in which case we're getting down to below it's on page 105 of the PDF, I think.

So we're getting down to margins of exposure reduced by 100 fold to 59.29, multiple parabens 8, not giving us very good margins of exposure there.

DR. SNYDER: Well, I can pose (inaudible). Here it says that the epididymal sperm counts were significantly decreased in all those groups, compared with controls. Histologic examination of the testes and epididymus which as put forth is considered, I believe I'm not a reproductive expert, but I believe I've heard in many, many discussions and summarized that the histology is way more a strong indicator of toxicity in sperm counts because of the things that discussed already.

And Curt, it says here that histologic examination of testes and epididymus and control of high dose show no difference between (inaudible). So I think it's probably an over interpretation of the data. In light of no histologic evidence, I'm not certain how strong or how much weight you can put in sperm counts, epididymal sperm counts.

DR. LIEBLER: And they also refer to the expression of this swarthily Ludwig cell marker NR 5A1.

DR. KLAASSEN: You know anything about that?

DR. LIEBLER: Nothing about that.

DR. KLAASSEN: It must be Stanford nuclear receptors. I don't know any of that. I found that interesting, but I didn't look it up.

DR. BELSITO: And just refresh my mind. The EU has recently changed their paraben regulations for probyl and isopropyl, right. They've reduced them in combination to like.4.

DR. BOYER: It was reduced from.4 to.19.
DR. BELSITO: Okay. For probyl and isopropyl?
DR. BOYER: Yes.
MS. LORETZ: Actually, it's probyl and butyl. Isopropyl they didn't go ahead and update it, so (inaudible).
DR. BELSITO: So probyl plus butyl with ethyl and methyl still staying
MS. LORETZ: staying at the yeah.
DR. BELSITO: at.8 or.4?
MS. LORETZ: At.4,.8 combination.
DR. BOYER: .4 for the combination, and.8 for single?
DR. BELSITO: Right. .4 for a single except for probyl and butyl which was.2 for a single?
MS. LORETZ: 19.
DR. BELSITO: .19. And that was based off of endocrine effects as well, right?
DR. BOYER: That was actually based on the DART study, the Nishi paper.
DR. BELSITO: Right.
DR. BOYER: And it's based on that NOAEL well, actually not NOAEL, no effect level of 2 milligrams per kilogram per day.
DR. BELSITO: Right. But repro.
DR. SNYDER: Right.
DR. BELSITO: Developmental and repro.
MS. LORETZ: Just a minor correction. Actually, they kind of rejected the Nishi studies, and they used another study, and the reason there was two was is that was the only dose level tested. And it was actually it was dosing not by dermal. It was subcutaneous. At the time, they didn't like either the Nishi studies or the Hoberman study, and, therefore, they said so this is what we're going to use.
DR. BOYER: Okay. We'll check on that, but my understanding was that they settled on the Nishi paper, one of the Nishi papers just simply to take a precautionary kind of approach for doing this calculation.
MS. LORETZ: I agree that they took a precautionary but I (inaudible).
DR. BELSITO: I think for many reasons, we need to be very, very careful with this document. I mean, it's not just Women for Earth, or whatever their group is. There are a huge number of NOGS, and public, and manufacturers who are very concerned about the safety of parabens, and I think that we need to be very grounded in our decision, and be able to justify it very, very clearly. So, I mean, I think that in the end it comes down to what we're going to do with these margin of exposures based upon the new data we have and how we're going to handle that.
DR. LIEBLER: I think we might need to get some input from somebody more expert in the use of these in the relative value of the end points that were used in this rat study. I mean, you know, if Paul feels comfortable with it, you know, and has more chance to review this carefully, he may be fine, but if Paul, if you have any concerns
DR. BELSITO: Guaiacum?
DR. LIEBLER: That's who I'm thinking of.
DR. BELSITO: Yeah, me too.
DR. LIEBLER: It's a colleague of ours on the expert
(inaudible) panel.
DR. BELSITO: Yeah. He's from Germany, from Hamburg. He's an incredible reproductive toxicologist. I think it might be good to table this, and ask him to review these studies, or review the whole issues of paraben and reproductive toxicity and address the panel.
DR. KLASSSEN: Another excellent person would be Paul Foster down at NIEHS. So what we're really talking about here is an environmental estrogen. Right?

DR. BELSITO: Right. Using the broad definition of environmental to include (indiscernible 4:01;34) exposures, but, yeah.
DR. KLAASSEN: So, in essence, he's kind of like taking a oral contraceptive drug?
DR. BELSITO: Well, except the effects seem to be more in male than female.
DR. KLAASSEN: But that's why we're seeing this is kind of decreasing the maleness of a male. All right.
DR. BELSITO: Right. Well, no. But there is epidemiologic data, I believe, that there is there is increasing incidents of hyperspatius among male children being born in the United States. There's a lot of that data, and then there was data on chemo to paraben levels in women of child bearing age too, wasn't
MS. FIUME: (inaudible).
DR. BELSITO: Yeah. I mean, so there's a lot of anecdotal data, you know, just like the phthalate, and adipose tissue increasing and all of that.
So I mean, it's a real hot button issue without clear answers, so I think we need to be as scientifically rigorous as possible. So, I mean, this guy that he's a repro tox person?
DR. KLASSSEN: Oh, yes.
DR. BELSITO: And, I mean, he's certainly closer than Hamburg, Germany and might be
DR. KLAASSEN: Well, two.
DR. LIEBLER: I think we talked to both of them.
DR. KLAASSEN: That's what I was thinking.
DR. LIEBLER: Yeah.
DR. BELSITO: Okay.
DR. LIEBLER: I mean, we know judging, you know, from our experience and working with
DR. BELSITO: Yeah.
DR. LIEBLER: he's excellent, and has really got broad knowledge, and he's got a great sense of what the relevance of different model animal model end points would be to possible exposure effects, and that's really important in interpreting, you know, from these studies in rats, for example, and but I think we get too reads from outside experts and be important.
DR. BELSITO: Okay. So my recommendation would be to table this, and to invite two different experts in reproductive and toxicity, specifically, to review with us the data that's available on parabens, and how we can interpret that in terms of safety as used in cosmetics.
DR. LIEBLER: Right.
DR. KLASSSEN: One of the problems with this is that what can you add (off Mic.). Correct?
DR. SNYDER: But we do have other repro studies. We discussed this before (inaudible) discussion before, there was another study with trimethylpental isobuterate where there were minimal reductions in sperm counts in the testes or epididytimies of treated male rats, but there was no treatment related growths or microscopic lesions, and no effect on reproductive performance. So I think it's the same story.
I think the sperm count thing is not a very good indicator because there's so many things that could affect that outside of toxicity. And so if all other parameters are normal, particularly gross and microscopic examination, and reproductive performance, I think it has to be kind of taken very, very lightly, and as a direct effect of the chemical that's been applied.
So I think that's what this what we need to ask the experts, but I'm pretty certain that's what's going to be the the bottom line on this.
DR. BELSITO: But it would be nice to have the expert explain it.
DR. LIEBLER: Yes, I agree. Well, because it is a very high risk use so we need to go to somebody who is considered a reproductive expert. So I'd like to hear more about this Swarthily Ludwig cell marker in our 5A1. I've looked briefly online, and I saw a series of there was at least ten references to that as a surrogate marker for Swarthily cell differentiation, and it's a apparently,

it's a it's a transcriptional regulator, and its expression is related to the downstream that are known to regulate differentiation of Swarthily cells.

But I don't know how reliable this is in different species, and what are the corks of using data based on this, so that's something that our experts can help us with, but that's one of the ones that was effective at all does in addition to the sperm counts.

And then there was also the issue of just the inner general distance measurements were affected at 100, and 500. So there is an adverse effect at 100. And so the next lowest dose is a 10, so that puts us back to 10 with these data, so again, I'd like to get (inaudible) know anything about interpreting that, but

DR. SNYDER: (inaudible) effective 10. That's not I mean, could be two.

DR. BELSITO: You can get that effect at 100. So that's what I was wondering about.

DR. BERGFELD: So my understanding is if these two people are cited and asked to come, they would have all the information ahead so that they could form an opinion ahead?

DR. BELSITO: We would provide

DR. BERGFELD: Yes.

DR. BELSITO: I would hope that we would provide them with all the information currently (inaudible) We would hope that they would provide us with all the information that are currently in these reports, in the old reports, and ask them if they were aware of any information that has not been included, or that might be relevant, and to present to us their opinions based upon scientific basis given how these are used in cosmetics in terms of their safety, margins of exposure for reproductive and developmental end points.

So basically, asking them almost like as adjunct panel members to weigh in on this issue.

DR. ANSELL: The issue of the specific paper, or the issue of

DR. BELSITO: The issue in general of parabens for reproductive and developmental toxicity as used in cosmetics based upon all the information that we have looked at over the many years we've reviewed parabens, plus any information that they may have that is not in our report that should be.

DR. BERGFELD: I gather that also they would have an opinion on the studies that we've quoted

DR. BELSITO: Right.

DR. BERGFELD: and the validity of those studies as well?

DR. KLAASSEN: Yeah.

DR. BELSITO: Yeah.

DR. KLASSSEN: Especially this one.

DR. BERGFELD: Okay.

DR. KLAASSEN: And especially this one.

DR. LIEBLER: So basically, external consultants.

DR. BERGFELD: Right.

DR. BELSITO: No.

DR. BERGFELD: Okay.

DR. BELSITO: You know, tasked essentially with looking at all of the data we have, plus any data they know, and in terms of, okay, here's how those are used, and in terms of, okay, here's how these are used in cosmetics. Can you weigh in on their relative safety, and what the margins of exposure would be based upon your opinion as to the NOALEs for the various parabens we're looking at.

And if you're discounting the NOAEL of 10, you know, is it the way Paul argues that, you know, sperm counts are not what you look at. You look at histology of the testes. Those were fine, so, you know what I mean, there are just too many things that can, you know, affect the sperm count other than a toxic effect on the chemical which you really want to look at and see what is happening.

DR. BERGFELD: I don't think we want this in printed form from these experts as well?
DR. BELSITO: Yeah, of course?
DR. BERGFELD: Something we can reference as unpublished documentation?
MS. FIUME: I was going to ask if you wanted it in written opinion, or in presentation.
DR. BELSITO: I think both. I mean, we would ask for a slide presentation with copies of their slides and opinion. But I think we need it for this. I mean, it's
DR. BERGFELD: Do you think it's necessary to pose some questions? It would seem to me that questions have come up during this conversation.
DR. BELSITO: Yeah, I mean, the questions are when you looking I mean, I think the questions that I've heard are Paul' questions, you know, are sperm counts what you look at, or is it histology of the testicle? And the other question is, you know, what is the NOAEL or LOAEL for these various parabens for reproductive and developmental toxicity as you read the literature.
And then once we have that, we can plus those numbers into our margin of exposure tables and see if we're comfortable.
DR. ANSELL: I'm just concerned that the scope is still a little fuzzy. If we're asking them to undertake a comprehensive review of the literature as it relates to reproductive effects of parabens, that's quite different than looking at the time papers which have been cited since the last review which would be very discrete. If we are interested in repro, then we're going to have reopen all the epi studies that my be relevant. I mean, it's just I think we just need to be ways are focused in terms of what the request is, not overwhelm these poor guys with a critical review of 50 years of reproductive toxicology.
DR. LIEBLER: On, I think that you can address this by providing them with the papers that we're currently considering, and also you could provide them with the previous reports with also cite, and you can highlight for on something highlight the papers (inaudible) cited.
And that's actually not a really big body of literature, and it focuses and we could provide them with questions regarding what is the, first of all, the assessment of the data of the base on which NOAELs or NOAELs are taken> And then what would they conclude in terms of NOAEL/LOAEL from the available literature, and are there reasons to include or discount any of the data that we're considering? Are there flaws in any of the studies that we're that we need to consider?
DR. BERGFELD: Three questions, basically.
DR. LIEBLER: Yeah.
MS. LIUME: And that does seem to be consistent with what has been going, and researching what Ivan looked at, what Europe looked at, and the papers presented to you all seem to be totally in line. I don't think there is any outstanding information that was true where and then if we focus it as Dr. Liebler said, it should get to the root of what you're looking for.
DR. BELSITO: Right. Okay. So Table (inaudible) some experts to give us a presentation, and a
MS. FIUME: Written opinion.
DR. BELSITO: a written opinion.
DR. FIUME: Before we (inaudible) the table and leave. I just want to check with Ivan. I know we had received comments from both industry and Women's Voices for the Earth. Did we miss anything that needed to discussed
(inaudible)?
DR. BOYER: I think the one other issue or suggestion was that we considered some biomonitoring that data, including more biomonitoring data. There's a very rich literature out there, oh, and studies that measured urine and carbon concentrations, and so forth.
And the council recommended that several references they would take a closer look at, and they would bring some
(inaudible) in scope, (inaudible) data from, (inaudible) data from those from those reports, and (inaudible) do that, but we're going to probably have to be very limited in scope as we attempt to

do that because there's just so much out there, and a lot of it may not be relevant, is not likely to be relevant specifically to exposure to parabens through the use of cosmetic products.

DR. LIEBLER: Sure. And I think that one of the issues that was raised in a letter from Alexander Scranton from Women's Voice for the Earth opposed the issue of parabens accumulating in breast tissue, which to my understanding, and I think you find out your draft response is that it's not that's commonly understood to mean more over time with more exposure over time.

DR. BOYER: Right.

DR. LIEBLER: And as opposed to just detecting the presence of parabens in a tissue specimen they get to analyze. And I think that we need to address the question of bioaccumulation because I think just detecting the presence of tissues, then we'd need to be very careful to try and restrict it to exposures that might be relevant to cosmetic ingredients, and address the question of whether it piles up over time.

DR. BELSITO: No, I don't think it does, because I thought one of the criticisms of measuring urinary parabens is they can vary from day to day, and that they don't really tell you about quantitative exposure over time. They tell you about what's happened in the last 24 hours.

DR. LIEBLER: Right. You need a longitudinal study

DR. BELSITO: Right.

DR. LIEBLER: to assess bioaccumulation.

DR. BELSITO: Right.

DR. BELSITO: The presence of the material in the tissue, or in biofluid is a separate issue and doesn't necessarily mean there's accumulation.

DR. BOYER: But I think there's a point of it is to a large extent a matter of semantics.

DR. BELSITO: Right.

DR. BOYER: It's a matter of how these trends are defined, and (inaudible) explicit about that.

DR. BELSITO: Okay. Anything else? (No response.) Anything else? (No response.) Biotin.

DR. KLAASSEN: Two tens.

DR. BELSITO: What?

DR. KLAASSEN: I thought you said buy a ten. I said two tens.

DR. BELSITO: I'm still not following it, Curt. I guess I'm a little punchy.

DR. KLAASSEN: Okay.

DR. LIEBLER: As opposed to uniten?

DR. BELSITO: Oh.

DR. LIEBLER: Kansas humor.

DR. KLAASSEN: It's getting light in the head after eating all those parabens. (Laughter).

DR. BELSITO: Okay. So 2001 we looked at this, issued a final report, and it was safe as used in cosmetics. There are no data proposed for inclusion. Is there absolutely any reason why we're desperate to add it, and I thought not unless Paul was concerned about the sperm studies. (Laughter).

DR. LIEBLER: (inaudible).

DR. BELSITO: You know, I guess the answer is

DR. SNYDER: No.

DR. BELSITO: no. Okay.

DR. LIEBLER: I concur.

DR. BELSITO: Okay. So we're not reopening.

Dr. Marks' Team

DR. MARKS: I'll first start with the May 19th memorandum from Ivan and Lillian with the subject "Re review of Parabens" and they said the Panel already agreed to reopen, so I take their word on it

for reopening this. And that's one bad new ingredients and then secondly, that assess any updates on that.

In 2008, the Expert Panel published a conclusion that seven parabens were safe. In this memo, it was proposed at 17 new ingredients, particularly sodium methyl paraben, et cetera. I think the assess updates would be relevant to addressing endocrine concerns in infant skin and then we received a June 12th memo from Ivan and Lillian concerning, one, Council suggests adding four hydroxaben, zoic acid, and they give reasons for that. The Council suggested recommending expanding the literature search relevant to exposures to parabens, including those not specific to cosmetic use. And then there was letter from Newman's Police for the Earth and Ivan and Lillian have summarized the responses to that, which were five responses. Very nice summary and then the letters relevant to those comments of

(inaudible). Let's start out with I guess now, we're up to 18

ingredients, so let's first start with the initial 17 we already saw and came to this meeting. Are there any concerns about adding those 17 new ingredients?

DR. HILL: No. MAN: No.

DR. MARKS: Okay.

MR. STEINBERG: I have a comment.

DR. MARKS: Sure.

MR. STEINBERG: First, we don't use para acid. It has no basis for use in cosmetics because the only way it functions is a preservative below a ph. of about two and half. And that ph., it's not an issue. I can preserve it almost blindfolded without putting anything in because it's so hostile. The second thing is, if you're going to have para if you're not going to use para acid as an ingredient, you're not going to use the source because it has no function then. So I don't know if you're adding I don't know how many different variations on it for ingredients that are never used.

MS. EISEMAN: For some reason, there is one report, sodium paraben.

MR. STEINBERG: I think it's a mistake.

MS. EISEMAN: Oh.

MR. STEINBERG: Because it's not commercially available. You do use sodium methyl parabenate . That's very commonly or more common

DR. MARKS: (Inaudible) difference.

MR. STEINBERG: It's a way to dissolve the parabens in water and then adjust the ph. and you get the methyl paraben because sodium methyl paraben is very water soluble when methyl paraben is not. But sodium I think that's mistake, that they just didn't know what they were doing because sodium para hydroxymandelic acid is just not a commercially available product. No one makes it.

DR. EISENMANN: We just thought it doesn't make sense to include the salts of parabens and not pentraxin benzoic acid itself. So if you're not going to include the calcium

MR. STEINBERG: Yes.

DR. EISENMANN: Potassi

MR. STEINBERG: If you're not going through the acid, then you don't include the salts in the acid.

DR. EISENMANN: Well, right now, the salts are in.

DR. HILL: No, they're not.

DR. EISENMANN: Yes, they are.

MR. STEINBERG: the salts of the esters are.

DR. EISENMANN: No, no. Calcium, paraben, potassium, paraben

MAN: Oh, yeah.

MR. STEINBERG: But that by definition

DR. EISENMANN: those three are in.

MR. STEINBERG: are the salts of the ester, not the salts of the acid.
DR. EISENMANN: No, by definition in the dictionary, they're
MR. STEINBERG: Then the dictionary is wrong.
DR. EISENMANN: salts
MR. STEINBERG: Then just the chemistry is wrong in the dictionary then.
MS. EISEMAN: Well, we have sodium methyl paraben is in there.
MR. STEINBERG: That's correct. That's correct, but sodium parabenate is not. We don't use that ingredient.
DR. EISENMANN: Sodium paraben right. But that's in the dictionary and that's in the report.
MR. STEINBERG: It makes no sense. You have a whole group of things which are just not used. Has no function whatsoever. It's not commercially available.
DR. EISENMANN: My feeling is if you include the salts of para I mean, sodium, calcium and potassium paraben, you would need to include pentraxin benzoic acid also because it's in the dictionary.
MR. STEINBERG: Well, we haven't gotten to that point yet.
DR. SHANK: It's a metabolite.
DR. EISENMANN: But and it's a metabolite of the esters.
DR. MARKS: That's why.
DR. SLAGA: Yeah, it's a metabolite.
DR. SHANK: So it should definitely be in there.
MS. EISEMAN: My original advice was if you don't include it in, it should at least be a search term because it's a metabolite of the esters.
DR. SLAGA: Right.
DR. MARKS: Oh, we're back to (laughs) David your comments are noted.
DR. MARKS: Team, do you want to include now, would be 18 instead of 17, do you want to do all 18? In the past, even though the dictionary may not be whatever, they're listed in the dictionary and they include them if they're in the dictionary unless there's a reason
DR. HILL: yeah and it's the metabolite and I agree. They should be down.
DR. MARKS: Yeah, but that's the one from the memo
DR. HILL: Yeah.
DR. MARKS: we just received. How about the previous 17? They're on this list. Is there any reason not to put them all on?
DR. HILL: If they're in the dictionary
DR. MARKS: Yeah.
DR. HILL: I would include them and then if there's a problem with one of them that can be, you know, discussed.
DR. MARKS: Okay. So we would ass in this case, sodium methyl paraben et cetera and it'd be a total of 18 new ingredients including
DR. HILL: Paraben hydroxyl, pentraxin benzoic acid
(inaudible)?
DR. MARKS: Yeah. Yeah, that's the four hydroxyl benzoic acid?
MR. STEINBERG: It's the starting material.
DR. MARKS: For
MR. STEINBERG: Its' also a metabolite.
DR. SHANK: Yeah.
MR. STEINBERG: When you got a few hydrolyzed methyl, the (inaudible) esters, that's how you would generate it, but
DR. SHANK: Okay.
MR. STEINBERG: we don't deliberately add
DR. SHANK: No.

MR. STEINBERG: a para acid.
DR. SHANK: Now, from a toxicology point of view, I think they're absolutely right. We should include that.
DR. MARKS: Okay and then I guess there was
DR. SHANK: Maybe you don't list it as paraben. You do consider the toxicology for hydroxyl benzoic acid.
DR. MARKS: Then would you change the title?
DR. SHANK: (Inaudible)
DR. MARKS: Parabens and four hydroxyl benzoic acid?
DR. SHANK: No. The review is in parabens.
DR. MARKS: Okay.
MR. IVAN BOYER: A lot of the literature that we pulled up includes studies that address multiple parabens, multiple ingredients and so forth. Some that are, in fact, aren't even listed as ingredients and often enough, that metabolizes included as well. So, the literature search has already brought forward some of that information. It's just that we didn't emphasize it in this particular draft of the (inaudible).
DR. HILL: Yeah, but you're right. It's there pervasively and some of the previous reports, discussions of that activity.
DR. MARKS: Is it going to change anything if we hear from Riffin that's it's a fragrance ingredient?
DR. EISENMANN: I doubt that you'll hear from Riffin. It's a claimant's ingredient.
DR. MARKS: (Inaudible).
DR. SLAGA: It's a metabolite. So it doesn't matter.
DR. MARKS: Okay.
MS. FIUME: I think the only difference would have been is to whether or not it's included as an ingredient in the review of the data were included without naming it as
(inaudible) the
DR. MARKS: That's sort of why I brought it up. It's an ingredient technically. If it's a fragrance, we shouldn't be reviewing it. Doesn't preclude having it in the document itself, but it wouldn't be one of the ingredients we make a conclusion on. Okay.
DR. HILL: And it isn't being used as a fragrance because it has no smell to speak of. It's if it's being used and that's actually Beth's memo here in what we got to base. Unlikely to be used to impart odor. It's probably there in a preserving function of some sort.
DR. MARKS: Okay, I think that ought address most of the comments from the Council. Team, any comments about
DR. EISENMANN: Our other comments
DR. MARKS: and that's what I'm going to. Number two, are we in?
DR. EISENMANN: was for the exposure, yes.
DR. MARKS: Because that was what I was
DR. EISENMANN: Because it's important some important studies, they're not in there. And one of them is this PBK model that was done by Harvey Crull's group that look at the in vitro concentrations that cause estrogen receptor. And then modeled it up and compared it to the endings. And they did sign an MOS for a combined three parabens of a hundred for men and four hundred for women. So that's important that they, not only did individual parabens, they did a combination of parabens. And they used the end Haynes , so it's not just cosmetic exposure, it's total exposure.
DR. HILL: My impression in reading all of this stuff and from the previous time when we looked at this and kept it to bed is the whole estrogen thing is a red herring. There are other biological effects with some of these, have nothing to do with estrogen. And that, that whole thing is a red herring, period. Unless with benzoic acid, you'd hydrolate that other benzene ring and then you have

something that's highly likely to have you look at the mechanism of action in combining the estrogen receptors.

If you've got enough scaffold in between and hydroxyl groups at the right distance, you can get high affinity binding to estrogen receptors. And I think two things about it. I think they're still a red herring, but I don't think the metabolites that could potentially have potent estrogenic action have never actually been looked at. Or if they have, I haven't found it. So that's something that needs a little more attention. That may have a lot to do with why the benzoate is essentially disappeared from use.

DR. BOYER: You have to go to the comment from the Council that the lurch for search be expanded to include biomonitoring data and so forth. There is a lot of data out there. It's a huge literature. There are lots of methods that have been implemented and there are there's a lot of data on parabens and urine samples and blood samples and tissue samples and so forth.

For many of these studies, the focus is not on carcinogenic exposure. Exposure to parabens is really the use of cosmetics. And so I guess the question for the staff would be if we're going to expand I can understand expanding the exposure and part of the safety assessment to include the pharmacokinetic model that Kapal just mentioned and maybe we can include some additional papers that were brought forward. They were identified in some of the comments that we received as well. But Enhaines again, does not focus specifically on cosmetic exposures. And the question

DR. EISENMANN: But it's the large populations I think is useful because I I'm reading your the conclusion from the last report. You were concerned about total exposure. At least that's the impression that I got.

DR. BOYER: That's right.

DR. EISENMANN: So I'm not saying Enhaines I mean, you can't put it all in.

DR. BOYER: It's huge.

DR. EISENMANN: Of course, it's huge. But, you know, a few 95 percentiles of can you see any trend because it's been they've been measuring it for a while. So I understand you can't put it all in, but I think you could probably put in, you know, say that it's there; where it can be found; maybe a few 95 percentile

DR. BOYER: That's perfectly doable.

DR. SHANK: That's a paragraph in the discussion, but an important one.

DR.BOYER: Right.

DR. MARKS: Would you repeat

MR. STEINBERG: As opposed to a full blown search for paraben data.

DR. EISENMANN: but there's a few other key ones I think you need to put I don't think we can I know there's a study you probably have heard of it. The Hermosa

(phonetic) in California where they gave they measured parabens in the urine of teens before they were before the start of the study. And then they gave them products without personal care products without parabens and then measured their values again. I don't think you can ignore that study because again, it was personal care products.

And I don't I'm surprised women's voices

(inaudible) didn't mention that study too.

MR. STEINBERG: Did they bring out the subjects by ethnic?

DR. EISENMANN: I think they were probably mostly Hispanic subjects.

MR. STEINBERG: The reason I'm asking, okay, this came up when Darby first (inaudible) published her paper and I was questioned about the use of parabens in foods. And we don't use parabens in foods in the United States. Even through it's approved for I don't know how many different applications, parabens have one major drawback for use in foods. They anesthetize of taste buds and that's not a good thing for foods.

There is one significant food use of parabens except we don't use it in the United States. It's limited to one country and that's Japan. And Japan uses parabens to preserve soy sauce which they inject by the gallon. So that's why if they are of Japanese origin, they might be using Japanese soy sauce.

DR. EISENMANN: So surprisingly, I bought tortillas recently that's preserved with methyl paraben.

DR. BOYER: Tortillas?

DR. EISENMANN: Yes, tortillas. They had methyl paraben on the label, so

DR. BOYER: That's strange. It okay, I'm going back 20 years when I was in the paraben business so (crosstalk)

DR. EISENMANN: They must occasionally show up in food

DR. BOYER: yeah.

DR. EISENMANN: because I was surprised to see that, but

DR. BOYER: It is commonly used in ingestible drugs and the one thing I believe you cited was the alcohol free mouth washes because there's very little that would work in the ph. of the mouthwash. You know, they throw in some parabens, which is not always the best of ideas, but they put so much (inaudible) whatever else they put in to mask it. But in general, you know, if you look at the federal regulations for use parabens in foods, jelly I've never seen jelly preserved with parabens. It just ruins it.

Tortillas, that's new. Again, my background basically stopped in the mid '90s when I got out of the preservation business, but in those days we just we thought there was this big we called on every approval the FDA had, so on paraben, they never bought any.

DR. MARKS: Ron and Ron and Tom, do you like I'm looking at page 58, is the discussion, you and Rachel.

DR. SHANK: In the original report?

DR. MARKS: 208, do you like the direction of that where it talks about if you look at starting on 57, the Expert Panel consider most important, available for endocrine disruption, that's what we're talking about here. That most weekly estrogen and then it gives calculations. Now, these are calculations, exposure to personal care products.

DR. HILL: Mm hmm.

DR. MARKS: You had said, Ron, just handle it by the paragraph. Have one paragraph. I guess it's to me, it's somewhat reminiscent of the phalox where we said the exposure is going to be from nails. And all the concerns about adding it all up from other exposures. We're dealing just with personal care products exposure. So I don't know.

It's and it also deals with infants, obviously. There's the calculation for infants too.

DR. EISENMANN: And see, now, there's some studies that found it in breast milk. So you have a statement that you're dismissing that. Well, it's very low. It's only 50 percent of the women unless they were measuring in urine, but there's new data on it in breast milk. There's a Canadian study.

DR. MARKS: Mm hmm.

DR. EISENMANN: I was thinking you'd probably have to deal with some more of these things than in required currently.

DR. BOYER: Carol, do women have upset stomach issues. One of the uses of parabens is it's in antacids. So it's quite possible if they're taking liquid antacids for an upset stomach or anything like that; chemotherapy for that matter. The amount of paraben you would find in tissues would be much higher than for someone applying a cosmetic.

DR. BOYER: Well, we certainly let me pull the paper that addresses the measurements of parabens in breast milk. But it's basically, you want to be able to show that we've done a complete review of the literature. We've included considered everything just about everything out there. Everything that certainly that's important. But still, it doesn't help us to tease out just what fraction

of the parabens that appear in breast milk or any other tissue that's been mentioned, what fraction can be attributable to cosmetic use. In fact, it probably represents a very small fraction of the overall exposure. So we can soon discuss that and see (inaudible).

DR. EISENMANN: We're of the inclination that you need to see this information before you can make a decision. So it's obvious that it would be tabled at this meeting.

DR. SLAGA: That's what I would I think tabling may be to do to clarify everything.

DR. HILL: Well, we have the dispute over the dictionary and how it was stated. I think we have to have all of that well defined.

DR. MARKS: That sounds appropriate because the session's going to be marketed different maybe not different, but enhanced. If we table it, the next what we will see is these studies included; a broader picture; someone will develop a new discussion. It's an interesting I kind of like that because otherwise, we would be moving on with a tentative amended report and maybe it's premature.

DR. HILL: Right.

DR. MARKS: Although I think we're going to come to the same conclusion, but a tentative amendment. I mean that's the alternative, a tentative amended report.

Ron Shank, which do you prefer? Do you want to move do you think tabling it and seeing this more or no?

DR. SHANK: All I was going to say is that if we're going to add para hydroxyl benzoic acid, then that has to be surveyed.

DR. EISENMANN: No, it already was surveyed.

DR. SHANK: It was surveyed.

DR. EISENMANN: Yes. I included it. No uses.

MR. STEINBERG: No uses, which is all right. I didn't know if okay, so I was going to say, then we'd have to take a look, but never mind.

DR. HILL: The toxicology of that is not included.

MS. EISENBAUM: Right, wasn't as far as I know, it wasn't used as a search a cage number.

DR. HILL: But it's not a matter of use, it's a matter of metabolite.

MR. STEINBERG: Metabolite.

DR. EISENMANN: Well, you may have found it when you discovered the other parabens. It wasn't actually used as a search term, is that correct?

DR. BOYER: That's correct.

DR. EISENMANN: So

DR. BOYER: It was not used as a search term.

DR. SHANK: I think it needs to be used as a search term. Because there are a lot of these where metabolite has already been reviewed. But if there's one para hydroxyl phonemic acid has not been reviewed, but that is a metabolite in one of these.

DR. BOYER: The main one is hydroxyl benzoic acid and it's not peculiar to carbons. There are many things that we're exposed that generate that particular (inaudible), so but again, if there is some toxicity test data, there's typically a metabolite. And there some (inaudible) information in the chosen. In fact, it's one of the primary metabolites and then the other one's one that you choose a

(inaudible).

DR. MARKS: So I think a lot of the data is actually already captured. Because what I as I was pondering this because it's been a couple of years since we looked at it, is what's the mechanism of antimicrobial activity and the gist of it is, everything I saw, it's (inaudible). And actually bacteria might have (inaudible), but they produce a cell membrane, potential very similar to what we do with mitochondria and that's the basis for which a high enough concentration is uncoupling their ability to generate AGP basically. So if you follow this down again. I think this is almost red

herring and then you see these others thing like, the antiseptic effect and so forth popping up in some of this.

And I actually think, unless there are metabolites that we haven't really ever because they look at binding affinity of parabens themselves and like I say, I teach at least once a year. Here is what the Pharmacofore is for synthetic estrogen, binding estrogen receptors and you need the hydroxyl group at both ends and the ones that aren't that way, get metabolized in the human body to generate the hydroxylated metabolites. And that's what binds. They're either selected estrogen or captor modulators or sometimes, antagonists or agonists. And that's metabolism on the other end of the molecule, not the ester cleavage, which is what everything's been focusing.

But looking back I've actually focused more on some of these things related to chromosomal aberrations that were never explained and that's not going to be the para hydroxyl benzoic acid metabolite. There's a lot of new information about estrogens focused on (inaudible) metabolites of even estradiol itself. And those generate electrifials which turn out to be kind of bad actors, both in the genome and some other places.

And I doubt that those will be formed there because you've got a carboxic group on the end here, but I began to wonder as I'm looking and saying, the mechanism's for those. I've never been explained. And then we see this gene expression profiling and the paraben specific effects that pop out of that on page 54 and 55, suggests that there's something specific. The parabens that we haven't yet captured in the biology. And then the issue with the high risk breast cancer cell studies that are new in the new report on page 50.

So I genuinely believe unless their activity with metabolite of these things that we haven't capture and I think some of it will be the benzoic which is, I think the use of that's come to almost nil by now. The benzyl paraben, I don't think that's being used much anymore. And I suspect

MAN: (Inaudible)

DR. MARKS: yeah, I suspect that that might have been one of the worse actors. I suspect that the others aren't so bad, that maybe there are others again, everybody's so oppressively focused, I think on the estrogenic activity, I guess probably because you see things like this

(inaudible) and hypostadia and think that must be estrogen or androgen. I'm not so sure. We're ignoring maybe some of the newer things that are showing up and so, particularly, I didn't get a chance to read in detail that high risk breast the HRVECs, the high breast cancer pool where there's a genetic difference. But I would like time to digest some of this new stuff that's come in the report, which I haven't yet had time to do. However you decide to deal with it, table it or keep on going, I don't know, but I like table because it provides time.

DR. MARKS: Ron Shank, do you like to table or move forward:

DR. SHANK: I think table because there's some more to be added.

DR. MARKS: Okay and then while we're discussing parabens, I think it's worthwhile to go look at the comments or (inaudible) Women's Voices for the Earth. This could be addressed since we're going to be tabling it, but we had the bioaccumulation; we have the fetal abnormalities; and then we have a suggestion that Noell 10 mgs per kilo for bile paraben, whereas, in the 2008 document, we used a hundred times that a thousand milligrams per kilo. Did you want a you would answer that Ivan, did you want to make any comments about that now?

DR. BOYER: Well, as far as bioaccumulation is concerned, the term accumulation is used in some studies. And really what it seems to mean, even in the studies that Women's Voices for the Earth, it mentioned it seems to me that they were able to detect parabens in tissues that they examined. So that you would find it in breast tissue; you would find it in ovarian tissue and so on. And it's not very surprising because it is absorbed through the skin and through oral ingestion and for forth quick. As we understand accumulation or bioaccumulation, you really don't get that kind accumulation with these substances like you would for dioxin or and sort of pcbs and so forth. Nothing, nothing like that.

As far as the fetal anomalies are concerned. In fact, we don't have any studies that show fetal anomalies as the term is used by erotologists, people who study birth defects and do that kind of testing. So I think that's a matter of semantics, although we very clearly do have in this report, studies that show that there are effects on sperm counts and male reproductive organ weights and so on and so forth, which really which we really need to take a close look at. And Women's Voices for the Earth particularly point out a paper by Bulberg , 2016 Bulberg, et al. 2016. So make sure that you all have a chance to look at the full version of that paper. It is already incorporated into our current document. And basically, they found a genital a distance to the altered at doses of doses rates of about a hundred (inaudible) kilograms per day and so forth.

They did indicate some effects at a much lower dosage, 10 milligrams per kilogram per day in this wrap study. And it's really going to be a matter of evaluating whether or not what they found in the study. And also, in terms of evaluating the quality of the study and the reporting and so on, whether or not this warrants using, for instance, as recommended in the comments, 10 milligrams per kilogram per day as Noell for (inaudible), MOS calculations. The SCCS, in fact, they used in their assessments several years ago, in their calculations they used two milligrams per kilogram per day. That was actually a noe, N O E actually and no effect level. They didn't call it an abserved effect level because of the nature of the end points that the looked at, at those very low doses.

They used two milligrams per kilogram per day as an M E L calculation. If we would use the Burberg as basis for setting a Noell, then we probably be around down in that range, milligrams per kilogram per day. Or as suggested in the paper, that lowest dose which was examined in that paper is 10 milligrams per kilogram per day. So this is this is something that the Panel, I think need to take a little bit closer look at.

And also take a look at the Hoberman paper very closely. Take a look at that again. That's where the 1,000 milligrams per kilogram per day Noell came from. A very well conducted industry funded to take a dark step and it is also pretreat in the SCCS report. So you might want to take a look at those three reports, people. SCCS opinion of the Burberg 2016 report. And well, at least you want to take a look a close look at those two reports. And the certainly (inaudible).

DR. HILL: It's a dark study, oral exposure Turrets where the third paragraph, this is on 48, says F2 pumps exhibited statistically, significantly greater mortality at post naval base 7. I was trying to what was going on on that either, it was a deal where they exposed them some gestationally let's see, females starting getting Isoproparaben at post PMB21, PMB40 let's see anyway it's on page 48 and the reference is Reference 65.

MS. BECKER: Spencer VC.

DR. HILL: Yes, Spencer VC. What year? 2015. So that one to me

DR. BOYER: And if I recall correctly there's not a lot of elaboration

DR. HILL: Yeah.

DR. BOYER: on that observation?

DR. HILL: That's what I was worried about.

DR. MARKS: Is there anything other than so I'm going to be setting on a motion tomorrow, presumably it will be tabled, but if it isn't, I will put forward our teams proposal that we table this and the reasoning is that we have new studies, we have new data, we have new concerns along with a new ingredient presented today, that was the Florydroximensoic Acid and our team felt we needed more time to review this before we would proceed. Does that sound reasonable?

DR. SHANK: Yes, it does.

DR. MARKS: And is there anything really in our discussions other than the endocryn and infant skin issues?

DR. HILL: Well, I was going to say that one of the things that jumped out at me and trying to take my focus off estrogens for awhile when estrogenic activity was if you look at places where you do

see some affects on either strand breaks or gene repair, in almost all cases you see higher activity under metabolic activation. So that's the other thing that sticks out in my mind is, metabolic activation would have nothing to do with estro raises and clinging to Parahydroxybensoic Acid, that would be metabolizing one end of the molecules or the other presumably for seeing differences between metabolic activation and not. Some compounds and not others, so some are clean, some are not going back to ames and then there are a few other agents. So, anyway.

DR. MARKS: Tom, were you concerned about any mutagenic or carcinogenic issues?

DR. SLAGA: No.

DR. MARKS: Am I right, the real issues are looking at endocryn particularly, but exposure of infant skin? Obviously, how much gets absorbed? Although I don't know if that's that will be we've already calculated margin of safety.

DR. SLAGA: Right.

DR. MARKS: I guess the question is, is the margin of safety correct?

DR. HILL: And the reason I was asking the question, in part is, because if I remember right we had that paper last time we looked at this where the concentrations in one area of the breast were higher than others based on deodorant use or antiperspirant use, which makes and so I think the assumption that this is estrogen stimulated breast cancer, but I wondered if that was why I mean, there was no clear association as I remembered, I didn't

DR. BOYER: And that's the Darby study? Is that one of the Darby studies?

MR. STEINBERG: That was the original Darby.

DR. BOYER: And there's just a lot of speculation.

DR. HILL: I know there is.

DR. BOYER: And the paper also

DR. HILL: That's the way I felt about it too.

DR. BOYER: and criticized because I mean, they didn't use proper controls and so forth and it's a very small sample set and so on. So I mean, it's basically the story that the authors of that paper developed based on

DR. EISENMANN: In general they're not used in antiperspirants?

DR. HILL: No.

DR. EISENMANN: Can be used in deodorants, but not antiperspirants?

DR. HILL: Well, so antiperspirants we don't consider okay, so what you're saying is, their correlation was with antiperspirants, not deodorants?

DR. EISENMANN: I don't think they distinguished.

DR. HILL: And see that's a problem. Because deodorants are under our purview, antiperspirants would be FDA.

DR. BOYER: And they weren't really able to make any of those distinctions, because they used the tissue from I expect them to use as they received them and that's what they analyzed, so as far as exposure is concerned, especially the question the source of the exposure, there's no way to

DR. HILL: I agree with you. The only reason I raised it at all because I didn't feel particularly worried by that paper the last time when I saw it was, we have this new data where they did a cell based study with these were patients sampled high risk breast cancer cells. Grant you the work was done in cells and then I'm looking at these strand breaks and DNA repair affects and saying, have people been focused so much on estrogen that they've missed these other mechanisms potentially for carceniginicty that we need to revisit or pay attention to because we have new information, before all this gets put to bed.

And it may be that none of that is of any issue, that's why I'm raising it when the toxicologists are sitting here, all of you, including Ivan, to have a look at this.

DR. MARKS: This has really been actually a really robust discussion and I think we'll table it. I have a feeling we'll continue where we left off the next time we see these ingredients. But we made progress in that we're going to add 18 new ingredients now and we started focused on where we go from now in addressing these issues that were raised, including biocummulation, margin of safety and some dysfunction and such.

Okay. Any other comments?

DR. HILL: Just that we need good preservatives and so I'm going to try intersect preservatives that are probably of high value and not dangerous, but we'd like to know that.

DR. MARKS: This is probably one of the few group of ingredients where irritation and sensitization isn't an issue.

DR. HILL: I know, right.

DR. MARKS: I get off the hook on this one. Okay. So our team will recommend tabling or we will second table it.

Okay. Any other comments? Okay. Ivan and Lillian, you have your work cut out for you, huh?

Full Panel

DR. BERGFELD: Then moving on to a larger item here, parabens. Dr. Belsito?

DR. BELSITO: Yes. So it's actually very good that we just had this discussion on spermatogenesis because we've decided to reopen this report to add in some additional parabens, including carboxylic salts which at least Dan felt could be included despite virtually no data on them that we could read across. However, we were very concerned over the new data on developmental and reproductive toxicity because before when we did our margins of exposure we were using a NOAEL of 1,000, and now at least, based upon spermatogenesis, despite the absence of any histopathological changes in the testes, it appears that the LOAEL may be 10. We don't have a LOAEL at least for spermatogenesis. And I think that given the issues surrounding parabens in terms of endocrine disruption, we really need to make sure that we get this really correctly, and our team recommended this be tabled and that we invite two experts Kurt identified one, Dan and I identified another to come and review with us their take on all of the various reproductive and developmental data that we have on the parabens before proceeding. So we're recommending that this report be tabled for now.

DR. MARKS: Second.

DR. BERGFELD: Second. There's no discussion on the table.

All those in favor of tabling? Unanimous.

(The motion passed unanimously.)

DR. BERGFELD: Any discussion to follow the table other than the invitation?

DR. BELSITO: The issue is the issue is repro development.

DR. BERGFELD: Okay. Bart?

DR. HELDRETH: Is the industry willing to make those invitations for the speakers?

DR. ANSELL: I think this was considered to be consultants to the panel and I think that would be a CIR staff obligation.

DR. BERGFELD: Okay. Well, I understand that their contacts are available to you via some of our panel members.

All right.

DR. BELSITO: I would just note in our meeting today that we did recognize the letter from Women's Voice for the Earth, and that raised some of these issues. So we're appreciative of that letter, and we thought Ivan's response was good, but we, our team had the same issues. Lots of new data, new studies, concerns, new ingredients. So tabling is the best way to proceed at this point.