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# Amended Safety Assessment of Parabens as Used in Cosmetics

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Status: Draft Final Amended Report for Panel Review  
Release Date: March 15, 2019  
Panel Meeting Date: April 8-9, 2019

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



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## MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Jinqiu Zhu, PhD, DABT, ERT  
Toxicologist

Date: March 15, 2019

Subject: Draft Final Amended Safety Assessment of Parabens as Used in Cosmetics

Attached is the Draft Final Amended Report of 20 parabens and 4-Hydroxybenzoic Acid, as used in cosmetics (*parabe042019FAR*). At the September 2018 meeting, the Panel issued a tentative amended report for public comment with the conclusion that the following 20 ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

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

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

The Panel also determined that the available data are insufficient to determine the safety of Benzylparaben. The data needed to determine safety of this ingredient comprise a no-observed-adverse-effect-level (NOAEL) derived from developmental and reproductive toxicity (DART) studies. This ingredient is not reported to be in use.

The Panel discussed concerns about the bioaccumulation potential of parabens. The Panel noted that recent studies with sensitive analytical methods have demonstrated the presence of parabens in various human tissues. However, the data are equivocal regarding cumulative storage in such tissues; and importantly, the available evidence suggests no significant association of parabens exposure with diseases or other adverse health conditions. The Panel also noted that refined aggregate exposure models suggest that cosmetic product use is a major source of parabens exposure. However, the vast quantity of biomonitoring data indicates that systemic exposure to these ingredients is very low.

The Panel discussed the safety of parabens as used in vaginally-applied cosmetic products. The Panel classified the currently available studies as illustrations of potential, general hazards, which fail to demonstrate risks relevant to cosmetic safety in the context of concentration of use.

The Panel's deliberations on the draft assessment report comprised extensive revisions to better identify, and explain the rationale for, the values utilized in conducting the risk assessment therein. The Panel further requested that the margin of safety (MOS) should be re-calculated, weighing the different use concentrations and exposures of Butylparaben in various cosmetic products category. The report was revised accordingly.

Since the September meeting, additional data on biomonitoring, endocrine activity, dermal sensitization and epidemiological studies have been incorporated into the report. The input of new studies as well as discussions regarding bioaccumulation, dermal absorption rate, and Physiologically Based Pharmacokinetic (PBPK) Model for the estimating the internal exposure of parabens, are highlighted within the text of this report.

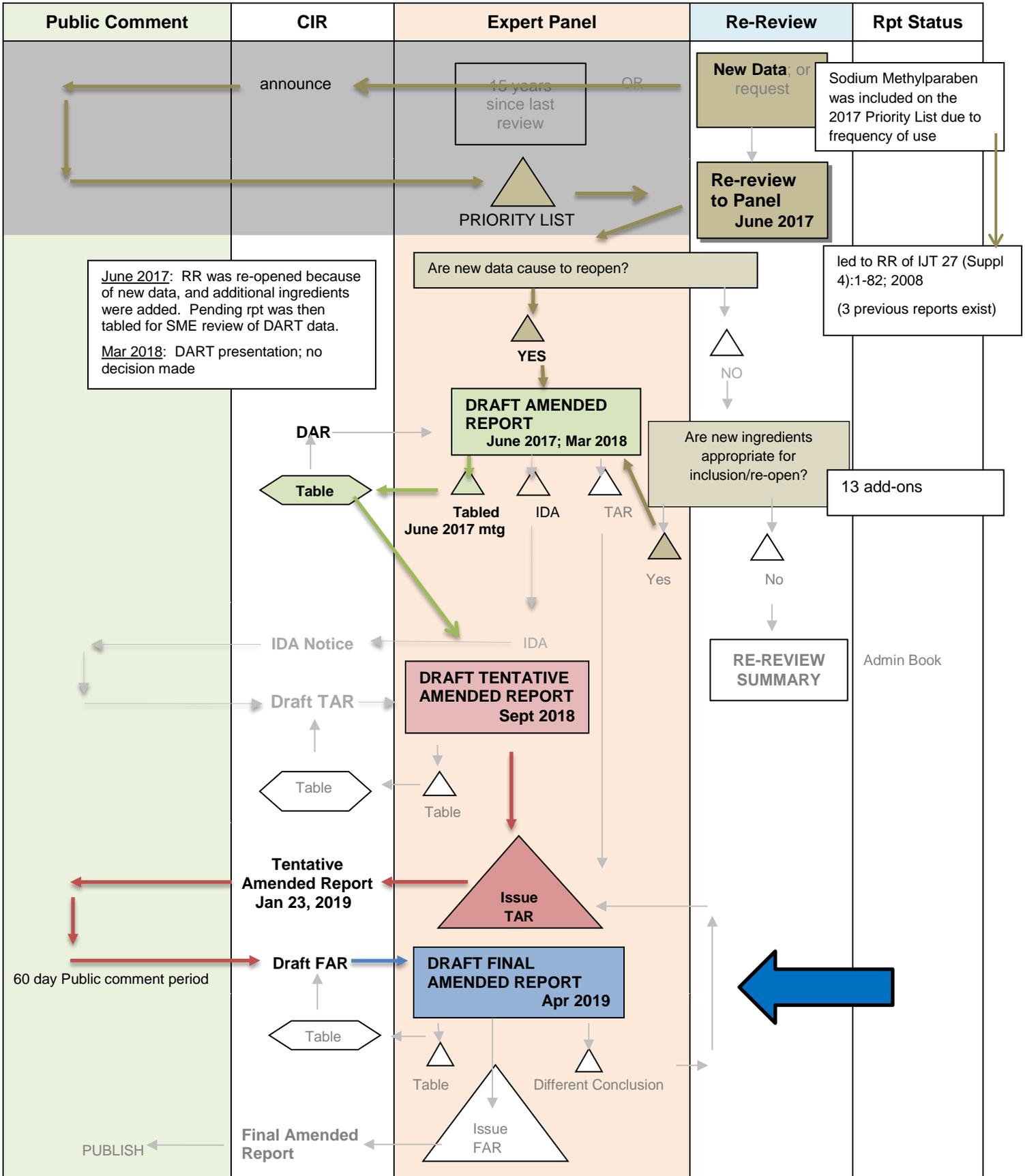
Also included in this package for review are the CIR report history (*parabe042019hist*), flow chart (*parabe042019flow*), literature search strategy (*parabe042019strat*), ingredient data profile (*parabe042019prof*), and previous meeting transcripts (*parabe042019min*). Comments provided by the Council prior to the September meeting and on the tentative amended report have been addressed (*parabe042019pcpc1* and *parabe042019pcpc2*, respectively).

The Panel should carefully review the Abstract, Discussion, and Conclusion of this report. If these are satisfactory, the Panel should issue a final amended report.

# RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Parabens

MEETING April 2019



CIR History of:

**Parabens**

**1984** – Report published for **Methylparaben, Ethylparaben, Propylparaben, and Butylparaben** with the conclusion that these ingredients are safe as cosmetic ingredients in the present practices of use.

**1986** – Report on **Benzylparaben** was published with an insufficient data conclusion. The data needs were:

1. UV absorption spectrum. If absorption occurs between 280 and 360 nm, a photosensitization study is required (in animals only, not in clinical assays).
2. Data detailing the possible presence of impurities.
3. Subchronic feeding study-90-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.

**1995** – Report on **Isobutylparaben and Isopropylparaben** was published with a conclusion of safe as cosmetic ingredients in the present practices of use.

**2008** – Amended report published. The ingredients in the three previous reports are included. The Conclusion was that these ingredients are safe as cosmetic ingredients in the present practices of use.

“The CIR Expert Panel considered exposures to cosmetic products containing a single parabens preservative (use level of 0.4%) separately from products containing multiple parabens (use level of 0.8%) and infant exposures separately from adult exposures in determining margins of safety (MOS). The MOS for infants ranged from ~6000 for single paraben products to ~3000 for multiple paraben products. The MOS for adults ranged from 1690 for single paraben products to 840 for multiple paraben products. 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) and support the safety of cosmetic products in which parabens preservatives are used.”

**March 2012** – “The Panel reaffirmed the safety of parabens as preservatives in the present practices of use and concentration in cosmetics.

At the request of the Personal Care Products Council, the Panel re-examined its 2008 published safety assessment of parabens. The Council cited new opinions from the European Commission’s Scientific Committee on Consumer Safety (SCCS) regarding (1) safe levels of parabens in cosmetics and (2) parabens in products intended for children under 3 years of age.

The SCCS updated opinion on parabens confirmed that methyl- and ethylparaben are safe up to 0.4% for one and a total of 0.8% for any mixture, but lowered the level in cosmetics considered safe for propyl- and butylparaben to 0.19% for any one or any mixture. This lowering appeared to be based on a re-evaluation of existing dermal penetration/metabolism data, not on new data. The Panel reiterated its very conservative value of 50% dermal penetration and the robust toxicity study it used as a benchmark to evaluate a margin of safety, i.e. how far below the exposure levels known to produce no damage in the toxicity study are the

levels found in cosmetics. The Panel stated that its published margins of safety are still valid and continue to offer ample assurance that parabens are safe in the present practices of use and concentration.

The second recent SCCS opinion addressed the Danish decision to ban parabens in products intended for children under 3 years of age. The SCCS opinion appeared to say that there is no real basis for the Danish ban, and the Panel agreed with that position. The SCCS opinion did note that additional data would be useful for children <6 mo of age.

The Panel agreed that infants are a sensitive subpopulation for risk assessment and has consistently considered the higher skin surface area to body mass ratio in infants when performing cosmetic ingredient safety assessments. The Panel believes that more data regarding dermal penetration through infant skin and potential metabolism in infant skin are available and should be brought to bear on this question. The Panel directed CIR staff to begin the process of pulling that information together in an overview report, with the intent of providing the information to the public, as was done for aerosols.”

**September 2012** – The Panel reviewed new publications to see if they warranted reopening the report.

“The CIR Expert Panel determined to not reopen the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben and benzylparaben. One new study suggesting that the preservative function of parabens might be linked to allergic sensitization, while other potential endocrine disrupting chemicals were not linked to this condition, was considered by the CIR Expert Panel. The Panel also reviewed a study that measured paraben concentrations as a function of location in breast tissue. In addition, an in vitro study of immortalized but untransformed human breast epithelial cells in culture reported cell transformation at concentrations that were considered to be comparable to the concentrations measured in some of the breast tissue studied. The Panel determined that these data are not relevant to the assessment of the safety of parabens in cosmetics. The Panel reaffirmed that parabens are safe in the present practices of use and concentration. The Panel suggested that their extensive discussion about these data would be important to communicate to the public and to the scientific community and that a detailed discussion should be prepared for posting on the CIR website, for a press release, and for a letter to the editor of an appropriate scientific journal.”

**2016** – Parabens put on the Priority List because of the number of uses of **Sodium Methylparaben**. Additional parabens were added to the report:

<b>Sodium Methylparaben</b>	<b>Potassium Paraben</b>	<b>Sodium Isopropylparaben</b>
<b>Calcium Paraben</b>	<b>Potassium Propylparaben</b>	<b>Sodium Paraben</b>
<b>Potassium Butylparaben</b>	<b>Sodium Butylparaben</b>	<b>Sodium Propylparaben</b>
<b>Potassium Ethylparaben</b>	<b>Sodium Ethylparaben</b>	
<b>Potassium Methylparaben</b>	<b>Sodium Isobutylparaben</b>	

**June 2017** – The Panel agreed to re-open the parabens report, and added 4-Hydroxybenzoic Acid to the group.

“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 also 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, and 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.”

**March 2018** – The Panel agreed to table the re-review of the parabens.

In response to the Panel’s request of further expert input on the topic of parabens and DART, Dr. George Daston, a Victor Mills Society Research Fellow at Proctor & Gamble, presented to the Panel on these ingredients. His briefing was titled, “Assessing the Developmental and Reproductive Toxicity of Parabens.” Dr. Daston acknowledged that there is a great deal of data on this subject that may at first seem quit conflicting. However, he stressed that much of these data 1) are irrelevant to the routes of exposure associated with intended cosmetic use, or otherwise did not account for the extensive metabolism of parabens to metabolites with no known DART activity; 2) are the result of poorly or uncommonly designed studies; 3) were not verified by other methods (as would traditionally be done); and/or 4) are not dose-dependent, and thereby likely erroneous. Indeed, Dr. Daston suggested, based on the relevant data, that a pragmatic no-observed-adverse-effect-level (NOAEL) of 160 mg/kg bw/day could be used to calculate a conservative margin of safety (MOS) for Butylparaben, and inferred to other members of the ingredient group. After careful consideration of all the new data in the category of endocrine disruption and from new DART studies, the Panel determined an adequate NOAEL value of 160 mg/kg bw/day for Butylparaben and requested margin of safety for parabens be re-calculated accordingly.

Additional references were submitted by various stakeholders or discovered by CIR, many of which were provided for the Panel’s consideration for inclusion in this report. The Panel reviewed the additional references and requested that all the new information be incorporated into the report before proceeding to the next stage.

The Panel discussed the EU Cosmetic Regulations and SCCS opinions on parabens and put into perspective the potential burden of parabens from cosmetics versus multiple other sources of exposure, e.g., food and pharmaceutical use. The Panel also discussed the bioaccumulation potential of parabens in human body and the estrogen receptor binding potential of Butylparaben, Isobutylparaben, and Benzylparaben metabolites.

**September 2018** – The Panel issued a tentative amended report for public comment with the conclusion that the following 20 ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

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

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

However, the Panel concluded that the available data are insufficient to determine the safety of Benzylparaben. The data needed to determine safety of this ingredient comprise a no-observed-adverse-effect-level (NOAEL) derived from developmental and reproductive toxicity (DART) studies. This ingredient is not reported to be in use.

The Panel discussed concerns about the bioaccumulation potential of parabens. The Panel noted that the presence of parabens in various human tissues. However, the data are equivocal regarding cumulative storage in such tissues; and importantly, the available evidence suggests no significant association of parabens exposure with diseases or other adverse health conditions. The Panel also noted that cosmetic product use is a major source of parabens exposure. However, the vast quantity of biomonitoring data indicate that systemic exposure to these ingredients is very low.

The Panel also discussed the safety of parabens as used in vaginally-applied cosmetic products. The Panel classified the submitted studies as illustrations of potential, general hazards, which fail to demonstrate risks relevant to cosmetic safety in the context of concentration of use.

The Panel requested extensive revisions on the draft tentative amended report to better identify, and explain the rationale for, the values utilized in conducting the risk assessment therein. The Panel also requested that the margin of safety (MOS) should be re-calculated, weighing the different use concentrations and exposures of Butylparaben in various cosmetic products category.



### Search Strategy for Parabens

- PubMed – February 15, 2019
  - Search for (benzylparaben OR butylparaben OR “calcium paraben” OR ethylparaben OR isobutylparaben OR isopropylparaben OR methylparaben OR “potassium butylparaben” OR “potassium ethylparaben” OR “potassium methylparaben” OR “potassium paraben” OR “potassium propylparaben” OR propylparaben OR “sodium butylparaben” OR “sodium ethylparaben” OR “sodium isobutylparaben” OR “sodium isopropylparaben” OR “sodium methylparaben” OR “sodium paraben” OR “sodium propylparaben” OR “4-hydroxybenzoic acid” OR “94-18-8” OR “94-26-8” OR “69959-44-0” OR “120-47-8” OR “4247-02-3” OR “4191-73-5” OR “99-76-3” OR “38566-94-8” OR “36457-19-9” OR “26112-07-2” OR “16782-08-4” OR “84930-16-5” OR “94-13-3” OR “36457-20-2” OR “35285-68-8” OR “84930-15-4” OR “5026-62-0” OR “114-63-6” OR “85080-04-2” OR “35285-69-9” OR “99-96-7”) AND (“acute effects” OR “acute toxicity” OR “ADME” OR “adverse effects” OR “adverse events” OR “adverse health effects” OR “allergic reaction” OR allergy OR anaphylactic OR anaphylaxis OR asthma OR “birth defects” OR cancer OR carcinogenesis OR carcinogenicity OR “case report” OR “chronic effects” OR “chronic toxicity” OR “clinical report” OR “clinical study” OR “clinical trial” OR “co-carcinogenicity” OR cocarcinogen OR “co-carcinogen” OR comedogens OR comedogenic OR comedogenicity OR cytotoxicity OR “dermal effects” OR “dermal exposure” OR ((dermal OR skin OR “mucous membrane”) AND (irritation OR sensitization OR penetration)) OR “dermal penetration” OR “dermal toxicity” OR “developmental toxicity” OR “effects on the endocrine system” OR “effects on the eyes” OR “effects on the skin” OR “endocrine activity” OR “endocrine disruption” OR “endocrine disruptor” OR “endocrine disrupter” OR “endocrine effects” OR “endocrine toxicity” OR “epidemiological study” OR “epidemiology” OR “eye exposure” OR genotoxicity OR “health effects” OR hepatotoxicity OR “liver toxicity” OR hypersensitivity OR immunotoxicity OR “in vitro test” OR “inhalation exposure” OR “inhalation toxicity” OR irritation OR “meta-analysis” OR “meta analysis” OR (metabolite NOT (bacterial OR bacteria)) OR “mucous membrane” OR “multicenter study” OR mutagenicity OR neurotoxicity OR “ocular effects” OR “ocular exposure” OR “oral effects” OR “oral exposure” OR “oral toxicity” OR “penetration enhancer” OR pharmacokinetics OR photosensitivity OR phototoxicity OR pigmentation OR “prospective study” OR “renal toxicity” OR “repeated dose” OR “repeat dose” OR “reproductive and developmental toxicity” OR “reproductive toxicity” OR “respiratory effects” OR “retrospective study” OR risk OR safety OR sensitization OR “short-term toxicity” OR “short term toxicity” OR “skin contact” OR “skin exposure” OR “skin penetration” OR “subacute effects” OR “subacute toxicity” OR “subchronic effects” OR “subchronic toxicity” OR “toxicity in vitro” OR “in vitro toxicity” OR toxicity OR toxicokinetics OR “tumor promotion” OR biomonitoring)

819 hits, reduced to 315 references of interest based on careful reading of the abstracts

- Scifinder – February 15, 2019
  - Substance Identifier: benzylparaben, butylparaben, calcium paraben, ethylparaben, isobutylparaben, isopropylparaben, methylparaben, potassium butylparaben, potassium ethylparaben, potassium methylparaben, potassium paraben, potassium propylparaben, propylparaben, sodium butylparaben, sodium ethylparaben, sodium isobutylparaben, sodium isopropylparaben, sodium methylparaben, sodium paraben, sodium propylparaben, 4-hydroxybenzoic acid; Combine with search for: 94-18-8, 94-26-8, 69959-44-0, 120-47-8, 4247-02-3, 4191-73-5, 99-76-3, 38566-94-8, 36457-19-9, 26112-07-2, 16782-08-4, 84930-16-5, 94-13-3, 36457-20-2, 35285-68-8, 84930-15-4, 5026-62-0, 114-63-6, 85080-04-2, 35285-69-9, 99-96-7

21 hits

Get References - Adverse Effect, including toxicity; Biological study: 27,952 hits

Refine by Document types- Biography, Book, Clinical Trial, Commentary, Dissertation, Journal, Letter, Report, and Review: 13,217 hits

Refine by:

**Acute toxicity; 90 hits**

Repeated dose toxicity; 6 hits

Subacute toxicity; 3 hits

Short-term toxicity; 4 hits

Subchronic toxicity; 11 hits

**Chronic toxicity; 31 hits**

Adverse health effects; 27 hits

**Allergy; 299 hits**

Anaphylaxis; 13 hits

**Asthma; 31 hits**

Hypersensitivity; 63 hits

Sensitization; 874 hits

Carcinogenicity; 541 hits

Cancer; 541 hits

Cocarcinogenicity; 2 hits

Tumor promotion; 6 hits  
Tumor progression; 1 hits  
Case report; 313 hits  
Case study; 313 hits  
Clinical trial; 25 hits  
Multicenter study; 13 hits  
Clastogenicity; 5 hits  
Genotoxicity; 50 hits  
Mutagenicity; 180 hits  
Comedogenicity; 0 hits  
Cytotoxicity; 420 hits  
Dermal absorption; 31 hits  
Dermal penetration; 14 hits  
Dermal irritation; 11 hits  
Dermal effects; 192 hits  
Dermal pigmentation; 0 hits  
Developmental toxicity; 114 hits  
Reproductive toxicity; 77 hits  
Endocrine toxicity; 59 hits  
Endocrine activity; 81 hits  
Endocrine disruption; 337 hits  
Epidemiology; 56 hits  
Hepatotoxicity; 42 hits  
Renal toxicity; 6 hits  
Inhalation toxicity; 8 hits  
Respiratory effects; 89 hits  
In vitro toxicity; 64 hits  
**In vitro test; 1571 hits**  
Neurotoxicity; 26 hits  
Ocular effects; 166 hits  
Oral exposure; 24 hits  
Penetration enhancer; 62 hits  
Phototoxicity; 12 hits  
**Photosensitivity; 5 hit**  
Risk assessment; 154 hits  
Safety assessment; 44 hits  
Toxicokinetics; 1253 hits  
Pharmacokinetics; 208 hits  
**Biomonitoring; 28 hits**

Combined: 3,528 hits (after duplicates removed), total; reduced to 485, all years, based on careful reading of the abstracts

- Consolidated and eliminated duplicates in PubMed and SciFinder search results
  - 412 references, all years
- Screened out:
  - Subcutaneous injection studies (with one exception of 1999 Fisher *et.al* paper which was used by SCCS for the derivation of Margin of Safety for Butylparaben and was requested by the Panel for the discussion)
  - Animal studies on mixtures of parabens and other test substances (e.g., parabens + phthalates administered together)
  - Studies covered in previous CIR safety assessments of parabens
  - A few older studies that are redundant with other studies covered in previous CIR safety assessments

Final tally: 75 references

### LINKS

#### Search Engines

- Pubmed (<http://www.ncbi.nlm.nih.gov/pubmed>)
- Toxnet (<https://toxnet.nlm.nih.gov/>); (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)
- Scifinder (<https://scifinder.cas.org/scifinder>)

appropriate qualifiers are used as necessary

search results are reviewed to identify relevant documents

### **Pertinent Websites**

- wINCI - <http://webdictionary.personalcarecouncil.org>
- FDA databases <http://www.ecfr.gov/cgi-bin/ECFR?page=browse>
- FDA search databases: <http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm>;
- EAFUS: <http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true>
- GRAS listing: <http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm>
- SCOGS database: <http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm>
- Indirect Food Additives: <http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives>
- Drug Approvals and Database: <http://www.fda.gov/Drugs/InformationOnDrugs/default.htm>
- <http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf>
- FDA Orange Book: <https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm>
- OTC ingredient list: <https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf>
- (inactive ingredients approved for drugs: <http://www.accessdata.fda.gov/scripts/cder/iig/>
- HPVIS (EPA High-Production Volume Info Systems) - <https://ofmext.epa.gov/hpvis/HPVISlogon>
- NIOSH (National Institute for Occupational Safety and Health) - <http://www.cdc.gov/niosh/>
- NTIS (National Technical Information Service) - <http://www.ntis.gov/>
- NTP (National Toxicology Program) - <http://ntp.niehs.nih.gov/>
- Office of Dietary Supplements <https://ods.od.nih.gov/>
- FEMA (Flavor & Extract Manufacturers Association) - [http://www.femaflavor.org/search/apachesolr\\_search/](http://www.femaflavor.org/search/apachesolr_search/)
- EU CosIng database: <http://ec.europa.eu/growth/tools-databases/cosing/>
- ECHA (European Chemicals Agency – REACH dossiers) – <http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1>
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - <http://www.ecetoc.org>
- European Medicines Agency (EMA) - <http://www.ema.europa.eu/ema/>
- IUCLID (International Uniform Chemical Information Database) - <https://iuclid6.echa.europa.eu/search>
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- <http://webnet.oecd.org/hpv/ui/Search.aspx>
- SCCS (Scientific Committee for Consumer Safety) opinions: [http://ec.europa.eu/health/scientific\\_committees/consumer\\_safety/opinions/index\\_en.htm](http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm)
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- <https://www.nicnas.gov.au/>
- International Programme on Chemical Safety <http://www.inchem.org/>
- FAO (Food and Agriculture Organization of the United Nations) - <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/>
- WHO (World Health Organization) technical reports - [http://www.who.int/biologicals/technical\\_report\\_series/en/](http://www.who.int/biologicals/technical_report_series/en/)
- [www.google.com](http://www.google.com) - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

### **Botanical Websites, if applicable**

- Dr. Duke's - <https://phytochem.nal.usda.gov/phytochem/search>
- Taxonomy database - <http://www.ncbi.nlm.nih.gov/taxonomy>
- GRIN (U.S. National Plant Germplasm System) - <https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx>
- Sigma Aldrich plant profiler- <http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html>
- American Herbal Products Association Botanical Safety Handbook (database) - <http://www.ahpa.org/Resources/BotanicalSafetyHandbook.aspx>
- European Medicines Agency Herbal Medicines - [http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/herbal\\_search.jsp](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/herbal_search.jsp)
- National Agricultural Library NAL Catalog (AGRICOLA) <https://agricola.nal.usda.gov/>
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- [http://www.seasoningandspice.org.uk/ssa/background\\_culinary-herbs-spices.aspx](http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx)

### **Fragrance Websites, if applicable**

- IFRA (International Fragrance Association) – <http://www.ifraorg.org/>
- Research Institute for Fragrance Materials (RIFM)

## **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  $\alpha$ -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 Oishi 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 methyl dibromo 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. BRESLAWEK: 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 unbiasedly 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 methylidibromo 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. BRESLAWEK: That is a drug.

DR. HILL: But not mouthwash?

DR. BRESLAWEK: The relevant use here is deodorant.

DR. HILL: Is what? Is deodorant?

DR. BRESLAWEK: The largest use for Triclosan is deodorant.

DR. HILL: Yeah. But there is some use in mouth rinses?

DR. BRESLAWEK: 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. BRESLAWEK: 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. BRESLAWEK: 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't?

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-dermally 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 sodiummethyl, 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 carboxylic 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 protonated, largely protonated 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 protonate 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. KLASSESEN: 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 propyl, 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. KLASSEN: 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 they 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 lines 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 lines and other cell lines. So it starts out, "Isobutyl paraben antagonize the estrogen receptor in Chinese hamster ovary cells. The effect was statistically significant at greater 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 heregulin which is a stimulator of the EGF receptor, or it's a possible stimulator of the EGF receptor."

And then glucocorticoid 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 carboxylic 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. KLASSESEN: 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 do 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 propyl 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 propyl and isopropyl?
- DR. BOYER: Yes.
- MS. LORETZ: Actually, it's propyl and butyl. Isopropyl they didn't go ahead and update it, so (inaudible).
- DR. BELSITO: So propyl 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 propyl 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. KLASSEN: 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 hyperspatus 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. KLASSEN: 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. KLASSEN: 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 epididymies 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 transcriptional regulator, and its expression is related to the downstream that are known to regulate differentiation of Svarthily 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. KLAASSEN: 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 NOAELs 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's 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 may 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 be 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 "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 phenolic 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 ATP 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 teratologists, 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 no effect level. They didn't call it an observed effect level because of the nature of the end points that they looked at, at those very low doses.

They used two milligrams per kilogram per day as an MEL 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 team's 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 Florydroximenoic 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 endocrine 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 effects 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 estrogen raises and clinging to Parahydroxybenzoic 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 endocrine 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 carcinogenicity 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.

**[Discussion of Parabens]**

**MARCH 2018**

**Dr. Mark's Team**

DR. MARKS: Here's the memo from Bart in February of this year. The updated draft, the review of 20 parabens. Last year we agreed to add sodium methylparaben to the priority list. Seven parabens had been reviewed in 2008. They are listed in the memo. In addition, the panel included 12 other paraben salts, which had not been reviewed. This was reopened. After the June meeting the panel also added for hydroxybenzoic acid.

As per the presentation this morning, thank you. The panel expressed concern about the new data from the developmental and reproductive toxicity, the DART studies, indicating reduced sperm counts, reduced expression of a specific enzyme and a specific cell marker in the testes of the offspring of female rats orally dosed with 10 milligrams per kilogram per day. Butylparaben during the gestation and lactation periods, reduction in anogenital distance and other effects at the 100 milligrams per kilogram per day, in that study.

There were the additional references, which we had presentations on. Then we're at the point now, do we move forward with a tentative amended report, safe and sufficient? Tom, Ron, your comments? Do you want me to read what Ron Shank has to say?

DR. SLAGA: Maybe we should have a little discussion about the presentation.

DR. MARKS: Sure.

DR. SLAGA: But overall, I think we should add the add-ons, the salts, and I think it's basically the same conclusion as it was before. I thought the presentation summarized, very well, all the data and it was good to hear someone give some results and discuss about subcutaneous injections of compounds, which, if you want to get a large amount of something in a body, that's the way to do it. It's much greater than even if you give something by gavage, which is still a tremendous amount that you would give to a -- it's much greater than even a dietary study. And if you compare it to dermal, I mean, dermal is so low compared to all of these.

The point I liked about the presentation is the human studies supported that there is really no effect. Of course, epidemiological studies are not infallible, but the one point he brought out about the esterase that I thought was very, very interesting, and that if they are down regulated during pregnancy and lactation, that can be a concern. But scientifically I can't come up with any reason why they would be, but I don't know if anybody else would think they should be, but I don't.

Anyway, I think there is a tremendous margin of safety here.

DR. MARKS: So, you feel that they're safe because the margin of safety and you like all 20 ingredients?

DR. SLAGA: Right.

DR. MARKS: Ron Hill, your comments?

DR. HILL: I have quite a bit. I spent a good bit of time. Since we started with the presentation, I'll make note that there is a result in here that I think needs to be explained. Since the pages aren't numbered, it's close to the end. It's from the Boberg study where they had the gene expression studies. And he did make the comment that they didn't do the follow up that would apparently be considered now de rigueur on these.

In the prepubertal testes, the one that jumps out is Cyp19a1 and that's aromatase. That's the enzyme that makes estrogen, and it seems to be pretty heavily suppressed even at the 10 milligram. And there is sort of a whiff -- not statistically significant -- of dose response between 10, 100, and 500. When I look at a result like that I say, well, we're already at saturating, then maybe we're seeing results actually well below 10. So, it's not clear. I think somewhere along the line that research ought to be followed up.

For me, the most significant study in this whole report that we got this time is buried in Table 10 on the top of page 45 PDF where they looked at 31 healthy women. Basically, there is some

commentary here that suggests that the SAR of esterases in skin are not the same for humans as they are for rodents. Now, it's interesting because they're in a couple places and I flagged them, where they suggest that as the lipophilicity increases for diffusion through the skin, the diffusion rate goes down. That's an incorrect conclusion. That's not what's going on here.

Diffusion through a lipid layer, which this is, is going to increase proportionate to the partition coefficient.

If the partition coefficient goes up by a factor of 10, the rate of diffusion or the rate of mass transfer is going to, in general, decrease by a factor of 10. But what else is here is, the other thing that comes into play in mass transfer through lipids is floppiness of the molecules.

So, when we got butyl, we've got a longer chain and so the effective diameter with that butyl group flopping around would be much larger than with a methylparaben. So, that's trading off in diffusion through human skin. But it's something I've been wondering for a long time, anybody who ever looks at the SAR for estrogen receptor -- and definitely people who have been teaching it, especially as long as I have and have been thinking about these parabens and estrogen effects since long before I was on the CIR panel is -- so, forgetting high affinity binding to an estrogen receptor, whether you have an agonist, an antagonist, or a selective estrogen receptor modulator, you need an aromatic OH, a phenolic OH on one end, ideally a fairly rigid scaffold in between, and a hydroxyl group that if the scaffold is long enough -- about 12-angstrom separation.

Now, in estradiol it's about a 10-angstrom separation, and so that distal OH -- the saturated OH at carbon 17 actually makes hydrogen bond to a bound water in the estrogen receptors, which then makes additional hydrogen bonds to both estradiol receptors A and B and then there are subtypes of those. In something like raloxifene, both of those hydroxyls are already in place.

And if you look at the earlier generation selective estrogen receptor modulators, the toremifene -- what's the other one I'm looking for? Tamoxifen. Those are actually not estrogenic, per se. They have to be hydroxylated so that you have a hydroxyl on both ends of the molecule, about 12 angstroms apart. Then you get big activity. If you go way back to the diethylstilbestrol -- which was really one of the first synthetic estrogens -- and you look at that, you've got hydroxyl groups on a rigid scaffold, x number of angstroms apart.

I've always been puzzled, and I wonder about the benzylparaben in particular, why people haven't been doing the studies on the metabolites that are hydroxylated as opposed to the others. And so, with rodent studies, what you see is exactly what you're saying, the esterase at either portal of entry is higher activity; but the SAR for skin esterase as it turns out are different. So, in rats as the chain gets longer, in mice as the chain gets longer, it seems that the esterase hydrolysis goes up. In humans, it appears like it's actually going in the opposite direction. But of course, our skin barrier is better.

There are a lot of things trading off here, but what I'm noticing is in this study that is in reference 51, which is a 2016 paper by Moos, is that some of these hydroxylated metabolites that I've been wondering about for a long time are actually showing up. And it appears in reasonably significant amounts from dermal dosing of these women. I didn't look up the original paper to find out how much skin area is actually being treated. But that got my attention.

So, you would expect any -- I mean, the chain isn't long enough with methyl or ethyl, or even isobutyl or propyl, but as soon as you get to butyl and definitely benzyl -- because we had an aromatic ring on the other end -- suddenly you've got chains that are long enough to bridge so that we could potentially have high affinity binding of these metabolites to the estrogen receptor. If this has been studied, I haven't been able to find it. I've been puzzling about this for a long time.

The other thing is that especially the liver port of entry when you're given orally, rats and mice are incredibly aggressive phase 2 metabolizers coming in through the liver. So, they make glucuronides and a lot more sulfation than humans. I remember this in detail way back in the early '90's, because I proposed doing a study that I wanted to do where that came into play in rabbits. They didn't want to let me house rabbits at the time, so I couldn't do the study I wanted

to do, and I wrote a different grant instead as it happened. That got funded and so the rest is kind of history.

The point is, now of course if you're giving by gavage at very high doses where we're saturating all the roots of metabolism, then presumably things will get in. But you've got two roots going on.

You've got esterases and you've got phase two conjugation; and in rodents, I think whichever way you go in -- skin or you go in orally -- you're going to take those suckers out.

It's not 100 percent clear to me, especially after looking at this 2016 paper that I think we need to spend a good bit more time on; because how much of these doses are showing up as metabolites at the other end of the chain. And the potential for those things to have significant estrogenic activity that I don't think has ever been studied.

Anyway, I realize that's long, but it captures most of what I was looking at here in looking at this and then seeing the suppression of aromatase, particularly in prepubertal testes. I don't know if there is any significance there or not. It got my attention that, well, we might be seeing in fact some estrogenic activity because this is butyl. I've never been worried about methyl or ethyl or propyl, and again, even isobutyl has a shorter chain. I've never been worried about those. But butyl and benzyl have been on my radar for a good long time, so butyl still is at this juncture.

DR. SLAGA: I thought NTP did a whole series of compounds. I don't remember --

DR. HILL: Binding studies?

DR. SLAGA: Binding studies. And even the longer chain ones were --

DR. HILL: As is, without hydroxylating at the other end, I wouldn't expect them to have high affinity at all.

The point is, until you hydroxylate, you won't get high affinity. It's amazing there is any estrogenic activity until you hydroxylate

DR. SLAGA: But even that I don't think would be super high affinity.

DR. HILL: You can look at the bridging differences; as I haven't put these on the computer myself, other than just on paper is good enough usually to get an idea. In the longer chain, when you get to butyl it's long enough. Now, it's floppy, so that's going to cost you a lot of binding entropy. There will be a lot of penalty for the rotational freeze out, but still you'd expect that to be substantially stronger than butylparaben itself. Somewhere down the line that needs to be looked at. I was hoping somebody else would dredge this up before I ever said it in any form.

DR. MARKS: So, bottom line, Ron Hill, 20 ingredients are still okay, correct?

DR. HILL: Yes. Parabens are parabens.

DR. MARKS: Safe or insufficient? I almost get a split -- when I heard you, I almost get a split decision on your --

DR. HILL: I feel like we're just -- on the butyl in particular, we're missing some science. And again, I think that 2016 paper is important because they're showing significant quantities of these metabolites popping up systemically that I hadn't seen any evidence of that before. Using a good robust LC-MS assays.

DR. MARKS: Dr. Daston, did you want to make any comments in response to that? Thank you for your presentation this morning.

In a minute I want to move over -- I'll read Ron Shank's comments. If you want to hear his first, maybe that will be helpful and then you can go ahead and comment. The other is, we need to deal with a margin of safety; before we used 1,000, now it's suggested using 160.

Ron Shank, page 13 DART, these studies all produced exposures far greater than would occur in cosmetic use, or gavage, a bolus effect versus dermal. The epidemiologic studies do not support an adverse effect on male reproduction systems. They carry little weight because of the inability to quantify the exposure to parabens.

Page 21. Discussion, the animal studies on butylparaben. They reported adverse effects on various parameters in male reproductive system. Administered the agent by oral gavage. This route of administration produces a more rapid and higher blood concentration, the bolus effect, than

would be achieved by topical application of a cosmetic formulation. In conclusion, add the paraben salts. Old conclusion is still valid. Which is safe.

If you wouldn't mind commenting to, perhaps, some of Ron Hill's edits, and then how do we deal with a margin of safety from the 2008 paper.

DR. DASTON: I guess in terms of the dermal metabolism and absorption, probably the best information we have still is that Janjua et al. paper where they used really, I think, heroic amounts of butylparaben, along with two phthalates that could also have been substrates, so you could have competition.

And even with those heroic amounts, they were able only to see a maximum concentration of 2 percent of the butylparaben in circulation. It just seems to me that, regardless of the fact that there probably are species differences in the esterase affinities and activities, that they are still active enough in humans that the concentrations that would be absorbed are going to be extremely low with any realistic kind of usage.

Then the other thing you were questioning about was the possible hydrolysis. I agree that would be interesting. I'm kind of at a loss as to understanding how that hydrolysis would occur with -- at the end of that --

DR. HILL: No, not hydrolysis. Hydroxylation. So, the P450 catalyzed hydroxylation.

DR. DASTON: So, again, I mean, that would be a very unusual reaction.

DR. HILL: No, no, no. P450's -- lipophilic compounds with aliphatic groups are very good substrates for P450's. So, a butyl chain and omega and omega-1 hydroxylation for a sufficient lipophilic compound is an easy reaction for P450's to do, and an array of them. So, hydroxylation at the distal end -- of course for benzylparaben, an aromatic hydroxylation -- is very common to put a phenolic. But that also occurs with aliphatic ones.

And they're showing these metabolites produced in these women that are getting it in orally; which surprised me because I would have thought that orally coming in through the liver, that we would take out either by combination of esterase, catalyzed hydrolysis, or glucuronidation first pass through the liver -- which is usually pretty aggressive for phenols -- that we would end up with not much in the system. But they're showing substantially detectable amounts and I don't have any reason to think that they're doing something squirrely here.

But butyl is really the only one I'm worried about and the ones that -- if benzyl is off the market, butyl is really the only one I'm worried about because we don't get the distance with the others. So, it's the amount of omega hydroxylation because I think even the omega-1 is on the short side to span the distance needed. We need 10 angstroms to get to that other water molecule from the phenolic hydroxyl, center to center on the oxygens.

DR. DASTON: My opinion is that it would be a very low concentration.

DR. SLAGA: What surprised me was -- I mean, small esters are usually metabolized more rapidly, but I would think that butyl would still be because really no steric hindrance and not much electronically going on, would be just as good. It struck me that maybe that length is sandwiched between really short chain esters and the longer ones that start to get picked up by the lipid carboxylesterases as soon as you get to C6 or something like that. I think it's something that humans and liver, and humans for skin bears some further research.

I think we need effective preservatives. I'm not anxious -- definitely not anxious to see any disappear at the moment from what we've got left. But on the other hand, there has been a lot of -- we have things that we need to be careful about with -- again, I think benzylparaben disappearing from the market, we're not sure why. But I wonder.

THOMAS SLAGA: It's not soluble.

DR. HILL: Solubility is an issue? Yes, but -- yeah, okay.

THOMAS SLAGA: You can't get it at the water base.

DR. HILL: That's where you need it for microbial growth inhibition? Sure, okay.

DR. MARKS: Ron, if you want to comment tomorrow about that, that would be good.

Let's go to page 23 after Bart's memo here, and that's from the 2008 paper where it talks about the CIR expert panel selected a NOAEL of 1,000 milligrams per kilogram per day; that's calculations for adults and then infants. Tom and Ron and Dr. Daston, if we change that from 1,000 to 160 -- if I heard you this morning for a NOAEL correctly -- how does that effect this calculation? And do we still have this confidence of safety that we use hard numbers in here and the calculations?

DR. HILL: The NOAEL is specifically for --

DR. MARKS: If you look on page 23, it goes through the reasoning. And if we don't use the same calculations and come up, obviously, with a new number, what do we do with -- why do we have this MOS, before feel confident, and now we don't feel so confident if we have less margin of safety?

DR. HILL: I don't feel any less confident about the male reproductive effects. I'm still fine with that.

DR. MARKS: What do you with the margin of safety then, that's going to come up?

DR. HILL: With the 160 versus 1,000?

DR. MARKS: Did I hear you correctly this morning? 160 is what you suggested to do?

DR. DASTON: That would be cautious.

DR. MARKS: Yes. Do we run the numbers and then see where they get us?

DR. SLAGA: Yeah, run the numbers and see what comes up.

DR. HILL: I'd still be okay with that, actually. Right now.

DR. MARKS: Still okay? Did you quickly look at this and in your mind calculate it?

DR. HILL: I mean, keeping it at 1,000? I don't know, maybe it needs to be maybe reduced.

DR. BERGFELD: It's going to be 160.

DR. HILL: That's still not going to be a problem is it, for in use products in most cases? We don't cover sunscreens, so when I think of whole body exposure and something that's probable, sunscreen comes to mind. We don't -- That's out of the cosmetic purview.

DR. MARKS: Okay.

DR. BERGFELD: Are you into the discussion yet?

DR. MARKS: I think this is part of the discussion, but I pull it from the 2008, and I think it's really important that in this -- which will be an amended safety report -- that we address that margin of safety calculation.

DR. BERGFELD: Primarily, because you're adding the salt and you're amending the risk assessment?

DR. MARKS: Yes. Well, and then we have the new studies that suggest that 160 perhaps is a better conservative figure than 1,000.

DR. BERGFELD: Well, I would like to add to the discussion, if I might, at this time, that you need to bring in the hydrolysis activity rather than subcutaneous activity and absorption. And you need to bring something in about the accumulation in tissue, which has been considered negligible, to fill out this particular discussion piece.

DR. MARKS: You're anticipating, Wilma. I was going to address that. I think for that -- so we still feel comfortable with safe -- we'll calculate a new margin of safety with a 160 figure. We know these are used in lots of products.

Now, what I wanted to do is -- this was at your desk this morning, so I don't know, Tom and Ron, if you had a chance to read it. This is a letter dated February 28, 2018 to the CIR from the Women's Voices for the Earth. And it's from Ms. Scranton. There's not an MD or a PhD, so I assume it's Ms. Scranton, who is the director of science and research for Women's Voices for the Earth.

She raised three issues as I saw it. The first one was on the bioaccumulation, which you mentioned, Wilma. It needs to be mentioned in the discussion. If I heard you correctly or interpreted what you said, Dr. Daston, the metabolism and excretion of the pharmacokinetics of the parabens would indicate bioaccumulation is really not an issue with these ingredients. So, that needs to be put in the discussion.

Then the second issue was the margin of safety. That's why I brought that up and we've discussed that. That will be in the discussion. Then lastly -- and this is the comment that Don Belsito had referring to a paper you mentioned -- is what is the impact of cosmetic use on the body burden of parabens. We know there are a lot of exposure from other sources such as foods and such.

DR. SLAGA: That should be in the discussion too.

DR. MARKS: Exactly. So, I think we should address that in the discussion.

DR. EISENMANN: There are a number of studies, too, that you could add on that. There was one in your packet that looked at the male, that it pulled out 10 products. And another study in teenagers where they took away products with parabens and looked at it. Then there's also this Campbell PBK model. It really needs to be in the report I think.

Because it's reassuring because they start with the in vitro levels and work back to estimate in vivo levels, and then compare with NHANES data which is accumulative exposure to everything. So, there's a lot of aggregate exposure there that would be reassuring to your NOAEL calculations, your MOS calculations.

I think that Campbell PBK model would be very important to put in. It's not in there. And then Dr. Daston also mentioned one more study that we'll have to get to you, looking at exposure.

DR. HILL: In the Women's Voices letter, she did flag something that I already flagged in here which was this -- it's near the bottom of the second page and it's butylparaben and again, it's in rats. Again, I think human skin in general -- adult human skin -- in most of our areas of skin is a better barrier, if I'm not mistaken, than rat skin. But it's talking about rats exposed to 100 milligram per kilogram and then there is a 10 milligram per kilogram. The language that's in our report right now says most of the dosage, greater than 46.4 percent, was not absorbed, and less than 26 percent was found in the urine.

She wrote the same thing that I wrote in mine, which is if 46.4 percent of the parabens were not absorbed, this implies that actually most of the parabens dosage, 53.6 percent was absorbed. And then they've got something else here, 52 percent and 8 percent of a single 10 or 100 milligram per kilogram body weight dosage of radiolabeled butylparaben was absorbed. So, there they're tracking radiolabel. So, there is absorption of butylparaben.

And again, as I said, human skin is a better barrier, but then we have this piece of information that was new to me that as the chain gets longer, our esterases in humans get worse. We don't hydrolyze as much. Whereas in rats it goes exactly the opposite direction, and mice too.

I think there are some pieces of information we simply don't have, and that's why this 2016 Moos, study that's talked about in Table 10, page 42, where they're showing butylparaben specifically, and what percentage. Like 80 percent of it was absorbed and that's a pretty substantial amount.

Then they're showing these metabolites, which I have never seen a paper indicating that those are there before; and that got my attention. Because in looking at the SAR for estrogens I've said well, yeah, has anybody looked at the P450 mediated distal hydroxylation so that we can get the two hydroxyls on either end and have high affinity binding to estrogen receptors. This is the first I've actually seen that those metabolites were there in appreciable amounts. I think it's something worth following up because a lot of concerns have been expressed.

I don't think, for me, in terms of male reproductive effects, yeah, we can calculate the margin of safety and maybe it's 160 instead of 1,000; but the male reproductive effects, I just don't think the estrogenic effects -- we're not going to be seeing androgen effects from that; because androgen receptors, once you have the aromatic phenolic group on the other end, they just don't bind. They're made not to bind with estrogen, I guess is the best way to put it. Similarly, even with progesterone receptors.

DR. MARKS: Any other comments by anybody?

MR. GREMILLION: The Women's Voices for the Earth letter brought up several studies that weren't included in the report; and I just wondered why there was that discrepancy. I think she mentioned

Ferguson (phonetic), Tahan (phonetic), Sezhi (phonetic), Wang, Gazin. There were several from her previous comments that still aren't in this report.

DR. MARKS: Thank you for bringing that up. I don't know that we specifically discussed -- sometimes we don't include studies when we feel they don't add anything, or scientifically they may not be valid. But Bart, do you have any comment?

DR. HELDRETH: The progress of this report basically stopped back in June, as we tabled it. We didn't bring in any new studies until we covered this issue that we talked about today with the developmental reproductive toxicity issues of parabens. If the panel feels that any of these articles or any of the data submitted does belong in the report, it will make it into the next iteration.

DR. MARKS: Is there any reason, Tom, Ron -- at least at this point we don't have Ron Shank's response -- but these studies shouldn't be included? We can always, as we've done in the past, if there's concerns about the conduct of a study, we could remove it. So, let's include those at this point.

DR. HELDRETH: Will do.

DR. MARKS: Any other comments? Anybody from Women's Voices for the Earth here? I've asked this before, and I certainly wouldn't want to overlook any comments from that group.

If no other comments, then tomorrow I'll be moving that a tentative amended report be issued with a conclusion of safe for the 20 ingredients. The discussion will be quite extensive covering the margin of safety calculations, based on the 160 milligrams per kilogram per day, the reasons why we feel the studies that we've reviewed and the ones that will be included support the safety of these 20 ingredients. We'll address the accumulation issue of the parabens and then also the body of burden issues with the parabens in the discussion. And we'll get to see this all again in the next rendition of this.

Any other comments? Tom? Ron? I think we've captured Ron Shank's then also.

DR. HILL: Let me look back.

DR. MARKS: I see you non-verbally telling me you want to say something more, Ron Hill.

DR. HILL: I'm not sure. I had written a number of notes to myself. I think I covered them all.

DR. MARKS: If you want to, you can review those this evening and bring it up tomorrow. I'm sure we're going to have another robust discussion tomorrow. I would hope we will.

DR. HILL: I was trying to minimize my remarks tomorrow by putting into the transcripts whatever needed to go in there today.

DR. MARKS: And thank you again for hanging around, Dr. Daston.

DR. HILL: I think that's it.

DR. BERGFELD: Can I ask a question? Does the FDA have a comment about the OTC sunscreens and the use of parabens today? Are they addressing this?

DR. KAPAL: I don't have that information. Again, from the cosmetics point of view, I can talk about it, but I'm not sure where OTC is going in that direction.

DR. BERGFELD: Okay. Thank you.

DR. MARKS: Thanks, Wilma. Any other comments. If not, we look forward to our review tomorrow.

#### **Dr. Belsito's Team**

DR. BELSITO: Okay. Perfect. Anything else? It looks like George has made it to our table, so we're going to move to parabens. Do we have the paraben writer here?

MS. FIUME: It's Bart, but I can sit in for him.

DR. BELSITO: Okay. Let's get to parabens.

MS. FIUME: Since he's here we're going to jump to parabens.

DR. BELSITO: This came up just as a 15-year re-review, and then we decided to add in a whole bunch of other parabens and take a look at their safety. And I guess also, in part, response to the

growing NGO agitation about parabens as endocrine disruptors. I have a lot of comments, but I don't think our conclusion at the end of the day changes.

DR. LIEBLER: Nope. It doesn't for me. I'm still okay with including the salts.

DR. BELSITO: Yeah. Include everything that we decided to add on and safe as used.

DR. LIEBLER: Yes.

DR. BELSITO: I guess the only issue when we're doing safe as used is, as you know, in the EU and -- I don't know if we ever did this. They have a total concentration at which a finished product -- I mean, a total concentration for parabens in a finished product. And we, I don't believe, addressed that at all.

MS. FIUME: The additive effect as --

DR. BELSITO: Yeah. I mean, they have, I think, it's 0.8 is the maximum limit of total parabens in any final finished product in the EU. And then I think they came back -- wasn't it last year or the year before -- where they took butyl and isopropyl and further reduced the amounts that could be present in the same product at once.

This came in Wave two, which I only got to yesterday. I didn't really get a chance to search for the SCC opinion in the EU regulations. But I know that they've set new regulations for, I think, it's isobutyl and butyl. And there is a total for all parabens. And we don't have that limitation.

DR. STEINBURG: Don.

DR. BELSITO: Yeah.

DR. STEINBURG: Is this mic on?

DR. BELSITO: I can't hear you George. I mean, David, sorry.

DR. STEINBURG: The European regulations are a total of 0.8 percent of parabens as the acid. They have restricted the maximum use of methyl or ethyl to 0.4 percent. And then they restricted the use of propyl and butyl total to 0.14 percent. They prohibited -- or they no longer have listed -- the isopropyl and the isobutyl parabens and benzyl parabens.

DR. BELSITO: They prohibited those?

DR. STEINBURG: Well, they moved them to Annex 2. The principle reason was the cost of the testing that they wanted done was about three times the annual sales of that. So, industry just was not going to run those types of tests.

MS. FIUME: PDF Page 35, does have a table on some of the history of SCCP's opinions on parabens. Is that what you're referring to?

DR. BELSITO: Yeah. And just my general knowledge of what's going on in Europe, with preservatives, as part of my involvement with Cosmetics Europe and DG SANCO, or whatever they call themselves now. DG SANTE, I guess, is what they changed their name to.

It doesn't state in here -- okay, so the use of butyl and propyl-- that was 2011 -- the sum of their individual does not exceed .19. But all of those have changed recently. In the past five years they've come out with new Regs.

DR. SNYDER: Yeah. That needs to be updated.

DR. BELSITO: My only comments was that -- well I had two. I don't know how you want to proceed, but perhaps we should table the issue and look at how they came up with those restrictions for totals and what their issues were. It was benzyl, isopropyl and isobutyl?

DR. STEINBURG: They're the three that were not supported, so they have been prohibited.

DR. ANSELL: But I believe you actually did review the SCCS opinion after it came out, concerning whether their conclusion of insufficiency on the iso's would have affected your opinion.

DR. BELSITO: I understand that. I guess my question and concern -- and perhaps, George, you can address this, is why they've set limits at .8? Because the way we say it's safe as used, you have a whole bunch of parabens with various ranges of concentration. And if you added them all together, at the ranges we said were safe as used, you would easily exceed the .8 limit that the EU has set.

I just want to point that out, that other authorities have set a total limit on parabens in any finished product. And we're not doing that in our conclusion at all.

DR. KLAASSEN: I guess. I think we're getting into territory that's probably way beyond the science. If you have two compounds that work through the same receptor, which we think they are, it might not be additive, it could even be competitive. And we don't know, from George's talk this morning and all the data that we've seen, if there's any effect in humans.

In laboratory animals it's very high. And then from that to say exactly what's the maximum concentration, I think is -- and adding two and three together, I just think that's way beyond our science. It would be nice if we could.

George, let me ask you this. Are you still here? There you are. Have studies been done in vitro where they had two or three of these "estrogen" type compounds? And do they add? Are they competitive or noncompetitive?

DR. DASTON: Yeah. Not with parabens that I know of.

DR. KLAASSEN: Okay.

DR. DASTON: I think that the prevailing wisdom would be that they would be additive.

DR. KLAASSEN: Do you really think that would be true?

DR. DASTON: I think it probably would. If you think about things leaving the receptor, and then you add something back on, I think adaptivity is a reasonable assumption.

DR. BELSITO: Do you have any clue how they came up with this .8 limitation?

DR. DASTON: I think it's a combination of they are using a very conservative NOAEL for toxicity for butylparaben. And that, along with essentially an aggregate exposure, and a marketplace approach that they take.

DR. STEINBURG: Don, just one comment on behalf of industry. When they propose this, this .8 far exceeds the solubility of all the parabens in water total. Industry just felt it didn't make any sense to argue a point in which whether they said .8 or .6 was academic, because the most you can get into water is about .4 of all the total parabens together. They're just not that soluble.

DR. BELSITO: I guess my point here, though, is that does this make us stand out as a scientific panel reviewing safety, that we have one scientific body on the other side of the pond saying they should be restricted; and this scientific body not making any mention of that. And there's nothing in the discussion as to why we have not made any mention about not restricting.

In other words, we're ignoring -- and first of all, I think that we need to look at the current regulations for parabens in the EU and bring that into the use section. And if we're not going to put a total restriction on parabens in finished products, we need a very robust discussion as to why we feel that's not necessary.

And I guess the last issue with all the parabens is now -- when we last look at this, benzyl paraben had one reported use, now there are no uses. I just want to point out are we still comfortable with that, since we don't know concentration of use other than just the range of concentrations per parabens in general.

I don't know the answers to these, but I do think we certainly need to come up with a very robust discussion if we're not going to put limits as to why we think those limits are not needed. From a dermatologic standpoint, you hardly ever see delayed type hypersensitivity of the parabens.

They are by far the safest preservative system we have; bar none.

This is not my area of expertise. It just gives me a little bit of pause that we're not addressing it in a discussion.

MS. FIUME: This is at the draft report stage.

DR. BELSITO: I understand.

MS. FIUME: Is there information that could go out in an IDA that would answer some of those questions? Or is it just more of crafting the discussion?

DR. BELSITO: First of all, I think what we should decide is, do we want language in the conclusion to restrict total concentration? If we don't, then I think that just maybe table it just to get a little bit more information as to why they've come up with these limitations. And craft a discussion as to why we don't think they need to be in our conclusion. I just don't think we can ignore the fact that the EU has set limits and we're not setting limits.

DR. SNYDER: Could we use the language that we used for constituents of concern in botanicals to say to be aware of it? Or maybe an additive affect and they should be aware of the formulation or something?

DR. BELSITO: But are we concerned about it?

DR. SNYDER: Because we don't have the data. We don't have the data. I don't think we have the data, do we, to come up with an additive.

DR. KLAASSEN: If we're going to give a number for this -- the maximum amount you should be exposed to -- then why don't we do it for every chemical? I mean, we do have a maximum -- I mean, while we don't give the number we say, as it's presently being used.

DR. ANSELL: Right. Current conditions of use.

DR. KLAASSEN: But I don't know --

DR. LIEBLER: We usually would not have the information to make that determination though.

DR. KLAASSEN: I agree.

DR. LIEBLER: So, we wouldn't have the data to be able to do that.

DR. KLAASSEN: And I don't think we do here.

DR. LIEBLER: Right.

DR. SNYDER: I don't think we have it at all.

DR. BELSITO: What are you suggesting, Curt? We don't have the data to make that determination.

DR. KLAASSEN: It think it would be a little bit more information on how the Europeans really came up with this number and read it in some detail. But I'm kind of against the philosophy of doing that.

DR. SNYDER: I mean, while our current use condition do cover the individual parabens, but I don't think it covers the multiple. Because we don't have total parabens, we just have measurements of individual from our use data. I think that if we think that's important, we probably need to address it.

DR. BELSITO: Well, obviously the Europeans do.

DR. SNYDER: Yeah.

DR. BELSITO: I just think we need to be aware of this, and if we don't set limits -- and perhaps we don't need to -- we need to have a reason in our discussion as to why we feel limits are not set.

My recommendation, perhaps, would be to table this. Or, I mean, it's early, go insufficient. And the insufficiency is we want to relook at the SCCS opinion. And look at the data they looked at to derive their reasons for saying that benzyl isobutyl and isopropyl use is not supported. That the total for parabens should not exceed .8. The total for methyl and ethyl should be not exceed this, and the total for butyl and propyl should not exceed this.

DR. KLAASSEN: Does their document describe this in some detail, how they came to these numbers? Or is it just people that just sat around the table know the answer, but it's not written down?

DR. STEINBURG: You have to go back to the origins that when they started the cosmetic directive, they established a positive list for colors, preservatives and UV filters. Now, UV filters in the United States have maximum levels set by the drug division, because they're regulated as drugs.

They just put maximum levels on preservatives. And you'll have to go back to 1975 documents, 1976 documents to find out how they came up with those numbers. They just were there, and no one's really questioned how they even came up with some of them back in the 70's and early 80's.

I know when we looked at some of the more controversial preservatives, such as the isothiazolinones, the manufacturer said maximum use level of 15 ppm for the methylchloro and methyl iso mixture was sufficient. Because that's all they needed to preserve.

The 100 ppm for the methylisothiazolinone, alone, was set strictly because the manufacturing process gave them a 95 ppm product, which they sold as a 10 percent solution, I guess, basically. So, it was easy to formulate with and there wasn't really a lot of science as to why they set that level. Reality levels are probably much higher and people would have used it at a, what, .5 instead of 1 percent as they were using it. Excuse me, .05 versus .1. You would have around 50 ppm in the active, not the 95, which caused so much sensitization.

DR. KLAASSEN: But I'm talking about specifically these paraben.

DR. STEINBURG: You'll have to go back to the early history.

DR. KLAASSEN: Is it written up in a nice document?

DR. ANSELL: In the last SCCS review, I do believe they iterate the studies they used on which to base these calculations.

DR. KLAASSEN: Okay. We need to read -- at least, I need to read those.

DR. BELSITO: So, how do we want to approach this? Table it, ask for the SCCS opinion and then relook at it? Is that fair?

MS. FIUME: There's several SCCS opinions. The 2011 seems to have most of the details. 2013 refers back to the 2011 except for the changes. We can provide you all of that; and look at it a bit more in detail as well.

DR. LIEBLER: We also received this letter from Alexandra Scranton, Women's Voices for the Earth dated February 28th, so obviously we're just seeing it this morning. And I've been looking through this mainly while you guys have been talking about this.

Most of the comments are about the issue of body burden and bioaccumulation of parabens and also margin of safety. The first page cites a paper -- first of all, the first page refers to the assertion in the report text that parabens don't bioaccumulate. I think that is taken actually from PDF page 10, under ADME.

The 1984 report language, summarized in italics, which only summarizes the 1984 report, but it says data obtained from chronic administration studies indicate that parabens do not accumulate in the body. So that is a paraphrase of a conclusion -- or not the conclusion, but of a statement from the 1984 report. And then also cites some discussion between myself and Don and Ivan, regarding the bioaccumulation.

There's a paper that she cites, Wang et al., which is in the bottom third of the first page of her memo, which I pulled up and I've been browsing at during our discussion here. It's actually a pretty good paper, but it's a study -- I mean, I think the analytical methodology is very sound.

But it's a study of a variety of heterocyclic compounds, environmental related phenols, everything from parabens to this bisphenol and other molecules.

And it's true that they can measure the parabens in liposuction and fat samples. And they refer to early work that they've been able to measure parabens in excised breast tumor fat.

The paper that she cites here, 2015, did measure parabens in concentrations in fat from older versus younger individuals. And show that there was no clear relationship between that. There's apparently no evidence in that paper for bioaccumulation.

Ms. Scranton cites a few other papers in the last page of her memo, that I would like to look at, that I don't think were in the report. But I think she has a point that we should evaluate to make sure that our report is very clear about the issue of bioaccumulation. Whether it actually impacts our assessment of safety is another question entirely.

While we're tabling this report and looking at that, I'd like to see those other references. I have the one paper from Wang et al. already. But I think we should distribute those, and look at those, as part of our evaluation.

MS. FIUME: So, summarized in the document itself?

DR. LIEBLER: I think so. I mean, I think the points that she raises in her memo are quite reasonable for us to consider. And I, and I'm sure others on the panel, would like to have a closer look at the literature on this.

DR. BELSITO: Okay. So, specifically, Dan, you want all of the references here?

DR. LIEBLER: Yeah. The reference on the first page and then on the last page. The Wang paper I already have, I can share with you guys. And then the others I didn't try to pull them up yet because I don't have the full references.

DR. BELSITO: So, we want to look at the references that Alexandra Scranton brought up in her --

DR. SNYDER: The most important one is the Boberg, because she's using the Boberg to come up with the NOAEL 10, of which I heard Bob say this morning that that's probably not good because it was a non-dose response --

DR. DASTON: George, you mean.

DR. SNYDER: George, I'm sorry. So, I think we need to consider that. That would be bringing in the non-dose response to epidermal sperm concentrations in an underpowered study and highly variable. And I think that the weight of evidence of all the studies -- you said it was -- 160 was what you would suggest would be conservative.

DR. DASTON: It would be a cautious number.

DR. SNYDER: I think we need to capture some language in reviewing that and see if we agree with George.

I had a question for you, because I read through the Garcia paper many times because I really had a hard time following that study. I mean, the parameters are highly variable in controls, which is -- even the sperm parameters in the rats, which are usually relatively stable, were all over the map.

Which led me to think, plausibly, what could be going on in that study, and how much does decreased bodyweight start to really effect the repro parameters. Or when do you consider bodyweight decline to really start to give you an unease about you're actually seeing a direct repro effect and not an indirect effect on bodyweight -- mediated through bodyweight?

DR. DASTON: You would have to have some pretty severe effects on bodyweight to get to infertility in the animals. My feeling on the Garcia study, is it's more of a methodological problem because you start looking at those standard deviations, which I didn't highlight, but are in that table. And they're much higher than what you would expect from other studies; and that's when we did the statistics, it was paralyzed, and it didn't come out the same way.

DR. SNYDER: Okay.

MS. FIUME: Regarding the Boberg study, you'd just like to have it --

DR. SNYDER: Well, no. What I'm saying is in our margin of safety, we use an older study that NOAEL was 1000. And we heard discussion this morning that maybe that more approximates, so maybe 160 can be justified. And the Wave two Earth people are saying 10.

And so, I think we need to figure out where we think scientifically it's plausible that we have a conservative NOAEL and go from there. Because if we use the 10, as they say, it's gets you down to a margin of safety of 1; we used 1000 and we had a greater margin of safety. I think we have to relook at that.

MS. FIUME: Okay.

DR. LIEBLER: We have to evaluate whether we accept using a 10, right?

DR. SNYDER: Based on an underpowered study.

DR. LIEBLER: Right. Exactly. Reason to be skeptical about using 10.

DR. SNYDER: Correct. And see if we agree with George in the assessment of 160. And even then, I was thinking 160 was --

DR. BELSITO: 140, wasn't it?

DR. SNYDER: 160.

DR. BELSITO: 160?

DR. SNYDER: Yeah. Because at 400 then you start having effects; so, there's nothing at 160.

DR. BELSITO: We need to determine what we think the NOAEL is?

DR. SNYDER: Yes.

DR. KLAASSEN: George, this study was done IP -- I mean subq?

DR. SNYDER: Oral. Oral.

DR. KLAASSEN: Oral?

DR. SNYDER: Zhang and Boberg were oral.

DR. KLAASSEN: Anybody done pharmacokinetics on blood concentrations after applying it on the skin?

DR. DASTON: Yeah. There's a study by Janjua et al. But it's a full-body application, early heroic levels, butyl paraben and a couple of phthalates at the same time. And they were able to show that about 2 percent of the butyl paraben is intact as a maximum concentration. And they also did some estimates of elimination half time, suggesting that's it' fairly rapid. And that, I think, is reviewed in a previous CIR.

DR. BELSITO: I guess the other thing I'd like to see brought into our document is the paper that George referenced before about the cosmetic use versus other uses. If we could get that paper to put into perspective.

And this is the same issue we had with the fragrance panel all the time. You know, where is the exposure coming from. Is it naturals? Is it flavor? Is it actually fragrances? I think it would be nice to put into perspective the potential burden of parabens from cosmetics versus multiple other sources of exposure.

Before we finish this off, let's just look and see -- so it does enhance penetration. There's also maybe something in the discussion that we would want to bring in as we look at this. It's on PDF Page 10, where it talks about the human liver microsomes having the highest hydrolytic activity. But then below that, it seems to be contradictive by a statement that was just the opposite.

In the rat liver micro and human liver, it says the hydrolytic activity is greater in humans. Then in cell cultures it says, butylparaben was rapidly cleared in hepatocytes from rats. It was cleared more slowly in hepatocytes from humans, which made no sense to me. This is PDF Page 10.

DR. LIEBLER: Yeah, but cultured liver cells, depending on how that was done, that may not reflect what you would get from microsomes that are freshly prepared from fresh liver, which is what the -- microsomal studies essentially represent the content of enzymes in the liver, at the time it's prepared. Whereas, when you make hepatocytes, you take liver cells and then they're cultured over time, expression of genes changes and adapt to --

DR. BELSITO: So, you think the in vitro studies, with the microsomes, are much more accurate than the cell culture studies?

DR. KLAASSEN: Yes. For that purpose.

DR. BELSITO: For that purpose.

DR. LIEBLER: Right. Yeah.

DR. BELSITO: Okay. So then from what we understand, parabens will be more rapidly hydrolyzed in humans than they would in rats.

DR. KLAASSEN: Well, part of the question is also, is some of this hydrolysis occurring in the skin and in the blood even before it gets to the liver, which is all possible.

DR. LIEBLER: This is all cultured hepatocytes or liver microsomes, right? And so, I think all you can say is that parabens are metabolized by animal and human microsomes and cultured hepatocytes. And I don't think, necessarily, there is a conclusion that you could draw like humans faster than rats, based on any of this.

DR. SNYDER: We have a sentence that says that, though, the last sentence.

DR. LIEBLER: Yeah, but I don't think that's really supported. If the sentence is about that study in what they report, then that's fine. But I think the sentence drawing that overall conclusion -- batch --

DR. BELSITO: Into our discussion would be reasonable.

DR. LIEBLER: Exactly. Batch to batch, liver/humans, it's just going to depend on how long it's been since death, how well preserved, blah, blah, blah. All those things are going to affect that.

DR. BELSITO: Right. You don't think we should bring that out in the discussion?

DR. LIEBLER: No.

DR. KLAASSEN: No.

DR. LIEBLER: Okay. The other question I had was on page 14 of the PDF where they say that -- this is the last paragraph above the genotox study. Where they were finding changes at 100 ppm.

And then it goes on to say the authors conclude that the NOAEC was the highest concentration tested, 10,000 parts compared to the NOAEL of about 1140 to 11,000 milligrams per kilograms per day. And I don't know how to do all of these conversion, but it seemed that the NOAEC therefore, would be much higher than 100 parts per million based upon those numbers and milligrams per kilograms per day.

I mean, they don't make sense to me although I don't know how you do those calculations. I mean, when you're talking about thousands of milligrams per kilograms per day, and then you're getting down to parts per million.

MS. FIUME: We can check it and make sure.

DR. SNYDER: That's the Hoberman paper, so.

MS. FIUME: We'll look into it and make sure the numbers are correct as reported.

DR. BELSITO: And then, Curt, I had a question for you on page 15 under the methylparaben. Where it says that maintenance of S-phase in OHT-treated cells, like apoptosis evasion, was correlated with increasing concentrations of methylparaben. Does that bother you at all? Is it significant?

DR. KLAASSEN: I think these in vitro studies are kind of like these clinical reports. You know, you have to be pretty careful in interpreting them.

DR. LIEBLER: Which page is this?

DR. SNYDER: It's under page 15. The bottom of the page, the last sentence above other relevant studies.

DR. LIEBLER: Oh, where you just dump chemical in a bunch of cells?

DR. SNYDER: Yeah. There are cells that were harvested from high-risk breast epithelial cell donors.

DR. LIEBLER: I think we have to note those things in our report, but they are not representative of in vivo exposures. Unless it's a well-designed study, where there's a cellular endpoint and exposure, it is representative of a testable hypothesis about in vivo action, these things are just chaff.

DR. BELSITO: Okay. Anyone else have comments on the parabens or questions for George? And then I can summarize where I think we are.

DR. LIEBLER: Just thanks for a great presentation.

MR. DASTON: You're welcome.

DR. SNYDER: See you in 2028.

DR. BELSITO: Where I have where our team is, just to recap; is we want to table the report for now. We would like that paper on the volume of parabens in cosmetics versus other sources of exposure. We would like to look at the relevant SCCS opinions regarding concentration limits on the various parabens.

We would like to review the references that Alexandra Scranton brought up in her letter and consider those in light of George's presentation today. And at the end of the day, we need to assess what we think the true NOAEL is for the DART studies based upon all of that.

DR. SNYDER: Yes. Perfect.

DR. LIEBLER: I agree.

DR. BELSITO: Okay. We're done with parabens, I think. Any other comments?

DR. SNYDER: Bile break.

DR. BELSITO: Bile break and Dan needs a bile break. Okay. Well, it's 11:15 so can we do a 5-minute bile break. Okay.

### Full Panel

DR. MARKS: Seven parabens were reviewed and published in 2008, with a safe conclusion. Last year we decided to add 12 more parabens, reopen that report as a re-review, and then also add 4-hydroxybenzoic acid for a total of 20 ingredients.

Also, at the meeting last year, the panel was concerned about new data for developmental and reproductive toxicology. Yesterday we heard a very complete and in-depth presentation by Dr. Daston. We felt that we could move ahead with a tentative, amended report with a conclusion of safe for the 20 ingredients.

There is a fair amount we would put in a discussion, but that's the motion from our team.

DR. BERGFELD: Is there a second? Seeing none, a discussion?

DR. BELSITO: I personally just wanted to table this for several reasons. First of all, Europe has put limits on the total amount of parabens that can be present in any one cosmetic product. And there have been a number of revisions to the SCCS reports and decisions regarding this.

I am somewhat familiar as to why they came up with those restrictions. I think some of them had -- I'm not sure -- were environmental. I get sometimes confused when they do environmental restrictions plus human health restrictions.

But be that as it may, they have total restrictions. And if we say safe as used, we're not putting those total restrictions in the final amounts of parabens that can go into a product. And I would like to understand that. I think we would need to address that in a discussion if we disagree with that.

They have restrictions not only on total parabens, but they have also said, I believe it's isopropyl, isobutyl, and benzyl should not be used. I would like to be able to discuss that in our discussion if we feel they're safe as used.

I think that this conclusion would differ significantly from the conclusion that's been issued in the EU, and we need to capture that data; we need to look at it and we need to decide, do we agree with them or do we disagree with them, and either way put that into our discussion. I would like to table it for that.

DR. BERGFELD: Is that a motion?

DR. BELSITO: Yes. And there's one other point that I would like to make. We were told that there is a paper out there that gives us a relative idea of the volume of parabens that are used in cosmetics versus the volume of parabens that are dumped into foods and drugs and other things. And I think that's a very important source on parabens when you start bringing in data into your report, saying oh, you know, this level of paraben is found in the urine of people, it's found in breast tissue, it's found in here, just to get a sense as to what are the other exposures. Because too oftentimes people want to blame cosmetics for the exposure to a specific chemical, when the greatest bulk of exposure is coming from some other source.

I would just like to table it to try and capture that information. I think it's going to come out safe as used. Do we want to put a restriction on total concentration, maybe, maybe not. But I would just like to get all of the data on here because it is such a controversial group of preservatives.

DR. BERGFELD: Is there a second to table, or another comment before that?

DR. MARKS: I'll withdraw my motion and I'll second the motion to be tabled. We --

DR. BERGFELD: There's no discussion with that, so I need a vote. All those in favor of tabling? Thank you. Unanimous. Go ahead, discussion.

DR. MARKS: In addition, Don, to what you mentioned, our team discussed -- and we expected we would see it in the next rendition of the report; and we will, but it will be tabled, and we'll see a more, I

think, robust report to look at and more data. But looking at the margin of safety again, using the 160 milligrams per kilogram per day, and calculate the safety of that margin of safety, we wanted to address the accumulation of the parabens.

This is again from the Woman's Voices of the Earth Letter, dated February 28, 2018. Our feeling was the metabolism, the excretion and the pharmacokinetics of the parabens made accumulation in the body not an issue; and the body burden. And I think that's what you were referring to, Don, when you mentioned how much comes from cosmetics versus other sources of parabens. And add those papers and make the discussion concerning that.

DR. BELSITO: Dan in particular wanted to review several of the papers that were referred to by Dr. Scranton and Women's Voices for the Earth, too, before signing off on these; and I think my other panel members also.

DR. LIEBLER: Thanks. That was exactly what I wanted to emphasize, that some of the literature that she cited was not in our report. There was one paper that she cited in the beginning of her letter that I manage to pull up during our discussion yesterday.

Actually, analytically, it's a very good study, but it's not just parabens, it's a lot of different molecules, some of which they presented data for bioaccumulation. And for the parabens, it was ambiguous at best, and apparently no bioaccumulation. But on the other hand, presence in the tissues examined.

I think we'd like to incorporate that other literature into our report, and at least be able to consider it, to address the points that she raised.

DR. HELDRETH: Just a matter of process, we typically table reports when the information that we're seeking is not going to be immediately available. Say if there is a study we know that another agency is going to be doing, we'll table it to wait for that. Or we tabled this report to wait for Dr. Daston to come and talk to us about this spermatogenesis and the other reproductive affects.

My suggestion would be that instead of tabling it, we just mark it currently as insufficient for the information that you've requested, and CIR staff will incorporate that information in here, and it will come back as a future iteration; and the report will keep moving forward in that way.

Because currently, we're only at the draft report stage. So, that means, even with that new information, the panel is going to get to see the report at least twice more.

DR. BELSITO: I'm fine with that.

DR. BERGFELD: I think that's a reasonable thing to do. I think everyone will agree.

DR. BELSITO: Okay. So, insufficient to bring in the SCCS opinion. Get that paper on relative cosmetic use versus non-cosmetic use of parabens. Get the original papers that Dr. Scranton referenced, and let's take a look at all of that.

DR. MARKS: Second.

DR. BERGFELD: Good. Everyone agrees, nod your heads. Okay. Ron Hill?

DR. HILL: For me, one of the most important papers in here appears somewhere down in Table 10, on page 45, which is the moos 2016 paper in our archive toxicology that's dealing with -- in humans -  
- dermal absorption and metabolites.

What I talked about yesterday was, what I know about the SAR -- and I've been teaching this for a long time and looking at it carefully -- the estrogen receptor binding to both alpha and beta and subtypes, is that for high infinity binding you need hydroxyls at both ends. And there's a metabolite of butylparaben that satisfies those criteria potentially.

And I needed the time to find out has that ever been studied in terms of estrogen receptor binding; because I would have thought, from all the information I had seen before now, that that would be potentially a problem with benzylparaben. And I wondered if we're potentially going to clear benzylparaben, even though it's no longer in use in our review. So, I'll see what's known about that.

But the point is, has anybody actually ever tested, rigorously, the binding of that butylparaben metabolite that could potentially meet the criteria for the SAR? Because up until now, I'd assume that some combination of glucuronidation or esterase metabolism would cause those to not appear systemically in appreciable amounts.

And then the other things was some information -- and it was in a different paper that suggested that in humans, going through the skin as the chain gets longer, the esterase metabolism slows down. We don't get as much biotransformation.

We've heard in past presentations, you don't have to go all the way through the skin, all you need to do is get to the valuable epidermis to where you have blood flow. We need to have a better handle on -- I was only concerned about butylparaben in this regard, but benzyl, if we're going to keep that into the report, what else is known.

I just call people's attention to reference 51, because it paints a different picture of absorption in humans of these things that I would have expected.

DR. BERGFELD: Thank you. Any other comments? So, we're moving the parabens to insufficient. And the data has been requested and will be incorporated according to what has been said.

Moving on to the next ingredient, which is probably phosphates --

DR. BELSITO: Wilma?

DR. MARKS: That means a tentative, amended report with a conclusion of insufficient is going to be issued.

DR. BERGFELD: I thought you would hold that.

DR. BELSITO: Yes.

DR. BERGFELD: I thought you were holding it for more information. Can you clarify, Bart?

DR. HELDRETH: We're going to take it forward and keep it in the process to a tentative report. It'll be insufficient --

DR. MARKS: Tentative amended.

DR. HELDRETH: Correct. And then the panel will get to see it the next time it comes, and then even one more time before it goes final. So, even with all the new information in there, you'll get two bites at the apple.

DR. BERGFELD: Okay. Good.

DR. BELSITO: I think the point, Wilma, was the data's out there. The SCCS's opinion are there and the paper on cosmetic use versus non-cosmetic, we were told yesterday.

DR. EISENMANN: I have one question. When you create the tentative report, it will have all the new additional information? In other words, it won't be released in a week, like this?

DR. HELDRETH: That's correct.

DR. EISENMANN: So, the 60-day comment period won't start until after you've added all the information that the panel provide?

DR. HELDRETH: That's correct. My plan is to certainly get all of that information in there. We're now going to have a staff toxicologist on board, I'd like him to go through it and set up the process. In all likelihood, this will come back to the panel in September. It will be issued with at least a 60-day comment period for input from any stakeholders.

DR. BERGFELD: Okay. Have we clarified what we're doing with this ingredient.

DR. MARKS: These ingredients, yeah.

DR. BERGFELD: Moving on then. Dr. Belsito, you're up again. The polyol phosphates.

**[Discussion of Parabens]**

**SEPTEMBER 2018**

**Dr. Belsito's Team**

DR. BELSITO: Parabens. Oh, my God. Okay. Where do we want to start with this? Do we want to start with the Wave 2, Women's Voices for the Earth? Or do we want to start with the document per se? Because there were just some issues that she raised in her paper that I really couldn't find in our report, and I was just curious why. The effect of parabens on sperm, that Samarasinghe paper.

DR. ZHU: What page -- paper?

DR. BELSITO: It's on Wave 2, PDF 407. Where she refers to an effect on parabens and generation of reactive oxygen species and human spermatozoa. And she gives a reference to a Samarasinghe paper. Wave 2, PDF 407, third paragraph down, last line. There's a reference to that paper that doesn't appear in our report.

DR. LIEBLER: I read all of these papers. The Samarasinghe paper, and there was --

DR. BELSITO: There also was a Mundy paper that wasn't in our report.

DR. LIEBLER: Yes. These in vitro studies, essentially, they took freshly-collected donor sperm. They incubated it with different concentrations of parabens, and then they measured a collection of different endpoints.

In the Samarasinghe paper, the most unambiguous results they had were for the effect on motility and viability of sperm. Not having worked in the sperm biology field, I have no idea if experiments that go up to 24 hours in vitro are valid; if they're still viable and you can take valid measurements. So, they have a lot of data at 24 hours, they had some at zero, two, and five hours. With that caveat I'm not sure how to interpret that.

But, they measured several other endpoints that were -- probes that indicate the formation of oxidants. They got really kind of inconsistent, ambiguous data with those, also at the highest doses and the longest time points. And they also measured a DNA damage endpoint, using an antibody for 8-Oxo-deoxyguanosine, which is an oxidation product in DNA.

And they did not see significant effects of the parabens on 8-oxo-dG. 8-oxo-dG is known to be an endpoint that is subject to confounding factors in measurements, particularly when you have an assay that have a high background. And they even note that in their paper. They note that this isn't a very good assay. Then they went on to this TUNEL and halo analyses. The TUNEL was for DNA breaks. The halo was another test that looks at DNA integrity indirectly. And these didn't really produce significant differences.

The thing that you would worry about in their kind of take-home headline was potential DNA damage to sperm by parabens. And the data for the DNA damage, per se, is the weakest part of the paper. And the data for the effect on sperm morphology, viability, and motility was more clear-cut, but I'm not sure how relevant that is to in vivo effects of parabens on sperm or male fertility.

Anyway, I put this paper into the category of a lot of papers we have, where they take some cultured cells and dump a chemical in, and they observe some deleterious things. It's usually never a driver of our decision process.

DR. BELSITO: Okay. Paul's comment was, "Exposure from vaginal product, very weak data, has not been reproduced. I don't see this as new, we already acknowledged the effect on sperm and our NOAEL is based on sperm effect, Reference 58." And the Mundy paper?

DR. LIEBLER: But, I should say I have no problem with adding this to the report.

DR. BELSITO: Right.

DR. HELDRETH: Add it in, essentially, in the reproductive section, and then comment in the discussion about how the data is weak?

DR. BELSITO: Yes.

DR. LIEBLER: Yes. Same for the Mundy paper, we can add it. Then there's a bioaccumulation issue.

This one, I read these papers pretty carefully. The first one was the Wang paper. It was actually in our report last time and we did discuss it last time. It might've been in Wave 2 data last time.

DR. HELDRETH: Yes, there was a brief discussion about it at the final meeting, but it didn't come to the full panel.

DR. BELSITO: I see it in the reference list, but we don't discuss it at all in the document.

DR. LIEBLER: The Wang paper and the other one from cohort in southern Spain, the Artacho-Cordón paper.

DR. BELSITO: That was not in ours.

DR. LIEBLER: -- that wasn't in ours. I think those should be added. The Wang paper and the Artacho-Cordón paper, as far as the analytical methodology for measuring the parabens, it's excellent. I mean, they're doing exactly the right kind of an analysis, given the state-of-the-art for analytical technology for these small molecules; and so they're measuring and quantifying these things accurately.

There are two problems with these papers, and they pretty much acknowledge it. They don't really have the longitudinal cohort, to type -- design in specimens that would allow them to really assess bioaccumulation over time. They have to try and infer that.

And then there's a major technical problem with the Wang paper, and it has to do with the source of their samples. They did this in liposuction fat. The idea was that the parabens would get into adipose tissues, so they used samples of fat from liposuction. And I didn't know anything about liposuction, but apparently when you do liposuction to vacuum out this fat, they use an alkaline alcohol aqueous mix to help solubilize the fat, so they can get it out through the hose. And the Alkaline PH will hydrolyze the paraben esters, so you get para hydroxybenzoic acid, and then methylol, or ethanol or propylol.

And, they point out that they measured very high levels of pure hydroxybenzoic acid relative to the parabens they detected in their samples. And they said, we suspect this is due to possible hydrolysis during the liposuction procedure. And so, they essentially acknowledged that it's likely that their samples are tainted or compromised with respect to being able to measure the paraben.

They were the ones that took a swipe at trying to measure the relationship of paraben abundance in the tissues with age of the subjects. And they had that curb -- well it really wasn't a curb, it was just a straight line across the top in that figure with no apparent age dependency.

I think that based on what we know about the effect of the sample treatment on what they're trying to measure, and the fact that they didn't really have any kind of longitudinal design, I would say that the data show that parabens are present in lipids, in fat, despite the fact that they are robustly metabolized. And this led me to edit the discussion in our report, to acknowledge that, rather than to try and take the position that because parabens are so robustly metabolized, that they don't "bio-accumulate."

I think that's an argument you can't win. Because, whether something bio-accumulates or not, really is a function of how good your analytical methodology is.

DR. ZHU: So, the limitation of the Wang study will be addressed in our discussion, or not?

DR. LIEBLER: Yes.

DR. BELSITO: And the Artacho?

DR. LIEBLER: That was my comment about those. I'm kind of going through Bart's memo with your question about, what should we do with respect to these points raised by Alexandra Scranton and her Women's Voices for the Earth.

DR. BELSITO: Paul said, "I don't see our scientific understanding as changed regarding bioaccumulation. The discussion will need to be edited to reflect our current understanding. The opposing findings of parabens in tissues stratified based on age needs to be strengthened in the discussion. The wildlife data is likely species-specific and related to metabolism of parabens and

not likely relevant to humans. If the data is to be included in the report, we'll have to address why it is not relevant to humans."

I guess I was perplexed where they -- I mean, these animals aren't using cosmetics; what is it, cosmetic runoff into the environment?

DR. HELDRETH: They're just saying any environmental source, so we don't even know what it's from. It could have been some other source of paraben.

DR. LIEBLER: I suggest that we leave the critters out, add the human papers.

DR. BELSITO: Okay.

DR. HELDRETH: You have that new discussion section verbiage in your version for Jinqiu?

DR. LIEBLER: I do. When we get to the report I'll talk about it.

DR. BELSITO: Okay, so then the next issue is personal care product use that's the most significant contributor to paraben exposure. That was sort of hit hard. And it does sort of seem that our report is saying that.

DR. HELDRETH: Essentially, the conclusions about what the sources were that we put in the report were directly from the papers we pulled. We weren't trying to make our own conclusion. We wanted to leave that to the panel to decide what they felt was the overall feeling.

DR. BELSITO: It seem to me that what we're saying is that cosmetics were the greatest source of exposure.

DR. LIEBLER: Yes, and that, again, all boils down to the wording in the discussion.

DR. BELSITO: Did you wordsmith that at all?

DR. LIEBLER: Yes. We'll come to that.

DR. BELSITO: Okay, so yes.

DR. LIEBLER: We're just going down the checklist for this memo here.

DR. BELSITO: Right. So, Paul's comment, "...was bothersome to me if, in fact, cosmetic use leads to the greatest exposure as we reported and thought that non-cosmetic use was the greatest exposure. This must be accurate..."

And then particle size. I guess this is a continuing debate, right, looking at particle size that you can make in a material versus particle size that comes out in cosmetic products.

DR. LIEBLER: Right. We just don't know it.

DR. BELSITO: And then margin of safety calculations, we're going to update that with a new concentration for butylparaben at 0.5 percent?

DR. HELDRETH: Jinqiu already did that calculation.

DR. ZHU: Yes, we did that calculation.

DR. BELSITO: Okay.

DR. HELDRETH: And the MOS is still above 200.

DR. BELSITO: So, we're going to include and discuss the Wang reference and the Artacho reference, and the Mundy and the other one. And we'll put those in the discussion. And, Dan, you've wordsmith those?

DR. LIEBLER: The discussion, yes, when we come to that.

DR. BELSITO: Okay. Then we're done with the Women's Voices for the Earth concerns. We'll write a very detailed letter addressing point by point.

Okay, so then let's go into the document. And Dan and I had a sidebar discussion with Bart about where the SCCS got this data. And, it's in one report that 0.19 for butylparaben, but I don't know how they derive that data.

DR. LIEBLER: Right.

DR. ZHU: Actually, for the margin of safety calculation, first they put 0.4 percentage into the calculation. And it was based on that value and the NOAEL at 2 milligrams per kilograms per day. And then you will get a margin of safety at 46. So, that means it's below 100, at least acceptable margin of

safety. So, and then go back and recalculate it. So, it should've lower the concentration to be 0.19, to get a margin of safety at a 10. That's what we did in that calculation. Basically, it's based on LOAEL -- actually it's a NOEL, not a NOAEL at 2 milligrams per kilograms per day. And then, it first included calculation at 0.4 percentage. And then it generated a margin of safety at 46. So, basically, it's not an acceptable margin of safety value, and then we recalculated it. When they decreased the use concentration from 0.4 percentage to 0.19 percentage, and then -- at least it get a margin of safety at 100. So, that's why -- how we derived that.

DR. BELSITO: For butylparaben?

DR. ZHU: Just for butyl. But, we don't have any clue how they derive the maximum use concentration from methyl, for ethyl and for the paraben mixtures. All of those measurements we don't have a clue.

DR. LIEBLER: It's hard for us to respond or to take into consideration, the SCCS approach, if we don't even understand how they did it.

DR. ZHU: Yes, actually when I go back --

DR. LIEBLER: What their reasoning is. I mean, I don't see how we either affirm it or take issue with it; we acknowledge it, I suppose. I don't know, Don, what do you think here?

DR. BELSITO: The two milligrams per kilograms was?

DR. ZHU: That's the (inaudible) they factored in. Actually, it's from a 1999 Fisher paper.

DR. BELSITO: Fisher study, right, which is not in our document.

DR. ZHU: Actually, there are a lot of limitations regarding that study. First, it's subcutaneous injections. And the second it's not an OECD (inaudible) study.

DR. BELSITO: Yes, but we don't even address it in the document. It's not part of our document. We did not see that data. I looked to see that study, I could not find it, certainly not in our reference list.

DR. ZHU: Yeah. That study is not included in our report.

DR. BELSITO: It should be.

DR. ZHU: Sure, no problem.

DR. BELSITO: If it's the basis of the SCCS opinion, we need to look at it.

DR. ZHU: Yes, basically it's a very (inaudible), you know, when it's a -- I looked at the details of the study, so like for the treatment in the controlled group only five rats. And in the paraben-treated group, only three rats. And that treatment period it only covered the postnatal period, not cover the gestation period. So, not like the principle study we based on, so to get to those value at 160.

DR. BELSITO: But it's the study that they used to set their value, and we're not looking at it at all. If we don't have that document, it's hard for us to criticize it, or critique it, or come up with our own value.

DR. ZHU: Sure, I will include that.

DR. HELDRETH: We need to add it and explain those issues with the study design.

DR. ZHU: Yes, okay.

DR. KLAASSEN: What page is that? I had this is supposed to be around Page 86, but I can't find it now.

DR. BELSITO: What we have is a 10 milligrams per kilograms for butylparaben, is that what you're looking for in our document?

DR. KLAASSEN: Yes, how we came up to ours.

DR. BELSITO: Ours is in a table. It's at the end; it's not in the text itself it's table --

DR. KLAASSEN: I thought we had it also in the text.

DR. BELSITO: No.

DR. KLAASSEN: That might be why I can't find it. Oh, here, it's Risk Assessment on 96.

DR. BELSITO: Yes, but the data itself is in endocrine activity for butylparaben. I think it's PDF 132.

DR. KLAASSEN: Okay, I have some questions about our calculations. First of all, how did we come up with the absorption? We used 50 percent, as I recall, in here; is that correct?

- DR. ZHU: Yes, we used 50 percent. And SCCS used 3.7 percent.
- DR. KLAASSEN: Yes. I think we should use 3.7. Why did we use 50?
- DR. ZHU: It was used in our 2008 report. And generally, it's a conservative assumption given it is recommended by the SCCS guidance.
- DR. KLAASSEN: Okay, so, you know why people use 50 percent, the real, real reason why people use 50 percent? Because you can't be off by more than 50 percent.
- DR. ANSELL: It's halfway between zero and 100.
- DR. KLAASSEN: Halfway between zero and 100, I mean, that's how scientific it is. And if you don't have any data, okay I could go along with that. But we have data. There are two to three studies here and 3.7 is what I think we should use, because that's what the data says.
- DR. ZHU: Okay.
- DR. KLAASSEN: And, that 50 is a goofy number. And then, that makes ours even safer. And then, we should state there why we use the 3.7, because that came from such-and-such a study.
- DR. ZHU: Yes, actually we can -- can we just cite that the SCCS uses this value, or we have to go back to look at all those studies? I think it's based on three studies and all these studies are based on population of data.
- DR. KLAASSEN: Well, I'm not talking about their calculations, I'm talking now about our calculation that we want to use. If I understand correctly --
- DR. ZHU: Yes, actually, this study is coming from the SCCS report; so, it's based on three studies to get to the 3.7 percent.
- DR. KLAASSEN: That's fine. That's fine. Okay. So, if you go to Page 97 of our report, you have how you calculated that. First of all, we state that we are using 17.76 grams per day. So, where did that come from?
- DR. BELSITO: Product use.
- DR. ZHU: Yes, probably products we use per day.
- DR. BELSITO: Products and practices.
- DR. ZHU: Maximum use dose.
- DR. KLAASSEN: Yes, okay. But I think we need a reference for that. Who says that people use about 17.8 grams of this a day? That came from somewhere, we didn't dream it up.
- DR. BELSITO: It came from us, right?
- DR. ZHU: It's from our 2008 report.
- DR. ANSELL: I think both SCCS and our data all come out about 18 grams; but including the reference is entirely appropriate.
- DR. KLAASSEN: Yes, that's what I'm saying.
- DR. BELSITO: You did that, Linda, right?
- MS. LORETZ: Yes, but that particular value is from the SCCS.
- DR. BELSITO: Okay. But does it concur with the value that --
- MS. LORETZ: Yeah. I mean, basically, they kind of use a similar approach and similar numbers, so it's consistent.
- DR. KLAASSEN: I think we should use our numbers.
- DR. BELSITO: It's about the same is what we're saying.
- DR. KLAASSEN: I know, but that's okay. If there're two numbers -- it's kind of like when you publish a paper; if there're two people that have reported this, and one of them is you, who do you cite if you don't use both?
- DR. BELSITO: Right.
- MS. LORETZ: But there's actually not two numbers though. I mean, we've kind of always used the SCCS because they're the ones who put together a number for a total number. And they actually did it specifically for preservatives.
- DR. KLAASSEN: Okay.

DR. BELSITO: And the 160 mg/kg, Curtis, the table on PDF 126. The two studies on butylparaben at the bottom.

DR. KLAASSEN: Yes, but I think right in this calculation you need to say where each of these numbers come from.

DR. ZHU: Okay.

DR. KLAASSEN: You know, this came from this study, this came from this study, and this came from this study. But, the only number I don't agree with is the 50 percent absorption, I think that should be the 3.7 or for one of those type studies.

Then, the other thing, we also have another calc -- it's this report, isn't it, that we have another calculation, or not? It's a different study. But so, our margin of safety is going to be much larger.

DR. ZHU: Yes, based on 3.7 percent.

DR. BELSITO: Assuming we keep the 160 mg/kg per day, as opposed to the two mg/kg per day that they used. And, we haven't refuted that paper yet, because it's not in our report.

DR. ZHU: I have one question, on PDF Page 96, and the last paragraph.

DR. KLAASSEN: The Risk Assessment Margin of Safety?

DR. ZHU: Yes. The last sentence in the last paragraph, we summarized the reason not to use a NOAEL at a 10 milligram per kilogram per day; it's from Boberg study. So, do you think it's enough, this reason, or any addition we should add to against not using a value at a 10?

DR. KLAASSEN: Which sentence are you --

DR. ZHU: The last sentence, last paragraph.

DR. KLAASSEN: Yeah, I got the last paragraph, but which sentence?

DR. ZHU: Last sentence.

DR. KLAASSEN: Last sentence?

DR. ZHU: Yeah. "Such dose-response relationship...." Yeah. That one.

DR. BELSITO: What is the other study you're referring to?

DR. KLAASSEN: Yes, I guess that's the 56?

DR. ZHU: Yes, 56. It's on PDF Page 196.

DR. BELSITO: That's the Boberg study.

DR. ZHU: Yes.

DR. BELSITO: And where do we discuss that?

DR. ZHU: Because in this study --

DR. BELSITO: What page of the document do we discuss that? Or we don't discuss it at all; we just say it didn't agree?

DR. ZHU: We kind of discussed it, but the only reason we summarized against the other value is there's no dose response relationship for the result, you know, between the butylparaben exposure and the epididymal sperm count concentration.

DR. BELSITO: So, we never discussed that study, though. We just say that it doesn't agree with another study; and we just reference that other study without ever discussing it in our paper.

DR. KLAASSEN: I agree with Don, that if we're going to say we're not using it, because there's not a dose response, that study needs to be described up above when you're talking about in the DART studies, right?

DR. BELSITO: Yeah. It's not there.

DR. KLAASSEN: And in there you can say what the results were, and that there wasn't a dose response.

DR. BELSITO: Just to go back to the DART studies, this effect on anogenital distance at 10 mg/kg of butylparaben, we're discounting; is that correct? And the effects on Sertoli and Leydig cells, that's Page 88, because we're using a NOAEL of 160. Do you see the one I'm referring to?

DR. KLAASSEN: You're saying which page, Don?

DR. BELSITO: PDF 88, under Oral. The first oral DART study.

DR. LIEBLER: And where is the 160 you're referring to?

DR. BELSITO: The 160 is in a table only. 160 is page -- PDF 126. It's the two butylparaben studies at the bottom of the page.

DR. LIEBLER: I see.

DR. BELSITO: Same studies.

DR. LIEBLER: And what did Paul say?

DR. BELSITO: He didn't really comment on that.

DR. LIEBLER: Call him up.

DR. BELSITO: He said, "Reference 58, a new NOAEL of 160 milligrams per kilograms per day for butyl." But he didn't comment on the other studies. He said, "Very good discussion, have to affirm previous conclusion including additional add-ons."

DR. LIEBLER: Okay. So, 160?

DR. BELSITO: But, how do we address that 10 milligrams per kilograms study?

DR. LIEBLER: Isn't this the one George Daston commented on?

DR. HELDRETH: Yes.

DR. LIEBLER: Was this the study, the 10 milligrams per kilograms dose NOAEL, I guess, was that the one that George Daston had commented on in our last meeting?

DR. BELSITO: I don't honestly recall.

DR. LIEBLER: I think it was in the notes.

DR. HELDRETH: Yes, and the 160 milligrams dose is what he arrived at, based on these.

DR. LIEBLER: I mean, I just don't know this area at all, so that's why I wanted to hear what Paul had to say. But also, you know, refer back to Daston's comment because this is really his field of expertise; and that's why we brought him in to help us evaluate the data.

DR. KLAASSEN: He definitely did conclude the 160 was the most appropriate. But I don't remember exactly what he said about the 10.

DR. BELSITO: His presentation starts on PDF Page 180.

DR. LIEBLER: Was the 10 based on the anogenital distance endpoint?

DR. BELSITO: And other endpoints.

DR. LIEBLER: Because one of the questions on PDF 192, CIR Question 2, it says is anogenital distance a relevant DART endpoint on which to base NOAEL?

DR. BELSITO: And he said no.

DR. KLAASSEN: He said no.

DR. LIEBLER: That's what I was remembering. AGD on its own should be considered a biomarker of effect and not an adverse outcome.

DR. BELSITO: But he said epididymal sperm concentration is highly correlated.

DR. ZHU: Yes, so the Boberg study, the 10, is based on the epididymal sperm concentration. So, at a 10 there are side effects observed.

DR. BELSITO: So, that would be a relevant endpoint according to what he's saying.

DR. LIEBLER: The epididymal sperm concentration?

DR. BELSITO: Correct.

DR. ZHU: But there is no dose response relationship, that's what we summarized on Page 96.

DR. HELDRETH: On PDF Page 206, that's part of George's presentation. He has effects on epididymal sperm concentration. And he says yes for Boberg, but there's no dose response. And then yes for Zhang, and that's where he gets to the 160.

DR. BELSITO: The Boberg there was no dose response. So, do we have that information in our paper?

DR. ZHU: We have, on PDF Page 96. That's my question.

DR. KLAASSEN: I don't think that's detailed enough. This study needs to be, again, put up high.

DR. ZHU: Okay.

DR. KLAASSEN: And, we need to say, in essence, that there appeared to be an effect at 10 and 100, but there was not an effect at 64 or 160. And it was only consistent after 400 milligrams per

kilograms, or something like that. I think we need to say more than it just wasn't a dose response. It wasn't even consistent until you got up to 400 milligrams per kilograms; and therefore, it isn't the lowest dose.

DR. BELSITO: We need to go into much greater detail on that study. It looks like we just blow it off.

DR. KLAASSEN: Yes, so they'll know why we didn't use it.

DR. HELDRETH: Right, we just have it in the table, where we should go into details in the text?

DR. KLAASSEN: It needs to be in the text as well.

DR. BELSITO: And it needs to be in our discussion as to why we discounted that study. Okay, so now I'm satisfied about the 10 milligrams per kilograms. Because in the text it looked like that was the NOAEL.

DR. LIEBLER: Yep. Okay.

DR. BELSITO: We need to add the Fisher study from 1999 that was referenced by the SCCS. We need to go into greater detail with the Boberg study, as to why 10 milligrams per kilograms isn't an acceptable NOAEL. We need to adjust our absorption rate to factual data that we can support from 50 percent. We need to up the dose of butylparaben to .5 percent.

DR. HELDRETH: And that brings me to a question I had, is how does the panel feel about the concentration that we chose for multiple parabens. In a previous review of parabens, we chose 0.8 percent for the concentration, in this calculation, for multiple paraben use. We carried that concentration forward, but does the panel feel that that's still the appropriate one to choose?

DR. LIEBLER: And this is for what, Bart?

DR. HELDRETH: If you look into the Risk Assessment, at the end of 96 and going into the top of 97. Of course, we did the MOS calculation for single use, you know, modeling butylparaben; but then directly after it we do an MOS for using multiple parabens for one formulation. The last time we looked at parabens, in the last CIR report, we used the 0.8 percent, and that's what we've carried forward here. Is that still an appropriate procedure?

DR. LIEBLER: Have we discussed any reason not to?

DR. HELDRETH: No. I mean, we just increased the max concentration of use from .4 to .5, does that have any effect on what we think the multiple of use. We don't have any data showing us that they used .4 of this and point whatever of another one. So, we don't really know what the maximum use concentration of two parabens in one formulation would be.

DR. LIEBLER: Well, if we changed it to anything, what might we change it to and why?

DR. HELDRETH: It looks like it -- and I don't know if this is the case -- it looks like when we looked at this we looked at the max use concentration for a single, that was .4. And if we're going to have two in there, well, it's essentially doubled to .8.

If you use two at the same -- now, I don't think we have any data to support that two are used together add up to 0.8 percent or higher.

DR. ANSELL: I think those two numbers come from the EU regulatory limit and not actual use concentration. And if we were to fold in actual concentrations, the amount would change substantively because the .5 was derived from mascara, which isn't used at 18 grams per eye.

DR. HELDRETH: But .5 is suggested because that's now the maximum use concentration reported.

DR. ANSELL: Right, in a mascara. So, if we go from these default assumptions to actual exposures, we're going to complicate it but come up with concentrations way way below these.

I mean, the .4 and the .8 are not derived from actual use concentrations, they're derived from regulatory limits. If we start folding in actual exposures, the .5 is going to drop to essentially none because it's only in mascara, which is not used at the 18 grams but .2 milligrams. So, we would end up with substantively larger margins of safety. I think that's why we have the .4 and .8.

DR. HELDRETH: You're suggesting that we don't increase our MOS calculation for single use paraben to .5?

MS. LORETZ: You could do so by taking in, but then doing a max in other products. I mean, .5 only applies to mascara, you can do it in other product types at different levels. It would be more accurate than making it all .5. It would just be an overestimation if you went to .5 across the board.

DR. KLAASSEN: Okay, I'm a little mixed up here. So, we made this calculation here and when we recalculate it, it's probably going to be a margin of safety of 500 approximately. But, in the bottom line in the document we're going to say it's safe as used, right?

DR. ANSELL: Yes. The question is where did the .4 and the .8 come from? They're not derived from actual use concentrations. If we were to use actual use concentrations the margins of safety would go much larger. So, the question on the table was do we change to .8? And our argument is that it's fine to use the maximum concentration; but we're going to consider changing to .8, then we're going to have to make a much more sophisticated analysis. And, I think the margins of safety --

DR. BELSITO: .8 to a higher limit.

DR. KLAASSEN: What page is this?

DR. HELDRETH: PDF Page 97.

DR. ANSELL: We are not arguing to change to .8.

DR. BELSITO: Okay.

DR. ANSELL: You consider changing to .8, I think you're going to have a much more complicated exposure scenario, which will result in our estimation of significantly larger margins of safety. And, as an example, the .5, which was suggested from the Women's letter, is limited solely to mascara, and mascara is not used at 18 grams per person per day. And so, it's okay at .5, but an actual concentration would come up with much much higher margins of safety.

DR. BELSITO: Okay. So, you want to keep the .8 and -- so, let's look at other EU issues. What about the .14 restriction for butyl and propyl? What about the ban for isobutyl, phenyl, benzyl, pentyl, and isopropyl?

DR. KLAASSEN: I would say no. Why?

DR. ANSELL: Yep. I mean, you guys have looked at that before, and disagreed with the conclusion that the data was insufficient.

MS. LORETZ: The .14 is based on that two milligrams?

DR. BELSITO: Right.

DR. ANSELL: Yes.

MS. LORETZ: So that's addressed.

DR. HELDRETH: So, then your recommendation, Jay, is that we stay with the .4 percent for single use and the .8 percent for a combined use, because it's in line with the expected use of aggregate per day.

DR. ANSELL: It's referenceable.

DR. KLAASSEN: So why are we talking about the maximum exposure, or the maximum amount that they should use? I don't understand what we're doing here. I'm completely lost. We said that there's a 500-fold margin of safety, therefore it safe as used period. What's all this crap about .4 and .8 and all of this, and it's irrelevant.

If we have a 500-fold safety factor for the butyl, which is supposed to be "the most toxic" and we have a 500-fold factor, why are we wasting our time? Other than the Women's don't like it, or some people don't like it. But, I think, from a scientific standpoint this is a nonissue.

And the more that we talk about the maximum amount -- we never talked about the maximum about you could use, especially when it has a 500-fold safety factor. Or am I missing something?

DR. ANSELL: We agree.

DR. KLAASSEN: I think a lot of this stuff can just be erased.

DR. BELSITO: But, I do think we have to put something in our discussion as to why we're disagreeing with the opinion of the SCCS. You know what I mean? That basically, there's another regulatory body out there that's come to a different conclusion, and to not address that in our discussion, I think, would set us up for more criticism.

DR. KLAASSEN: Okay. Well then, do we know how they came up to their --

DR. BELSITO: Well, we know that they came up with a .4 for butyl and propyl by saying that the NOAEL was two mg/kg. We've already discounted that once we bring that information into the report, which apparently it's not.

And then once we discuss the 10 milligram butylparaben, which we really didn't discuss adequately in this report, and put the reason why we're coming up with a NOAEL of 160. And then come up with, also in the report, why we're changing our factor from 50 to 3.2, because that's what the scientific data supports. Then that gets rid of the restriction at .14 for butyl and propyl.

The Europeans have banned isobutyl, phenyl, benzyl, pentyl, and isopropyl, not because there's any specific data to ban it, they said there's not sufficient human data to approve its safety. That's their argument there. They basically said, you didn't show us the data and therefore we'll ban it. That's the way the SCCS works.

I think we need to come up -- I mean, again, this is not my area of expertise, but we need to come up with some argument as to why we feel the data supports those parabens as well. And, I don't know what that --

DR. KLAASSEN: Well, I think you just explained it well. I mean, I think we can do that now. Why did we use our numbers? They used these numbers. Why didn't we use those numbers? Well, we know about the dose and we just need to explain it, but we have to have their data in there that they used, and we interpreted it differently.

DR. BELSITO: This needs a lot of rewriting.

DR. KLAASSEN: Yes. This needs a good rewrite.

DR. BELSITO: And, a lot of new data brought in that wasn't fully brought in.

DR. HELDRETH: Yeah, much of it was in the table and not brought into the text.

DR. BELSITO: Not in the discussion. Several other papers weren't really -- the Wang paper -- all of those needs to be brought in and discussed as to why we're discounting them.

DR. HELDRETH: Yeah. We would appreciate as much verbiage for the discussion as you can provide in your notes so that gives Jinqiu --

DR. BELSITO: This is a huge consumer issue. It's not just Women's Voices of the Earth, it's Environmental Working Group, there are a huge number of NGOs, it's huge in Europe.

DR. LIEBLER: When we get there, I do have some substantial edits to the discussion that I wanted to just paraphrase for you. Sorry it's hard to edit very well in a PDF, even with the latest software, so, I've been trying to do it for you. But if I could just walk you through.

My edit start on PDF 105, fourth paragraph I have small edits for readability I won't dwell on here. The fifth paragraph is the one that starts, "The panel noted that both in vitro and in vivo studies indicate rapid and effective metabolism." And, I think the problem with this paragraph, as written, is it suggests that the metabolism is so rapid and extensive that any measures of the intact parabens are result of exposure that are regular and frequent. I didn't know how to interpret that. I rewrote that paragraph. I kept the first sentence as is. And then I simply said, parabens are further metab -- okay, I kept the second sentence largely as is. And then, the last two sentences, I deleted, and I replaced with, "When applied to human skin, parabens are extensively metabolized to 4-hydroxybenzoic acid." And then I went on to say, "Whereas older studies suggested that unmetabolized parabens are not excreted, recent studies with more sensitive analytical methods have measured unmetabolized parabens and the metabolites, following dermal exposures."

And then the next paragraph is the one that addresses bioaccumulation, and it also ends with epidemiology, so it covers a lot of ground. One of the points, I think, that Alexandra Scranton

raised in her memo was having to do with the very last sentence on PDF 105, which was, “however, the metabolism excretion pharmacokinetics of the parabens made accumulation in the body not an issue.” Okay, so that’s not true. And it implies that there’s no accumulation.

I just took another swing at that whole paragraph and I said, “The panel noted that parabens are relatively lipid soluble documents that have been measured in adipose tissues.” And, second sentence, “Recent studies with more sensitive analytical methods measure parabens in breast, adipose, and placenta tissues...”

And then I deleted that, “however, the metabolism excretion... not an issue.” I deleted that sentence, and then I have a new sentence for you. “Thus, despite extensive metabolism, these lipid soluble chemicals may accumulate in tissue. Paraben exposures are attributed to cosmetic products, foods, medicines and other sources. Refine aggregate exposure models suggest that dermal exposure from cosmetic product use is a major source of parabens in the body.” Which I think is true based on the available information, and we don’t need to get into the argument is it “the” major source, or is it gold, silver, or bronze medalist?

Anyway, my notes, I’ve been kind of cleaning them up for you. Hopefully, it’s clear from the document and if you have a problem you can email me back.

DR. ZHU: Okay, thank you.

DR. LIEBLER: And I don’t have any other substantive edits in the discussion.

MS. LORETZ: Can I make one comment about the discussion?

DR. BELSITO: Yes.

MS. LORETZ: Certainly, it’s very important that the literature that Dr. Daston presented be in the discussion report and discussed; but I’m thinking the reference to the fact that he made a presentation is probably best left in the minutes and not in the report.

DR. BELSITO: Not be in the discussion.

MS. LORETZ: Right, not in the discussion.

DR. BELSITO: Right.

DR. KLAASSEN: I was wondering about that too.

DR. LIEBLER: That second paragraph of the discussion.

MS. LORETZ: Right.

DR. LIEBLER: The first two sentences of that paragraph about, “In response, Dr. Daston...” And then, “He provided expertise.” So that could be deleted and reframed. “The panel considered which data represent the most appropriate DART endpoints.”

Should we explicitly state the reason why we didn’t go with the 10 mg/kg, based on the anogenital distance, because that was essentially George’s comment?

DR. BELSITO: No. We didn’t go with it because there were effects on spermatogenesis as well, but there were no dose response effects. There was one at 10, there was none at dah, dah, dah. And as Curt said, it was only when you have to 400 milligram that there was consistency.

DR. LIEBLER: But that needs to be brought into the discussion.

DR. BELSITO: Right. It needs to be brought into the data. There needs to be a paragraph where we discuss the Boberg study. Discuss the fact that there was a complete lack of dose response. And then brought further into the discussion as to why we discounted that 10 mg/kg as in the NOAEL.

DR. LIEBLER: But that part of the discussion can go in that paragraph that did refer to George Daston’s comments; and take the George part out and expand that with what Don just said.

DR. BELSITO: We’ll just go back to another comment that was made by Women’s Voices for the Earth about, “Our current safety assessment claiming little or no unchanged paraben is excreted in the urine.” That’s what you changed in the discussion, is that correct?

DR. LIEBLER: Right.

DR. BELSITO: So, we’re addressing that by your --

DR. LIEBLER: Correct.

DR. BELSITO: Okay. Since I'm reporting on this, what I have so far is we need to bring in the --

DR. LIEBLER: The in vitro sperm effects papers, the two papers; the Samarasinghe paper and the Mundy paper.

DR. BELSITO: Samarasinghe, Mundy, and the Wang paper.

DR. LIEBLER: Yes, and that's the bioaccumulation paper.

DR. BELSITO: Right. And the Artacho.

DR. LIEBLER: Correct.

DR. BELSITO: We are not bringing in the Xue paper on animals?

DR. LIEBLER: Right. I think the key thing about those papers that I'm referring to as bioaccumulation is that we're citing and discussing those papers, but I don't think we can agree with the assertion by Women's Voices for the Earth, that these papers necessarily demonstrate a lifelong bioaccumulation of these chemicals. They're detectable. The attempts to measure a relationship between age and abundance in tissue is complicated, as both papers discuss in their own discussion sections.

But, I don't think we can argue that there's no accumulation of parabens in tissue, because the data shows otherwise. Whether and how much it goes up with lifetime exposure is not possible to say with the currently available data.

DR. KLAASSEN: So, what does the word accumulation mean?

DR. LIEBLER: It's an interesting question you raise, Curt, because it could mean that it's there. And it could also mean that it's there more and more.

DR. KLAASSEN: Yeah.

DR. LIEBLER: And it depends on what you intend it to mean. I think that the memo from the Women's Voices for the Earth is saying, it's there more and more. And I'm saying the data show it's there, and I think it's plausible to expect, to hypothesize, it might be there more and more; but you need data to test the hypothesis. And I don't think the data allow a test of the hypothesis. It's a reasonable hypothesis.

DR. ANSELL: Biomagnifying as opposed to deposition.

DR. KLAASSEN: Right. In fact, the half-life would suggest it doesn't accumulate.

DR. LIEBLER: But it's there. So, it's there and the methods are not ambiguous.

DR. KLAASSEN: You got my point, you know, what the word accumulate means. It would be nice if there were two words that one could use instead of just -- to separate those two phenomena.

DR. LIEBLER: At that point you need to back off and say, okay, what is it that we're arguing about here?

DR. KLAASSEN: Well, it distributes to the fat.

DR. LIEBLER: It's in the tissue.

DR. KLAASSEN: It's in the tissue. It distributes to the fat. If it continues to accumulate with time, we do not know.

DR. LIEBLER: And we don't know how much, and we don't know if there's any health impact of that at all.

DR. KLAASSEN: No.

DR. LIEBLER: It's there, that's all we can say at this point.

DR. ANSELL: Well, maybe we need to say that as a simple declarative.

DR. LIEBLER: We can go so far as to say, because sensitive analytical techniques demonstrate the presence of the unmetabolized parabens in adipose tissue, one might hypothesize that it accumulates with exposure in time. But the available data from the two studies do not provide an unambiguous test of this hypothesis, for reasons that are articulated in those papers.

DR. KLAASSEN: Right, I think that's a good thought, except for that middle point. I mean, we can hypothesize all sorts of things. I think the parent compound can be identified in adipose tissue, but we cannot go farther than that statement, as the authors point out in their discussion.

DR. LIEBLER: That's pretty much what I've written in my revised discussion.

DR. BELSITO: The two mg/kg that they were limiting, that came from what study? I don't remember.

DR. KLAASSEN: It was at 58 or something -- or was it -- it was Study #56. And 56 is -- somebody help me.

DR. HELDRETH: 56 is Boberg.

DR. KLAASSEN: A little louder.

DR. HELDRETH: Boberg.

DR. BELSITO: It's the Boberg study?

DR. KLAASSEN: That's what they said, it's number 56. And they say 56 is the Boberg study.

DR. BELSITO: But I thought that was 10 mg/kg. But you said the calculation of .14, as a limit, came from a two mg/kg restriction?

DR. ZHU: Yes.

DR. BELSITO: And where does that come from?

DR. ZHU: You mean the two we were talking about today?

DR. BELSITO: When we were discussing how they came down to a 0.14 for butyl and propyl, I thought you said that it was based off of 2 mg/kg.

DR. ZHU: Yes.

DR. BELSITO: Where is that number coming from?

DR. ZHU: It's from a study, Fisher 1999.

DR. BELSITO: Oh, that's the study that we don't have.

DR. ZHU: We don't have, yes. We will include that.

DR. BELSITO: Okay, so we need to bring in the Fisher study as well and address that.

This is what I have so far. We need to bring in the Samarasinghe, Mundy, Artacho, and Wang papers and discuss those. We need to not even bring in the Xue paper on animals and not even discuss that.

DR. LIEBLER: Correct.

DR. BELSITO: We need to bring in the Boberg paper and discuss why we dismissed it, because there's no dose response. We need to bring in the Zhang paper into the text where we get our NOAEL of 160 milligrams per kilograms, rather than having it hidden in a table, and why we've gone with that.

We need to use the .5 percent butylparaben, but point out it's used only in eye makeup, which would have limited body burden at that level. We need to bring in the Fisher study and discuss why we've dismissed that and are not limiting a combination of butyl and propyl to .14 percent as the Europeans do. We need to rewrite the discussion, which Dan has done, and get rid of the reference to Daston per se.

And then, we need to come to a conclusion. Is it simply safe as used? Oh, we need to say why we're not banning isobutyl, phenyl, benzyl, pentyl, and isopropyl. We need some documentation to support that. Do we have it in the papers? What data do we have on that?

DR. KLAASSEN: Well, I thought the reason would be that as far as -- these compounds theoretically work through the estrogen receptor and since the --

DR. BELSITO: I thought benzyl had even a higher effect than butyl on endocrine disruption, no? And I thought when we originally did parabens we didn't even look at benzyl, did we?

DR. HELDRETH: The 2008 report was on methyl, ethyl, propyl, butyl, isopropyl, isobutyl, and benzyl.

DR. BELSITO: It was. Okay.

DR. LIEBLER: If we have data to support them then we keep them; if we don't have data then we're insufficient.

DR. KLAASSEN: Unless we use read-across.

DR. HELDRETH: I don't think we have enough information to read across to the higher molecular weight parabens beyond propyl. I mean, we have data for up to propyl and butyl, pretty much, and then we don't have much of anything.

DR. KLAASSEN: It's generally thought, I thought, that the longer the less effective.

DR. LIEBLER: Well, these are all easily absorbed, even up to benzyl, I think.

DR. BELSITO: So, we have a dermal short-term. There were no significant changes in body and organ weights, isopropyl and isobutyl for 28 days. And then we have a short-term for isopropyl and isobutyl, NOAEL at 600 mg/kg and 50 mg/kg. And then we have a DART study on isopropyl. Females were exposed to 1000 mg/kg methyl or 250 mg/kg isopropyl on postnatal day 21 to 40, exhibited delays in vaginal opening, 1000mg decreases weight of the ovaries. And then that's it. Then for benzyl I don't think we have anything. Produced an estrogenic response in the uterotrophic assay, but the potency was like 100,000 times lower than estradiol.

DR. LIEBLER: And I think we're okay up to and including butyl.

DR. BELSITO: Um hmm.

DR. LIEBLER: And beyond that we really don't have data.

DR. BELSITO: And the data we need?

DR. KLAASSEN: I think what we're probably interested in is the DART effects.

DR. LIEBLER: Yes.

DR. BELSITO: Okay. So, the data for --

DR. LIEBLER: Yeah, I think that's the main issue, really. It's not sensitization. It's not 28-day dermal.

DR. BELSITO: The data are insufficient for isobutyl, phenyl, benzyl, pentyl, and isopropyl. And what we need are NOAEL for the DART. Correct?

DR. LIEBLER: Yeah.

DR. KLAASSEN: Yup.

DR. BELSITO: Okay. So, is our conclusion safe as used?

DR. LIEBLER: Yes.

DR. BELSITO: For --

DR. HELDRETH: Insufficient for benzyl, or?

DR. BELSITO: Yes, insufficient for isobutyl, phenyl, benzyl, pentyl, and isopropyl. That's what we're saying right? We don't have a NOAEL.

DR. LIEBLER: Right. Safe as use, again, for methyl, ethyl, isopropyl --

DR. BELSITO: Isopropyl?

DR. KLAASSEN: Well, there's propyl for sure.

DR. BELSITO: Propyl and butyl.

DR. LIEBLER: Okay. And butyl. If you can support propyl, I don't see any reason to exclude isopropyl. And if you can support butyl, I don't see any reason to exclude isobutyl at the same molecular rate, virtually the same chemical properties.

DR. BELSITO: Okay.

DR. LIEBLER: That's a read-across if there ever was one.

DR. BELSITO: Okay. So, we're saying safe as used methyl, ethyl, propyl and butyl. And read-across safe for isopropyl, and isobutyl. Correct?

DR. LIEBLER: Yes sir.

DR. HELDRETH: Are you including the salts of those?

DR. LIEBLER: And the salts are fine.

DR. BELSITO: And their salts. And then, insufficient for phenyl, benzyl and pentyl. Yes?

DR. LIEBLER: Yes.

DR. BELSITO: And the data we need for that are NOAEL for DART.

DR. LIEBLER: Yes.

DR. BELSITO: Okay.

DR. HELDRETH: And since all three of those are not reported to be in use, we'll probably just go insufficient zero.

DR. BELSITO: Fine.

DR. LIEBLER: If that what happens.

DR. HELDRETH: I'm just pointing out. We probably won't get data on them if nobody's using them.

DR. BELSITO: And we're not going to talk about limits at all in terms of .8s and .14s and all of that. Is that correct?

DR. KLAASSEN: That's what I think.

DR. LIEBLER: Right.

DR. BELSITO: Let me just run through this again to make sure I have everything. We're bringing in the Samarasinghe, the Mundy, the Artacho, and the Wang papers, and we're discussing those. We're not bringing in the Xue paper on bald eagles. We're bringing in the Boberg paper to point out the lack of dose response. We're going to bring in the Zhang paper into the actual document, rather than just the table as to why we pick the 160 milligrams per kilograms. We're going to go with the actual absorption and not just random 50 percent number.

We're going to point out that, yes, butyl is used at .5 but only in an eye makeup, in the discussion, so that lowers the body burden. We're going to say something as to why we're not limiting the butyl and propyl to .14 as the EU did, based upon our NOAELs. We need to rewrite the discussion to eliminate the reference to Daston.

And in the end our conclusion is going to be methyl, ethyl, propyl, butyl, isopropyl, isobutyl and their salts are safe as used. And the data are insufficient for phenyl, benzyl, and pentyl. And the data that would be needed would be a NOAEL for a DART --

DR. LIEBLER: I don't think we have -- we only have benzyl, there's no phenyl -- I don't even see it. I'm looking at the introduction, the list of the ingredients, I don't see a pentyl. We have Benzyl. And then we have butyl, isobutyl.

DR. BELSITO: We don't have phenyl?

DR. LIEBLER: We don't have any phenyl.

DR. BELSITO: Okay.

DR. LIEBLER: So, those are nonissues. Benzyl is on the list. And it was in the 2008 safety assessment.

DR. BELSITO: Okay.

DR. HELDRETH: And in the original assessment, 1986, I believe it was, the panel went insufficient on the safety of that.

DR. LIEBLER: Insufficient? Oh, so it's always been insufficient?

DR. HELDRETH: But then in 2008, it was brought in to the full parabens report and was part of the safe conclusion at the time.

DR. BELSITO: Now we're changing it.

DR. LIEBLER: Okay, we can do that.

DR. HELDRETH: But it's also not in use, so.

DR. LIEBLER: Yes. But there's no pentyl, there's not phenyl.

DR. BELSITO: Okay. They're not in the dictionary? Because why would the Europeans --

DR. HELDRETH: Yes, there're a number of other ones. There's isodecyl. There's phenyl.

DR. LIEBLER: In the dictionary, but not in this report.

DR. HELDRETH: In the dictionary, but we don't know if those are in use and we didn't propose to bring those in to the re-review.

DR. LIEBLER: Okay. So, anyway it's not our problem if it's not in the report, so.

DR. BELSITO: But should it be, and then should we say it's insufficient?

DR. LIEBLER: Yes, I know what you're saying. I look to Bart on that and Jay; you want it in, so we can kick it out?

DR. HELDRETH: If we're going at it from the re-review standpoint, if we don't think the data that we have in the report right now would support the safety of those ones that we would add on, then normally we would suggest not doing the add-ons.

It looks like the isodecyl is not reported to be in use. And the phenylparaben also not reported to be in use.

DR. BELSITO: Just getting back to the whole way that we're now going through things and saying oh, not reported to be used, data not supported; so we can at least have some stance against people who say, there're 16,500 cosmetic chemicals out there and you've only reviewed 3,000.

DR. HELDRETH: Right, so looking in the VCRP data, and subtracting out those names that are duplicates in there, looks like maybe there're 6,500 ingredients in use in the U.S.

DR. BELSITO: Right.

DR. HELDRETH: The CIR has evaluated the safety of over 5,000 ingredients.

DR. BELSITO: I understand. So, if we don't bring them into our report and say they're not used, are you creating a document of materials that are in the dictionary, that aren't used, that we haven't reviewed? You see what I'm saying?

DR. HELDRETH: There're essentially two precipitating factors of why we looked at this report. One was one of the parabens salts had a very high frequency of use, so that came up on our priorities list for that rational. Additionally, it's coming close to time to re-review the original parabens.

DR. BELSITO: Right.

DR. HELDRETH: And so, we looked at this as a re-review of those ingredients with essentially no-brainer add-ons, which we thought the salts would be, the ones that are in.

DR. BELSITO: Right.

DR. HELDRETH: And so that's why we added the salts as potential add-ons and not these longer chain analogues that we presumed that the panel would not find as no-brainers.

DR. BELSITO: Right. No, but my point is, to make it to the not in use category, do we have to review it? Or, is that something you --

DR. HELDRETH: Yes. But if we haven't looked at it at all it doesn't get categorized at all.

DR. BELSITO: It doesn't get categorized?

DR. ANSELL: It would be two separate questions. And I certainly agree as it relates to the re-reviews, that it's a limited universe of what's in the report in terms of the re-review. In terms of new reports, our position has been that we should only review materials whose data contributes to the assessment of the family. And if the material is not used and has no data, which is relevant to the assessment of the ingredient, it should not be considered.

I don't think there is an obligation to review the entire INCI dictionary. Our obligation is to assess materials which are used, to act as a validation of industry's responsibility to substantiate the safety of ingredients. We're not obligated to substantiate the safety of anything which has a harmonized name.

DR. BELSITO: I understand. But you are criticized by the fact that these ingredients occur in your dictionary, and there's no statement as to their safety.

DR. ANSELL: We're also criticized for not having banned materials that have no potential use. We're criticized comparing it to Annex 2, why you guys aren't reviewing jet fuel and industrial by-products waste. I don't think that within this venue --

DR. BELSITO: If it doesn't bother you, it doesn't bother me. I'm just saying, you know, that you're the ones that deal with, you know, there are 15,000 materials out there and you haven't looked at 9,000 of them.

DR. ANSELL: Yes, that is one of the criticisms.

DR. LIEBLER: So, there's an answer to that.

DR. BELSITO: It's fine.

DR. LIEBLER: I think it sounds like it's still a question. It's sort of a judgement issue of what to put in a report. Because if you had a simple rule that if it's in the dictionary, and it's in that chemical family, it goes in the report. And then we deal with it as the data allow. And, we can do that. I think that's what Don's saying, is that we can do that and that would make sure that we get a look at everything.

And we maximally deal with the issue of your reviewing everything that's in your use universe. I understand that many of these things are just not used and never are going to be used, but they're in the dictionary.

Anyway, I feel the same way you do, Don. We could do it. I wouldn't argue if we had a bunch in that turned out to be insufficient because we don't have data, at least we're being thorough. We could include anything that's in the right chemical family. And if you have a reason for not including them, that are sort of judgement issues like as we've been proceeding, I'm not going to argue; but I certainly wouldn't object to including them.

DR. ANSELL: Well, I think, we would.

DR. BELSITO: Okay, so let's not. Let's move on.

DR. LIEBLER: We had the discussion.

DR. BELSITO: We had the discussion. Anything more on the parabens?

DR. KLAASSEN: I have one point. In this document, I questioned before that we have 17.76 grams of the cosmetic that's used per day and exactly where we got that. But I would like to note, at least, there is a manuscript that CTFA was the senior author on, in 2005, that I guess we would include this as a body lotion. It says that it's 4.42 grams, which is a marked difference from 17.76 grams. And you said that the Europeans used 17.76 grams. And this says 4.42, why the difference?

DR. BELSITO: That's just for body lotion.

DR. KLAASSEN: But, what else is it going to be used for. I mean that's the highest use.

DR. BELSITO: Shampoos, conditioners, makeup, suntan lotions. And the 17.76 is aggregated across all the cosmetic products.

DR. KLAASSEN: That's where the 17.76 comes up?

DR. BELSITO: Right.

DR. LIEBLER: Right.

DR. BELSITO: It's like a 95 percentile use, right? It was done -- consumer study across all various products.

MS. LORETZ: Yes, it's all spelled out in the SCCS guidelines.

DR. KLAASSEN: Could someone send me that couple pages, or whatever it is, from that guidelines?

MS. LORETZ: Yes.

DR. BELSITO: Anything else? Okay, 10:35 we'll resume.

BREAK

DIALKYL DIMER DILINOLEATES

DR. BELSITO: Okay. In 2003, we issued a safety assessment on these dialkyl dimer dilinoleates. It was time for a re-review. We decided that we wanted to open it up to add a few more in. And then it would be a slam dunk safe as used, and that's the document we got back to us. Everyone happy with the abstract, discussion and conclusion?

DR. LIEBLER: Yeah, I am. I think it looks pretty good. I have a lot of little edits, but nothing substantive.

DR. BELSITO: Curt?

DR. KLAASSEN: Same.

DR. LIEBLER: One point I do want to raise from the discussion, PDF Page 25, near the end of the third line. It says, "And lack of dermal penetration." I would say, we don't really have data to say that

there's absolutely no dermal penetration. I would change that to maybe minimal dermal penetration.

DR. BELSITO: Okay. Anything else? Okay

#### XANTHINE ALKALOIDS

DR. BELSITO: Okay, xanthine alkaloids. This is the first time we're looking at the safety assessment. Three xanthine alkaloid ingredients, comprising of caffeine and two structurally related analogs. The function is skin conditioning agents. Where are we? One question I had was what do we do with all the positive genotox studies? Paul said, negative carcinogenicity study; that's his answer.

DR. KLAASSEN: That's my answer also.

DR. LIEBLER: Yeah, I did look at Table 7, the genotox studies. And as you kind of eyeball the table you see probably about two-third negative and a third positive, roughly. The text made it seem like it was more like fifty-fifty. And so that was one point. And it's not to say that the third positive don't need to be addressed.

The other thing I noticed, from looking at the table, is the larger number of in vitro genotox systems that are not widely used today, or have been superseded by AMES -- for example, E.coli K12. And honestly, I don't know what to do with the data from those. And I was hoping to hear actually what Tom Slaga would think about these. But I didn't review all of these individually.

DR. BELSITO: Okay. Well, Paul's comment was, "Poorly soluble, low tox profile, negative carcinogenicity studies. Theobromine has testicular affect only at high (lethal) doses. Safe as used."

Just a comment on page 17; you have under, other relevant studies, male reproduction impairment, shouldn't that go under DART?

MS. CHERIAN: We were thinking it, but we moved it. And we were wondering if it belonged there or back into DART studies. We just wanted to see where you all would prefer it.

DR. BELSITO: I thought it belonged in the DART Study. No?

DR. LIEBLER: Which one is that?

DR. BELSITO: Page 17, male reproductive impairment with theobromine and theophylline.

DR. LIEBLER: Theophylline.

DR. BELSITO: Theophylline, are under other relevant studies.

DR. LIEBLER: Yeah, reproductive.

DR. BELSITO: Rather than DART.

DR. LIEBLER: Yeah, DART.

DR. BELSITO: And then in Wave 2, we got the 6 percent data that clears caffeine for an HRIPT. So yeah, I thought safe as used.

DR. LIEBLER: I did too. And also, under the other relevant study, why couldn't we put tumorigenicity in with carcinogenicity?

MS. CHERIAN: That was another one for everyone to see it.

DR. LIEBLER: Okay. Curt, xanthine alkaloids, I guess it's used -- I was thinking of like Goodman and Gillman's, you know, pharmacology text, would refer to these as the methylxanthines.

DR. KLAASSEN: Um hmm.

DR. LIEBLER: I don't really care that much. Is there a CIR dictionary or industry-common terminology reason to use xanthine alkaloids? I think methylxanthines is sort of a more current descriptor for this class of compounds.

DR. HELDRETH: We could change it.

DR. KLAASSEN: And, you know, we know a lot about these chemicals. Some of the us have even taken them purposely today. Most of us. And it brings up a nice point in here -- I don't know if you people noticed it -- but dogs don't metabolize theobromine very well. And so there is this story

out that, you know, if your neighbor's dog is always barking, you just happen to dump a bunch of Hershey bars across the fence and the dog dies.

DR. LIEBLER: I would never do that, Curt. Just remember caffeine/coffee, theophylline/tea and theobromine/chocolate.

DR. KLAASSEN: Right.

DR. LIEBLER: You got your major food groups right there.

DR. BELSITO: Okay so safe as used. Do we want to change the name of the report to methylxanthines, is that correct?

DR. KLAASSEN: Yes.

DR. LIEBLER: Yup.

DR. BELSITO: Okay. So what discussion points do we need to bring up? The genotox, the positive DART, why we're dismissing them?

DR. LIEBLER: Yeah. I'd like to have a little discussion tomorrow with Tom about the genotox, how to approach that. Because I would really appreciate his perspective on it. He's probably got the most experience with in vitro genotox.

DR. BELSITO: Okay.

DR. LIEBLER: Because it's a strikingly large number of positive studies.

DR. KLAASSEN: Yes. No question.

DR. LIEBLER: Particularly in the endpoints involving like sister chromatid exchange as oppose to mutagenesis. And I don't know what that means, but those appear to have been at the high doses for all of those. And I don't know if that's one of these things where when you get to a toxic concentration in some of those in vitro modules, you get that effect. Or you can get that effect, and it compounds the test. I'd like to hear from Tom, if he could he help us craft the discussion language for that.

DR. KLAASSEN: In general, sister chromatid exchange is very difficult to interpret, they tell me.

DR. LIEBLER: Okay. We definitely need to talk about that. But I think the other points, obviously, are widespread frequent dietary exposure, lack of carcinogenicity; and these do nothing in skin.

DR. BELSITO: And what about the reproductive effects?

DR. KLAASSEN: That's only at a very high dose.

DR. BELSITO: Okay. But we need to point that out in the discussion.

DR. KLAASSEN: Yes.

DR. BELSITO: Anything else in the discussion? Aerosol? Does it have aerosol use? I don't remember. Incidental inhalation spray, yes. So, the aerosol boilerplate? Anything else? Okay. So, it is brown algae time.

DR. LIEBLER: It's brown algae time.

## BROWN ALGAE

DR. BELSITO: Oh my God.

DR. LIEBLER: Kelp.

DR. BELSITO: Wave 2. Now here -- we're getting Wave 4.

DR. LIEBLER: Wave 4 is just the greatest hits of Waves 2 and 3.

MS. CHERIAN: It's just a summarization of the sensitization and dermal to make it easier.

DR. BELSITO: Okay. Well, we definitely need to limit arsenic. We need limits on heavy metals. What about these extractions? Methanol, hexane, chloroform?

DR. LIEBLER: You know, so I thought we actually had a lot of information about the different prep methods, and they seem to me to fall into a couple of categories. Maybe two or three categories to get these ground-up powders, to get these alcohol extractions or these aqueous extracts.

And I wonder if it might not be possible to prepare a kind of a map diagram that just shows the major ways in which brown algae is converted to cosmetic products. Maybe not so much with a high level of detail in the map, but under method of manufacture it could be right there. I would imagine maybe sort of an inverted pitchfork trident thing, you know, with three pathways. Because then you'd have a table with lots of information for the individual ingredients.

MS. CHERIAN: Okay.

DR. BELSITO: Okay. Now, we know a lot about the impurities, we know a lot about the method of manufacture, we know zilch about composition.

DR. LIEBLER: Yeah. I had a more specific question about composition, which was do we -- because of Wave 2, we now have data on the actual cosmetic ingredients, not just on some representative algae from the literature.

DR. BELSITO: Right.

DR. LIEBLER: So, that's good. And I had a question about constituents of concern with respect to sensitization for example. And we don't have data on those for any representative, at least -- I might have missed it in the blizzard of Wave 2 or Wave 3.

DR. BELSITO: Well first of all, the two biggies are *Laminaria digitata* and *macrocystis*. Those are the ones that are most frequently used, right?

MS. CHERIAN: Yes.

DR. BELSITO: And we have an HRIPT on 46 humans for *laminaria*, but we have no data for *macrocystis*.

MS. CHERIAN: We have some data for that ingredient --

DR. BELSITO: We have no sensitization data.

MS. CHERIAN: -- either in Wave 2 or 3.

DR. BELSITO: I didn't see it.

MS. CHERIAN: Okay. Let's see.

DR. BELSITO: And all of the times that these were irritant, it was always with propylene glycol. And I thought propylene glycol was the irritant there. I was okay with the irritation, but we have no sensitization data for *macrocystis*. And we have just an HRIPT on 46 individuals for *laminaria*. And we also have no tox data for either one of them. And at most, we have 28-day tox data. And that raises the whole issue of iodine concentration and thyroid effects.

DR. LIEBLER: You're talking about *macrocystis*?

DR. BELSITO: Yeah. Now the thyroid issues with ingestion of these kelps were extremely high amounts, but we don't have absorption data. And then we don't really have good genotox data. And then we have some endocrine effects. We don't have photo, we don't have composition, we don't have 28-day dermal absorption. We don't have sensitization on *macrocystis*, we don't have photo. The genotox, there's some report of endocrine affects.

DR. LIEBLER: Yeah. I'm trying to get some idea of how widespread food consumption is with the ones that we're using. *Macrocystis*, *laminaria digitata*, *laminaria saccharina* approved as food additive or direct food addition, food for human consumption as a source of iodine or as a dietary supplement. I don't know to what extent that factors into our need for dermal tox or additional tox data.

My hunch with these is that we may be treating these more the way we treat other kinds of botanicals, where our major concerns is going to be sensitization and constituents of concern. Maybe that's not accurate, but that how I first approached these.

DR. KLAASSEN: Well, they are considered food additives, especially for animals to quite a high extent, without apparent toxicity, which gives me some support.

DR. LIEBLER: In the acute oral toxicity study, it's Table 21, PDF Page 55, we have a relatively small selection of brown algae compounds that have been tested -- or brown algae that have been tested. For our report, the *fucus vesiculosus*, there are three different studies in Swiss mice.

But if you look at all the brown algae that have been tested there's, let's see one, two, three, four, five, six, seven, eight studies, all of which have oral LD50s in the thousands. These are sort of the profile of nontoxic substances.

As far as dermal absorption, you know, it's basically a botanical. So, it's got sort of a wide variety of chemical substances, many of which are not absorbed at all.

DR. BELSITO: But we don't even know what they are.

DR. LIEBLER: That's a concern I have is the chemical composition of these. But I would say, particularly with respect to constituents of concern relative to sensitization. And of course, I didn't realize that these tended to accumulate arsenic so much.

DR. BELSITO: Right.

DR. LIEBLER: I found that interesting and surprising. Think of all the kelp in the world. This could actually be a major reservoir of arsenic other than the earth's crust.

DR. KLAASSEN: I think that arsenic form is not so toxic. It says in here some place that they're arsenic sugars. And I know at least fish, also, concentrate arsenic and puts it in a form that's not toxic like the inorganic form is. But I'm not entirely positive about this. But yeah, that's kind of interesting.

DR. LIEBLER: Paul have comments?

DR. BELSITO: Brown algae. "Extracts to 36 percent. Powders to 40. Juices no concentration. Water no concentration. Many uses with no concentration data provided. Plant-like, seaweed, protozoa, unique kingdoms -- very diverse group, too diverse?? Impurities; phytosterols, alginic acid, heavy metals, especially, arsenic, and phthalates. No data on composition. Tox data limited, but no level of toxicity. This one is touch with such a diverse number of sources and ingredients; don't know where to begin other than composition and impurity data base on some sort of plausible grouping." And that was my problem. We're just sort of assuming these all have the same composition.

DR. LIEBLER: Well, yeah. I mean, I suppose implicitly we're assuming that they have similar enough composition to be grouped together. If we did play the mental exercise of deciding to break these up, how would we break them up?

DR. BELSITO: I don't know.

DR. LIEBLER: With what would seem to be anything other than arbitrary.

DR. BELSITO: But wouldn't it be nice if we had composition on a couple different -- like at least the two that are primarily used for laminaria and the macrocystis?

DR. LIEBLER: Right. No, I agree. That's one of the notes I had, is that we need data on composition for the representative of the major groups. Particularly, I thought constituents of concern. Maybe you're not as concerned about sensitization with these, Don?

DR. BELSITO: I don't know. I mean, that's was one of my needs. I raised to you was an HRIPT of 46 sufficient for the laminaria, but we have nothing on macrocystis, which is the other one that has a high concentration of use.

DR. LIEBLER: I think we definitely need that. And I think of these as botanical. And with botanicals, we almost always are looking for constituents of concern. Flavonoids, terpenoids, things like that. And at least if we have representative data for the different classes, along with safety data on sensitization, then we can draw a conclusion.

We don't have genotox on major -- we have genotox on a couple of fucus vesiculosus?

DR. BELSITO: Mm-hmm.

DR. LIEBLER: But we don't have it on any of the laminaria, do we?

DR. BELSITO: Nope.

DR. LIEBLER: Or the macrocystis?

DR. BELSITO: Nope.

DR. LIEBLER: I think we need that.

DR. HELDRETH: Is there one for laminaria saccharina extract? At least according to Priya's table, it looks like there's genotox for Number 55.

DR. LIEBLER: I might have buzzed by it.

DR. BELSITO: The genotox is not on laminaria though.

DR. LIEBLER: We have laminaria digitata, prep method concentration not specified, AMES assay with and without metabolic activation. There's a reference, I didn't look at it. Is that what you're referring to, Bart?

DR. HELDRETH: In Priya's cheat sheet table here, number 55 in the table says laminaria saccharina extract.

DR. LIEBLER: Oh, sorry.

MS. CHERIAN: Oh, it's in Wave 2.

DR. HELDRETH: So, data came in Wave 2.

DR. LIEBLER: I think the other problem in reviewing this report is the data are spread out over so many reports, that I just was missing stuff.

MS. CHERIAN: And I think fucus vesiculosus was the highest number of uses and concentration. But the concentration might have gone down.

DR. LIEBLER: Okay, so the cheat sheet's only for the skin endpoints, right?

DR. KLAASSEN: Right.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: Yup.

DR. HELDRETH: No. It has repro, geno.

DR. KLAASSEN: Oh, he's talking about the one she handed out 30 minutes ago.

MS. CHERIAN: That's the data profile.

DR. HELDRETH: Yeah, the data profile.

MS. CHERIAN: Yeah. So, it's not on there. The genotox data is not on there, it's in Wave 2. That's only skin sensitization and irritation.

DR. LIEBLER: Alright. I think -- it's hard to tell what we have at this point.

MS. CHERIAN: Yes. Yeah.

DR. BELSITO: But the genotox data is on laminaria saccharina and not digitata?

DR. HELDRETH: True.

DR. LIEBLER: And where are you getting that, Don?

DR. BELSITO: Wave 2.

DR. HELDRETH: So, on Page 6 of Wave 2, it says for laminaria saccharina extract, the genotox says, tradename mixture containing this ingredient in seawater and methylpropanediol AMES test, salmonella strains. It lists five of those with and without metabolic activation in dose 50 to 5000 micrograms per plate, non-mutagenic.

DR. LIEBLER: Okay. But I think we need to have representative genotox for the major classes. And it looks like we've got it for laminaria.

DR. BELSITO: But does that take care of laminaria digitata?

DR. LIEBLER: In addition to the Wave 2, there is what was in the report, Table 23, which said laminaria digitata -- this is PDF 60 in the original report. And it's an AMES assay with and without metabolic activation. But it doesn't specify concentrations.

DR. BELSITO: Right.

DR. LIEBLER: It's probably not a great study. So, it's thin and nonexistent for macrocystis.

DR. BELSITO: Right.

DR. LIEBLER: But we've got two fucus vesiculosus in the report, Table 23, with concentrations. One is a common assay, which isn't the best; it's not very sensitive. And the other is the chromosome aberration OECD GL 487. So, we really need more on fucus vesiculosus unless that's in Wave 2.

MS. CHERIAN: There's no genotox.

DR. LIEBLER: None?

MS. CHERIAN: For that ingredient, no.

DR. LIEBLER: Okay.

DR. BELSITO: Macrocystis.

DR. LIEBLER: Fucus I was talking about. And then macrocystis. So, we're lacking genotox for both of those. We don't have any AMES for fucus.

MS. CHERIAN: No.

DR. LIEBLER: I mean, relatively to the number of ingredients is really spotty.

DR. BELSITO: Okay. So insufficient, is that fair to start with?

DR. LIEBLER: Yes. Right.

DR. BELSITO: Okay. And do we have enough on the residual impurities? Or do we just simply say restrict arsenic, heavy metals and extraction solvents?

DR. LIEBLER: I think actually we've got a lot of data on the residual metal impurities, or arsenic and metals. And we obviously should treat that in a discussion and say restrict. I'm more concerned about the lack of data on the organic constituents of concern.

DR. BELSITO: What do you mean, the extractants?

DR. LIEBLER: No.

DR. BELSITO: The solvents?

DR. LIEBLER: Like terpenoids and flavonoids. Not the impurities, the constituents of concern that could contribute to sensitization.

DR. BELSITO: Okay.

DR. LIEBLER: All the data we have so far are non-sensitizing?

MS. CHERIAN: Yes.

DR. KLAASSEN: It looks pretty clean.

DR. BELSITO: We don't have a lot of sensitization data.

DR. LIEBLER: I mean, how comfortable are you with the sensitization?

DR. BELSITO: I don't know what's in them.

DR. LIEBLER: Well, okay. If you were concerned about sensitization with these, then that increases the need for data on the constituents of concern that are associated with sensitization.

DR. BELSITO: Right.

DR. LIEBLER: If you had a very thorough list of studies that were to show non-sensitizing in humans, at use concentrations, then I wouldn't be so concerned about having data on terpenoids and flavonoids and so forth.

DR. BELSITO: So, we need composition on laminaria and macrocystis?

DR. LIEBLER: Yes.

DR. BELSITO: We need a 28-day dermal? Or are you happy with a grass status?

DR. LIEBLER: I think the grass status helps. We've got Table 22, Oral repeated dose. We hardly have any studies in which there's evidence of toxicity, either in acute or repeat dose.

DR. BELSITO: (Inaudible) dose with the extract for iodine.

DR. LIEBLER: Yeah.

DR. BELSITO: Thyroid affects.

DR. LIEBLER: Right. I mean, because it's such a big group, we don't have a comprehensive data set for toxicity with all of them. But for what we do, it's a pretty consistent message; these aren't really toxic.

DR. BELSITO: So, you don't need a 28-day dermal?

DR. LIEBLER: I don't think we need the 28-day dermal. If you take that information, plus the widespread use of these as dietary supplements or food additives.

DR. BELSITO: Okay, so we're not worried about dermal absorption because we have all of this grass status, dietary supplement, et cetera.

DR. LIEBLER: Right.

DR. BELSITO: Okay. And then we need composition on laminaria, macrocystis, sensitization and irritation and concentration of use for macrocystis. And we're okay with the 46 for laminaria?

DR. LIEBLER: If you're okay with it, I'm okay with it.

DR. BELSITO: Well, I guess we'll see what the composition looks like. Photo absorption?

DR. LIEBLER: Photo absorption?

DR. BELSITO: Yeah.

DR. LIEBLER: Oh, I'm sure they all absorb. I mean, they're complexed, you know, botanicals. They all absorb.

DR. BELSITO: So, then we need photosensitization/photo-irritation?

DR. LIEBLER: I don't think that necessarily follows. Do we have any photosensitization on any of them?

DR. BELSITO: Nope.

DR. LIEBLER: I mean, complexed organic mixtures all absorb, but not all of the absorbing materials -- I mean, most of the absorbing materials are not photo allergens or photosensitizers.

DR. BELSITO: Right. But some of them are.

DR. LIEBLER: I mean, with pure compounds, absorption tells you something.

DR. BELSITO: Right.

DR. LIEBLER: With mixtures, absorption doesn't tell you anything. So, the kind of logic use in RIFM where if it has absorption above or below the benchmark, clears it, that doesn't apply in mixtures like this.

DR. BELSITO: Right. So how do we deal with that?

DR. LIEBLER: If we had --

DR. BELSITO: Composition.

DR. LIEBLER: -- composition. Again, constituents of concern, including known photosensitizers. Flavonoid, terpenoid sensitizers. That's why I kept coming back to that point. If those are low, or minimal, or at least documented and the measured amounts are present in ingredients that have been tested, at least for sensitization, then I think we're okay.

For photo, that's really hard to predict for mixtures. For pure compounds, sure. But for mixtures, it's really hard to predict. And then I don't know that we're going to get very far by saying we want photosensitization on everything. I mean, we can ask for photosensitization on representative ingredients from the major groups.

DR. BELSITO: So, photosensitization, phototoxicity for laminaria and macrocystis, or concentration of use?

DR. LIEBLER: Yeah. And if we don't get that and they respond with data on constituents, particularly organic constituents that might be associated with photosensitization, then we can take that into consideration.

DR. BELSITO: What about genotox?

DR. LIEBLER: Based on what I've seen so far, I think the data are thin. We'd like more genotox data. Particularly for --

DR. BELSITO: For laminaria.

DR. LIEBLER: On the laminaria.

DR. BELSITO: On macrocystis.

DR. LIEBLER: Macrocystis, right. Yeah.

DR. BELSITO: Anything else? Developmental repro? No?

DR. LIEBLER: I really doubt it. I mean, I don't think we're going to need it.

DR. BELSITO: Are we clear on the genotox, on the idea that they're used as foods?

DR. LIEBLER: What do we have on carcinogenesis?

DR. BELSITO: Nothing.

DR. LIEBLER: Nothing.

DR. KLAASSEN: Well, you know, this is our first time around. I think we should ask for genotoxicity.

DR. LIEBLER: Yeah.

DR. BELSITO: Okay. For again, laminaria and macrocystis?

DR. KLAASSEN: Right.

DR. LIEBLER: I agree with you, Curt.

DR. KLAASSEN: And regarding phototoxicity, that's -- you know, these chlorophyll-type compounds and chlorophyll degradation products are photosensitizers. So therefore, to request those there is some reason.

DR. LIEBLER: I think we agreed on that. I think we agreed we're going to ask for that.

DR. KLAASSEN: But all I'm saying is it's not just grabbing out of nothing. There's a kind of a reason for it.

DR. BELSITO: The list I have so far is we would like some information on the composition of laminaria and macrocystis. Sensitization and irritation and concentration of use for macrocystis. Phototoxicity, photosensitization at concentration of use for macrocystis and laminaria. And some genotox on laminaria and macrocystis. That it?

DR. LIEBLER: Yes.

DR. BELSITO: Anything else?

DR. KLAASSEN: That should be good enough.

DR. BELSITO: Any other comments on brown algae? Okay.

DR. LIEBLER: I think this will be easier to deal with next time when we can have it all in one document.

DR. KLAASSEN: Yeah.

DR. BELSITO: Oh, well, then we still get Wave 7 and 8. Okay.

## HYDROGEN PEROXIDE

DR. BELSITO: Hydrogen peroxide. Oh, that's an easy one.

DR. KLAASSEN: At least it's one chemical.

DR. LIEBLER: Right.

DR. BELSITO: And it's safe as used.

DR. LIEBLER: Yes sir.

DR. BELSITO: Just some edits that I have.

DR. LIEBLER: Same. I have numerous small edits for clarity, but it looks good.

DR. BELSITO: Okay. Ginkgo biloba.

DR. KLAASSEN: I thought in regard to the hydrogen peroxide, as far as the discussion was concern, I don't think we had too much about being a mutagen and not a carcinogen. In essence, I don't think we had that discussed. And if we don't, I think we need that.

DR. LIEBLER: At the end of the first paragraph we have, "The panel noted positive genotox studies, but determined results are not relevant to cosmetic use due to --"

DR. BELSITO: The bactericidal nature and rapid hydrolysis.

DR. LIEBLER: Due to rapid -- it's not hydrolysis, I reworded this. "Rapid consumption of hydrogen peroxide by reaction with skin proteins."

DR. KLAASSEN: So, that is in the discussion.

DR. LIEBLER: It is at the end of the third paragraph. You know, unless you wanted something more elaborate than captured in a sentence.

DR. KLAASSEN: I guess maybe the reason I kind of missed it, it wasn't kind of a major paragraph by itself. What's said there is sufficient. I don't feel strongly about that. It just didn't knock me over the head. And so therefore, I thought it wasn't in there.

DR. LIEBLER: Yup. It's there. We're good.

## GINKGO BILOBA

DR. BELSITO: Okay. Then we're down to ginkgo biloba. At the last meeting we went safe as used when formulated to be non-sensitizing for all the leaf components. And insufficient for the biflavones, the meristem cell, the nut extract, the root extract and the terpenoids.

We wanted method of manufacture, composition, impurities, 28-day dermal tox, dermal irritation and sensitization, ocular. We didn't get it. So, we're staying with the same conclusion I presume?

DR. LIEBLER: Correct.

DR. BELSITO: I think Paul pretty much said the same thing. Let me see. "Okay to proceed with five safe as used and five insufficient."

DR. LIEBLER: I had no edits. Looks great.

DR. BELSITO: Yeah. Okay. We're up for Wilbur now with polyols.

MS. BURNETT: I think they might close to being done with him over there, but I'm not quite sure.

DR. BELSITO: What do we do here? He's got polyfluorinated. He's got the polyols, titanium, vinylpyrrolidone. Alice?

MS. BURNETT: She's over there.

DR. HELDRETH: We can continue with Wilbur's and I'll take notes for him.

DR. BELSITO: Okay.

## POLYOL PHOSPHATES

DR. BELSITO: Polyol phosphates at the June meeting we reviewed safety of ten of them and issued a tentative report with a split conclusion that the phytate, phytic acid, phytin and trisodium inositol triphosphate were safe.

The data were insufficient for disodium glucose phosphate, manganese fructose diphosphate, sodium mannose, trisodium fructose diphosphate, xylityl phosphate, zinc fructose diphosphate.

The panel determined the needs for those were manufacture, impurities, absorption, distribution, metabolism and excretion. We got some data on manufacture and impurities of sodium mannose phosphate in Wave 2, but nothing else.

DR. LIEBLER: I think it looks great. I didn't have any edits. I agree with the discussion and the conclusion.

DR. BELSITO: So, no change to the conclusion?

DR. LIEBLER: No changes for me. What did Paul say?

DR. BELSITO: "Four safe as used. Six insufficient. No new data received. Minor edits to the report submitted to Kevin. Okay to proceed. Four save, six insufficient." Okay.

DR. LIEBLER: Let the record show Curt put his thumb up.

DR. BELSITO: Wave 2 we got some impurities. And I was okay with a split conclusion.

DR. LIEBLER: Yup.

DR. BELSITO: Okay. Moving on.

DR. ANSELL: One of the insufficient was the sodium mannose phosphate, in which we have provided the manufacturing information, impurity information, skin irritation, eye irritation and a negative AMES in Wave 2. We're hoping that that may be sufficient to move that one insufficient.

DR. BELSITO: Yeah, Wave 2, I just have we have manufacture and impurities. Did we get anything else? That's all I have. I don't have any sensitization irritation.

DR. ANSELL: Oh, they were already in the report?

DR. BELSITO: What?

DR. ANSELL: They were already in the report.

DR. KLAASSEN: And this is for which chemical?

DR. BELSITO: Sodium mannose phosphate.

DR. KLAASSEN: Okay.

DR. LIEBLER: Yeah, what's in Wave 2, Page 422 of PDF for Wave 2, it strikes me as ambiguous.

Particularly the impurities. The possible impurities .1 to .5 percent are the following. Does that mean that they actually have measurements for these and they're below a certain amount? Or what? Is it just that somebody thought, well this was what would probably be in there which is how I read it, which doesn't really answer our question.

The method of manufacture is adequate. I mean, I supposed if I was being curmudgeon I would say, no, this isn't really very good for impurities. But it might just be the way that it was phrased. It looks like somebody typed up a memo and sent it to you; or somebody called somebody on the phone and somebody wrote down some notes. I don't know.

DR. BELSITO: Yeah. And also, we don't really have sensitization, Jay. What we have is one negative KeratinoSens assay. That hardly counts for sensitization. You don't have the DPRA. You don't have an hCLAT, you don't have a LuSens.

DR. LIEBLER: But I think our discussion made it clear that the sensitization data in aggregate were satisfactory.

DR. BELSITO: For sodium mannose phosphate?

DR. LIEBLER: Let's see. For phytic acid, for sodium phytate --

DR. BELSITO: Yeah, I know. But we're talking about the ones in the group that were insufficient. And he's saying he provided us with data on impurities and method of manufacture for sodium mannose phosphate and was hoping we could move that into the sufficient.

DR. LIEBLER: Mm-hmm. Our discussion only says that the group of insufficient, were insufficient for method of manufacture and impurities. We don't refer to sensitization. If you look at PDF 38.

DR. ANSELL: You have sensitization in 35, sodium phosphate KeratinoSens up to 1000 ppm. Non-sensitizing --

DR. LIEBLER: All I'm saying is that we didn't point out, in our previous report, that we wanted sensitization on sodium mannose phosphate.

DR. BELSITO: Right.

DR. LIEBLER: We said we were short for method of manufacture and impurities. And then the ADME, that came from a comment Ron made at the end of our vote, I think. And I didn't agree with it, but nobody argued it, it ended up as a bullet in here. But I don't think that we're really insufficient for those issues.

DR. BELSITO: Okay.

DR. LIEBLER: So, method of manufacture and impurities is the shortcoming for these ones that are listed. And, I think, arguably we can take what we have for sodium mannose phosphate and move that over into the safe as used group. And that leave the disodium glucose phosphate, the manganese fructose diphosphate, the trisodium fructose diphosphate, xylityl phosphate and zinc fructose phosphate as insufficient for method of manufacture and impurities.

DR. BELSITO: Okay. What you're saying, Dan, is that we're moving sodium mannose phosphate up with the other four?

DR. LIEBLER: Correct.

DR. BELSITO: And then the other five are remaining insufficient?

DR. LIEBLER: Correct.

DR. BELSITO: I'm okay with that. Curt?

DR. KLAASSEN: Yes. I have no problem with that. In fact, I'd go so far as to put them all into the top group.

DR. BELSITO: We don't have method of manufacture and impurities.

DR. KLAASSEN: Yeah, I know. But I just don't think that's a problem with these.

MR. JOHNSON: So, the ADME data are not needed?

DR. LIEBLER: You know, the way that came up, when I reread the minutes from the full panel discussion, that was kind of an afterthought mentioned by Ron Hill. It was, you know, one of Ron's things he mentions. We didn't really talk about it because we were already moving on to the next ingredient.

I recall, that was at the very end of the discussion. I think we had already taken the vote. And I don't agree that those data are lacking. And it wasn't clear to me why Ron thought that that was a problem. If that comes up in the discussion tomorrow, we can have that discussion. But I don't think we're lacking for ADME or that that presents a problem to arriving at a conclusion.

MR. JOHNSON: So, just the method of manufacture and impurities data?

DR. LIEBLER: Right. I mean, that's the insufficiency that carried through from the prior report.

DR. BELSITO: I'm highlighting that in the discussion that we felt that it's not needed.

DR. LIEBLER: Right.

DR. BELSITO: I'm actually saying Liebler doesn't need -- okay. We're moving sodium mannose phosphate out. We're moving it into the safe as used, and so we're having five and five. Five safe as used, five unsafe. Okay? Anything else?

#### POLYFLUORINATED POLYMERS

DR. BELSITO: Okay, polyfluorinated polymers. At the June meeting we issued a tentative report that PTFE and hexafluoropropylene/tetrafluoroethylene copolymers were safe in cosmetics in present practice of use and concentration described in the safety assessment.

The data for all of the others were insufficient. And what we needed for those ten were method of manufacture and impurities, skin sensitization data at the highest maximum use concentration, and we've not gotten anything.

And Paul said, "Data needs not met. No change in comments."

DR. LIEBLER: Yes. I agree. I had suggested a way that a risk assessment based on the PFOA exposure could be incorporated, based on the EPA drinking water limit. And you had done that calculation.

MR. JOHNSON: It's in here. Dr. Zhu did.

DR. LIEBLER: Okay. And I thought that looked fine; it was very helpful.

DR. KLAASSEN: Except one thought I had on that is, you know, the -- what page is that on?

DR. LIEBLER: PDF 52.

DR. KLAASSEN: Okay. I guess my point was here that when you talk about -- I think it should be emphasized that the EPA's advisory level on drinking water already has 100-fold safety factor in it. Otherwise, people might get the idea that it's something other than that. You know, so there's already 100-fold safety factor. EPA uses 100-fold safety factor to come up with that number. Just so people understand that.

DR. LIEBLER: Then as a follow up to that then, Curt, on PDF 53, the end of that first paragraph. The first paragraph ends, "Which is essentially 100-fold lower than the EPA's advisory level." You could add another sentence to point out that that EPA estimate already includes 100-fold safety factor.

DR. ZHU: Sure.

DR. KLAASSEN: That's exactly where it probably is the best to place it.

DR. BELSITO: Where are you putting that?

DR. LIEBLER: End of first paragraph, top of PDF 53. PDF Page 53.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: Right after .14 microgram per day.

DR. BELSITO: We're saying like comma, which is already 100-fold lower? Or where are we putting?

DR. LIEBLER: Either that or a separate sentence. That the EPA --

DR. BELSITO: It should be noted that the EPA --  
DR. LIEBLER: Already contains 100-fold safety factor.  
DR. BELSITO: EPA recommended level?  
DR. LIEBLER: Right.  
DR. BELSITO: Okay. Anything else on these?  
DR. ANSELL: Yeah. We have a comment on PDF Page 51 on the pneumoconiosis. It's the first case report under clinical studies. It's not actually a clinical study.  
DR. BELSITO: Pneumoconiosis?  
DR. ANSELL: Yeah. PDF Page 51. The first case cited under clinical studies.  
DR. BELSITO: What was the issue?  
DR. ANSELL: It's not a clinical study. It was an occupational exposure with ten hours per day, six days week. No respirator and --  
DR. HELDRETH: So, it just needs to move down the page?  
DR. ANSELL: Yeah.  
DR. BELSITO: So, where did you want it? Under other clinical reports or --  
DR. ANSELL: Well, no. Like that section under occupational exposure.  
DR. BELSITO: Okay.  
MR. JOHNSON: But it's still a case report, it just belongs, in your opinion, in the occupational exposure section?  
DR. ANSELL: Yeah. Twenty-eight years of ten hours a day would strike me as an occupational exposure, not relevant necessarily to cosmetics.  
DR. BELSITO: Anything else?  
MR. JOHNSON: Does the discussion need to be revised in any way?  
DR. BELSITO: I didn't have any comments on it. Dan?  
DR. KLAASSEN: I don't either.  
DR. LIEBLER: No, not really.

#### TITANIUM COMPLEXES

DR. BELSITO: Okay. Titanium complexes. At the June meeting, an insufficient data announcement was issued for isopropyl titanium triisostearate, 28-day dermal tox. Depending upon those results, addition systemic tox and mammalian genotoxicity. And we got 2 percent isopropyl titanium triisostearate with black iron oxide, acute oral toxicity, skin irritation, ocular irritation, but we did not get what we requested.  
We also requested use concentration data, manufacture and impurities, 28-day dermal, skin sensitization and irritation on the citrate, ethoxide, isostearates and salicylates. And we didn't get any data on that. Correct?  
DR. LIEBLER: Right. We have the same data needs still.  
DR. BELSITO: Right.  
DR. LIEBLER: I think we are still where we were. Yeah, we're on our way to being insufficient for everything.  
DR. BELSITO: Well, we do have data on triisostearate and citrate. No?  
DR. LIEBLER: I meant that we don't have our data needs met completely for any of the ingredients.  
DR. BELSITO: Right.  
DR. ANSELL: But we would suggest that perhaps the isopropyl titanium triisostearate we do.  
DR. LIEBLER: You have 28-day dermal?  
DR. ANSELL: No. But we have genotox. We have acute ocular dermal --  
DR. LIEBLER: You have mammalian genotox.

DR. ANSELL: -- sensitization and an application, which shows that it isn't actually -- there's no actual exposure to the material as such as it's a complex -- it's color coating, so it's complex to the colorant.

DR. LIEBLER: Is that a chemistry part that we don't have, Jay, that description of the chemistry?

MR. JOHNSON: It's in the unpublished data. PDF Page 40.

DR. LIEBLER: Forty?

MR. JOHNSON: Yes.

DR. LIEBLER: Oh, the sideways PDF PowerPoint presentation.

MR. JOHNSON: Yeah.

DR. LIEBLER: This isn't really incorporated in our text at this point?

MR. JOHNSON: No. Actually, the reaction is on page 41, I'm sorry.

DR. LIEBLER: Right.

DR. BELSITO: Where it, 41?

MR. JOHNSON: On page 41.

DR. BELSITO: Yeah.

MR. JOHNSON: Does this mean that there's no isopropyl triisostearate in the formulation?

DR. ANSELL: That the toxicity on iron oxide would be a better predictor of the toxicity. You know, it's a surface coated colorant. It's a surface coating for colorants.

MR. JOHNSON: What does the 1.5 percent use concentration refer to, you know, given that chemistry?

DR. ANSELL: I would have to ask what the dispersant is.

DR. LIEBLER: I think we really have a shortcoming in this report, with respect to an accurate description of what the chemical entities as used in cosmetic products are. These are represented as simply these coordinate complexes of, you know, like titanium ethoxide, isostearates, et cetera.

And this presentation of this as essentially a coating for a pigment, raises the question of what is the nature of the full molecular species on which the titanium isostearate is attached. And I don't know is that pigment a big molecule? Is it a little molecule? Do we know, Jay?

DR. ANSELL: It's titanium dioxide.

DR. LIEBLER: No. No. The pigment that it's attached to.

DR. ANSELL: Yes.

DR. LIEBLER: Oh, that's just titanium dioxide --

DR. ANSELL: With a surface coating.

DR. LIEBLER: -- with the titanium isostearate coating on it. But it's a titanium dioxide particle?

DR. ANSELL: Right.

DR. LIEBLER: So that's not absorbed.

DR. ANSELL: Right.

DR. LIEBLER: Okay.

MR. JOHNSON: And it's bound to black iron oxide, is that right?

DR. ANSELL: I don't know about the black part.

DR. LIEBLER: I thought that was another thing.

DR. HELDRETH: The data on acute oral tox skin irritation was on a 2 percent isopropyl titanium triisostearate on black iron oxide.

DR. LIEBLER: So, that's yet another -- black iron oxide is another particle on which this stuff serves as a coating.

MR. JOHNSON: That's the pigment though, is it no?

DR. ANSELL: Right. That's my understand.

DR. LIEBLER: So, the black iron oxide is a pigment. The Titanium dioxide is a pigment. And then the titanium isostearates are coatings around the outside of the pigment, both of those.

DR. ANSELL: It's a surface treatment for the colorant.

DR. LIEBLER: See, that's not at all clear from the description of the chemistry. It sounds like we're just talking about titanium coordinate complexes sort of free floating by themselves. And those are then relatively small molecules. And then we think really differently about their absorption distribution and toxicity.

For example, if the form of use of these is just in this pigment coating, then I think the 28-day dermal goes away because there's no absorption.

DR. BELSITO: Do we know that?

DR. LIEBLER: Well, that's what I'm asking.

DR. ANSELL: Well, and that is our position, is that these are just surface coatings, they're not free material. It's very much like the hydrogen peroxide or hydrochloric acid discussion. It's kind of philosophical to call that a cosmetic ingredient when, you know, it's completely reacted or in this case --

DR. LIEBLER: But that's how it was presented to us.

DR. ANSELL: Yeah.

DR. KLAASSEN: And that has to be majorly stated, what's going on. What really is the chemical?

DR. HELDRETH: Right. There's no description of it as being part of this particle in the dictionary description. And we have no data or information to suggest that the other ingredients in this report are also on particles.

DR. ANSELL: Well, and we're not supporting the other ingredients.

MR. JOHNSON: So, does that mean that the data that we're providing on black iron oxide, with 2 percent isopropyl titanium triisostearate, cannot be used to evaluate the safety of isopropyl titanium triisostearate?

DR. ANSELL: It can be used to assess the safety of it as a cosmetic ingredient because it isn't used.

DR. BELSITO: But nowhere in this report does that say that that's how it functions.

DR. HELDRETH: Right. Because that's not what's described in the dictionary.

DR. BELSITO: Right.

MR. JOHNSON: Just a surface modifier.

DR. HELDRETH: This data submission was my first understanding --

DR. LIEBLER: We need to table this and re-derive, first of all, the description of the chemical entities, and confirm that. What is the chemical form of these? Because Table 1 just shows the titanium isostearate or citrate complexes as if they were just these low molecular weight molecules that were added to whatever cosmetic product. It doesn't make any mention of the fact that they're actually bound to these larger pigments. And that changes the way we would approach this entirely.

We need to essentially re-derive the chemistry section, the descriptions. These idealized structures really aren't relevant unless they're attached to something else. The molecular weights aren't informative, they're misleading.

DR. HELDRETH: But as far as we know that's only true for the isopropyl titanium triisostearate.

DR. ANSELL: Right. That's the only data we have.

DR. LIEBLER: Okay, and that's the one that's being used. I don't know if you have any information in the dictionary as to whether or not the other ingredients that are listed are actually parts of some other complexes. The listing in the dictionary would tell you that, maybe?

DR. HELDRETH: It does not.

DR. LIEBLER: It doesn't tell you that.

DR. HELDRETH: And we've run across this same issue with other ingredients in the past where the dictionary is rather vague, but it leads you to believe it's just a small molecule. And then we find out, as we get information and process it, oh this was applied to a particle.

Now some of these other ingredients however, have functions that make it seem unlikely that they're used in that way. For example, titanium ethoxide is a binder.

DR. LIEBLER: Right. But that would leave those things all insufficient for the things we've already said they're insufficient for.

DR. HELDRETH: Right.

DR. LIEBLER: As long as they're in the report, and we don't have data, then they're insufficient for method of manufacture and composition, et cetera. And then you have a major correction for the isopropyl titanium triisostearate.

And I just want to know if there's any form of that that's just that molecule, not attached to a pigment. If there is any use for that, then we need to consider the 28-day dermal, et cetera. If the only form is the form that's attached to these larger pigment particles, then that dermal absorption issue completely goes away. And then we're really basically down to sensitization and irritation, things like that.

DR. BELSITO: Okay. So you're suggesting we table for clarification as to the true function of the --

DR. LIEBLER: The isopropyl titanium triisostearate.

DR. ANSELL: You know, we can discuss this tomorrow. But it went insufficient. We went out to the manufacturers and this is the answer we got back, is that it's used as a surface coating agent up to 1.5 percent. And I agree, absolutely, that that changes the types of data needs one would need to assess the safety of the material. But we have gotten no information back on any of the other materials and they should appropriately be carried forward with the insufficiency.

MR. JOHNSON: So, surface coating agent and surface modified are one in the same? Because the dictionary says surface modifier.

DR. HELDRETH: We've had a similar situation like this where we thought they were small molecules and found out in the process that it was actually a coating of the particle. And ultimately, what the panel concluded was safe when used as one of these coating things, and the data remained insufficient for all other uses.

DR. LIEBLER: I can't remember what it was. It was within the last year or so.

MR. JOHNSON: So, they're the same thing?

DR. ANSELL: I could not distinguish one from the other.

MR. JOHNSON: Okay.

DR. ANSELL: A coating versus a modifier.

DR. LIEBLER: So, in the irritation and sensitization section, as it's currently written we have -- on PDF Page 28, we have the yellow highlighted sentences under irritation which are the test results with the black iron oxide particle with apparently that coating. And that was no irritation in rabbits.

And then if you scroll down -- I'm sorry, at the bottom of that paragraph is a concealer containing isopropyl titanium triisostearate in an occlusive patch test with 23 human subject, no irritation. What I want to know is, is that stuff that was tested there, is that the pigment particle with this coating, or is that the isopropyl titanium triisostearate?

And then when you get to the next paragraph on sensitization I have the same question. What was tested? You have nonirritating, non-sensitizing, is that the pigment particle with the isopropyl titanium triisostearate?

I think we can't interpret the sensitization irritation data, or any of the other data for that matter, without some confirmation of what is the chemical nature of what was tested. The cosmetic ingredient was either a titanium dioxide or a black iron oxide with this coating on it. If it was, then I think we can take the data into consideration. If it wasn't then it doesn't represent the cosmetic ingredient that's in use.

DR. BELSITO: Right.

DR. LIEBLER: The reason I'm saying table it is that, I think, if we were to sit there tomorrow as a group -- I doubt this would happen -- but that we would make all these assumptions about what it is and how we can interpret the safety data and then go safe as used. I'm not there.

DR. KLAASSEN: Yes. Every time we mention this compound, in any experiment, we have to say was it the pure compound or was it this coating.

DR. ANSELL: Yeah. I think I will check with -- as you guys -- but that's my assumption from our discussion, is that these were colored cosmetics in which this was used as a surface modifier. And that those concealers and colorants, and eye products, were actually compounded -- you know, compounded products which contained pigments, which had been surface coated, surface modified with this ingredient.

DR. KLAASSEN: Okay. We just need to be sure.

DR. ANSELL: Yeah. Yeah.

DR. KLAASSEN: That's all.

DR. ANSELL: And I'm hoping we can get that resolved tonight. We'll ask.

DR. BELSITO: Anything else on this one? It's 11:56, do we have time for vinylpyrrolidone polymers?

DR. LIEBLER: Let's have lunch.

DR. BELSITO: Let's have lunch?

DR. LIEBLER: Yeah. We got parabens and brown algae behind us. We have the wind at our backs.

#### **Dr. Mark's Team**

DR. MARKS: The next ingredient I have are the parabens. And I think we all know the controversies associated with the parabens, especially the endocrine activation concerns and the development and reproductive toxicity concerns. So Jinqui, in a memo dated August 29th, has an amended safety assessment for parabens; a tentative amended report of the 20 parabens and for hydroxybenzoic acid. Last year we agreed to reopen it.

At the March meeting, we reviewed the new data. There was concerns about the EU banning five parabens, so dealing with that. We also wanted to, at the March meeting, put into perspective the potential burden of parabens from cosmetic versus multiple other source exposures.

And in March we had the presentation by Dr. Daston, reviewing endocrine disruption. Our team felt that at that meeting we would move on with a safe conclusion for the 20 ingredients, with a robust discussion about the margin of safety calculation, the bioaccumulation, et cetera. We decided to hold off with moving forward because the Belsito team wanted more details of the EU ban, the concentration limits, et cetera.

I could first ask for Tom and Ron's comments, but, Bart, I thought maybe it would be best that you address the Wave 2 letter from the Women's Voices for the Earth. I think there are four or five points and you addressed them. It's a pretty lengthy letter and you addressed them in your response. Do you want to make any comments about that? Because obviously, that's very important.

Is there anybody here from the Women's Voices for the Earth? I ask that every time we review the parabens, to be sure that if somebody were in the audience they could comment. Let me see, one, two, three, four, five. Parabens exposure in vaginal products, bioaccumulation, source of exposure, particle size, and margin of safety calculations. Bart, I think it's important we hear your responses before I ask Tom, Ron and Ron to weigh in.

DR. HELDRETH: Women's Voices for the Earth first proposal was that there was a couple cellular studies that may impact the panel's decision on safety for these ingredients. One of them being specifically directed towards DNA damage in sperm. And the other affecting the ability for a yeast strain to adhere inside the vaginal wall.

Both of these studies are a little bit pointing to endpoints that are a little bit different than maybe we are always looking at. But the question that we're asking is, do you want to include these studies in

the report and explain their relevance or lack of relevance? Or do you just not feel that these are worth inclusion at all?

MR. GREMILLION: Are the vaginal product discussed in the letter considered cosmetics? I mean, are they under the purview of this body?

DR. HELDRETH: It's a good question. They don't lay out exactly which products we're talking about here. And we're not given information whether or not it's purely cosmetic in that situation or it's a drug.

MR. GREMILLION: Well, I mean, it's talking about a lubricant for the sperm study and a douche.

DR. HILL: Those are personal care products.

MS. KOWCZ: Douches are cosmetic.

DR. EISENMANN: Lubricants are medical devices as far as I understand.

MS. KOWCZ: It depends how they're actually marketed.

DR. KATZ: For the lubricants, some of the lubricants may not actually be devices. It would really depend upon how they're labeled, but the douches are cosmetics.

DR. SHANK: Okay.

DR. KATZ: They cleanse.

DR. MARKS: Bart, your question was should those studies be included?

DR. HELDRETH: Right. If the panel feels that these studies somehow impact the safety, or they feel that it would be important to explain how they do not impact the safety, it would be helpful. I didn't know if the panel wants these included or if they're just not relevant.

DR. BERGFELD: Well, my opinion, if they're cleansing agents, then they have to be included. If that's how they're labeled, as a cosmetic cleansing agent.

DR. HELDRETH: Then we would want to know -- what should we add to our discussion section to explain the relevance or lack thereof for these? So that Jinqiu knows what to put in there, regarding whether these are relevant or not or how?

MR. GREMILLION: My understanding of the study that was cited for the cleansing agents, represented that parabens in those products would increase the risk of a yeast infection. I'd like to understand better when a lubricant is and isn't a personal care product. Because the other study, that was cited, kind of represented that using a lubricant could either maybe affect someone's ability to conceive. And maybe a consumer should be aware of that kind of risk.

DR. HILL: It's interesting that we ran into that kind of gray area when we did the group that included nonoxynol not too long ago.

DR. MARKS: Well, I don't think we can ignore the studies because they are relevant to personal care products. The question is how do we handle the results of the study? The one referring to DNA damage in sperm; and then the second one to the potential of yeast infection, which is no insignificant issue. And I'm not sure how to address those.

DR. SLAGA: What was the concern about DNA damage?

DR. MARKS: Yes. It says here -- do you have the Wave -- and I'm reading Bart's paraphrase here. "Products could potentially induce oxidative stress-associated DNA damage in human spermatozoa." When I read that, just face value, it raises a concern. Am I going to have some sort of -- end up with some sort of developmental problems because we have altered spermatozoa DNA.

MS. BURNETT: Or in infertility.

DR. MARKS: Huh?

MS. BURNETT: Or infertility.

DR. MARKS: Yeah, either one.

DR. EISENMANN: This is a direct sperm exposure study. And I think all of those are a little kind of sketchy sometimes. I think there are studies on olive oil that show similar effects. If you expose sperm to olive oil, you get some effects of motility. So yes, in a petri dish you could expose

sperm to parabens, you'd probably get effects. But I think if you do it to other things, I'm not sure it's a unique effect to paraben.

And I'm not saying you shouldn't put the study in. I think you could put it in and discuss it. But I'm not sure -- if it was a different type of exposure maybe it would be more of a concern than a direct exposure of sperm.

MR. GREMILLION: Why is olive oil -- that's not indicated as a --

DR. EISENMANN: I guess some people use olive oil as a lubricant.

MR. GREMILLION: It doesn't say on a bottle of olive oil to do that. You buy this lubricant, it says use it as a lubricant. And people buy it with the impression that they can use it for intercourse and conceive a healthy child with it. And the study is saying that you use it, it's going -- you know, there's significant risk of impairing the sperm and use as directed.

DR. HELDRETH: I think part of Carol's point is, this study didn't test a cosmetic lubricant and determine that it caused DNA damage. They just took parabens and applied it to sperm in a petri dish. And then are making assumptions about what that means for cosmetic products.

DR. SLAGA: You know we have a very good DNA repair system, both in nucleus and the mitochondria to take care of DNA damage. It's only when it's overwhelmed with large doses of something bringing about DNA damage is there's a problem. Where, you know, if you get a mutation from it later on. But the DNA repair insights are very effective.

DR. SHANK: Do sperm have DNA repair?

DR. SLAGA: Yes.

DR. SHANK: Sperm do?

DR. SLAGA: Yes.

DR. EISENMANN: Well, I gather it's insufficient to make a declaration on what it does. And so maybe we have to go insufficient and ask for more information or clarity.

DR. MARKS: So that would be -- yes. Because I think it's very significant that we need to have some sort of margin of safety determination. I'm not sure. You know, you have in vitro testing, but what happens with in vivo.

DR. SLAGA: But it has to be whole cells. I mean, or whole animals to have --

DR. MARKS: Pardon? I'm sorry, Tom.

DR. SLAGA: I said it has to be where you have cells -- most cells that you put in culture, do have DNA repair capacity. In vivo, it's very, very effective.

DR. MARKS: Ron Shank, if I were a consumer and I saw this, what information would I need to say, okay I'm not worried, it's not insufficient, we can move forward with safe?

DR. SHANK: I would think a margin of safety calculation. How you would do that with the data given is hard to say. What data do you want? I guess you would want in vivo data in female rats that have had impregnation.

DR. EISENMANN: These are relatively high millimolar concentrations that they're using, so I'm not sure how it's that relevant. I mean, the concentration products are relatively --

DR. SHANK: I couldn't get the paper to read, but was there a no-effect level?

DR. EISENMANN: I haven't read the paper either. I just looked at it and it says it's a direct exposure to sperm and that's not terribly --

DR. SHANK: Okay. But arguing dose concentration used right now, I think would be hard to handle.

DR. EISENMANN: In case of parabens there's like oodles of biomonitoring data now that I think you can also rely on for safety.

DR. SHANK: Well, I agree with that. But how do you handle these specific papers that show effects that would cause concern to the public?

DR. MARKS: Yeah. No, I agree. I don't think we can ignore it. And I think Wilma's suggestion of insufficient -- actually, I think it may be, we would be moving to a tentative amended report with

an insufficient conclusion at this point, with the ability to perhaps move on to safe. Right now, it's the vaginal products which are creating the issue.

We need to get more information/data, see if there are other papers. You were talking about the concentration in the in vitro testing for this DNA damage was so high, it wouldn't be relevant to the actual vaginal products. If I understood you, Carol.

DR. SHANK: Could you say these are safe for use and insufficient for use in vaginal products?

DR. MARKS: Yes. That would be another way of doing it. Thank you, Ron. Insufficient for vaginal products. And then it'll go out that way, and if we get the information we need, we can -- because we have two. I mean, it's not only just the DNA damage, the second paper referred to -- if I understood it correctly, the enhancement of yeast infections.

DR. HELDRETH: The enhancement of potential adhesion.

DR. MARKS: Adhesion which, again, your next inference, if there's increased adhesion of yeast to the vaginal epithelium, then potentially increased incidence of yeast infections. And there should be some sort of data maybe from, again, the producer of those products. What's the incidence of reports, so on and so forth of yeast infections?

DR. HELDRETH: But I'd also point out that this, again, was another cellular study.

DR. MARKS: Yeah.

DR. HELDRETH: There's a little bit of difficulty relating this to actual cosmetic use.

DR. MARKS: Yeah. Same as we have with the DNA. But I think, Ron Shank, your suggestion, insufficient for vaginal products. And then the manufacturers of vaginal products have got to give us the data to reassure us that it's safe, along with the public. That's obviously, whom we are.

DR. HILL: And beyond that, calculations can be done. Are the concentrations tested in these in vitro studies anywhere near the range that could be developed when you use those products as labeled for vaginal use? If it's 100 times different, probably not relevant. If it's in the same range, it might be relevant.

DR. BERGFELD: One of the interesting things about mucosa in skin is that it absorbs most everything very quick.

DR. SHANK: Could you say that again? I didn't hear you.

DR. BERGFELD: I said one of the absorption problems in the skin, in the mucosal area, is almost everything you put on a mucosa, whether it be oral or genital, absorbs very readily. And there have been measurements by Mabox four times of what on the glabrous skin.

DR. HILL: The question is, though, if the sperm have direct exposure to the parabens in the vaginal canal at the wrong time, you could make conservative assumptions. This much in there reasonably -- this much concentration would develop transiently. Intercourse occurs, sperm are exposed, what's the possibilities there? I think those calculations could be done with a little bit of conservativeness.

If we've got good dose response -- you need first what's the concentration versus response affect -- or response versus concentration in those in vitro studies. Because sperm aren't as well protected, metabolically, as many other cells in our body and in our system in general. But if it just kills the sperm, then the consumer is warned that this might affect your ability to conceive. If it's resulting in mutated sperm that can still fertilize the egg, that's a big, big difference in concern.

DR. BERGFELD: Well, I see that as one problem; the second problem was the yeast. But the third problem is that the paraben is more readily absorbed from that area. And there is a question here about bioaccumulation and this lipophilic.

DR. MARKS: Okay. Good, Wilma. Great segue into the next -- I think we've dealt with the vaginal products and how we're going to handle that. Insufficient at this point unless we get a margin of safety for the DNA sperm damage, and we get a margin of safety for the yeast adherence. Some sort of -- more data on that. Otherwise, it's insufficient.

And number two in the memo is bioaccumulation. Bart, do you want to address that? I know we've discussed that previously. Is there anything more in this report that we/Priya should add, to answer the letter from the Women's Voices for the Earth, concerning bioaccumulation? Taking into fact just what Wilma mentioned, it's found in vaginal products. We know absorption through mucosa is higher oftentimes than just skin.

DR. HELDRETH: Right. I think if we're looking at the accumulation, it's kind of two definitions of accumulation here. It goes into the fat cells; or it goes into the fat cells and we keep adding to it over time. Do these studies adequately demonstrate that they keep adding to that concentration in the fat cells over time? That's something for the panel to determine.

And then additionally, the Women's Voices for the Earth suggested some studies on wildlife environmentally exposed to parabens; suggesting that those concentrations found in the wildlife are somehow relevant to cosmetic safety.

DR. MARKS: I don't know. To me, the wildlife is an easier one, because we usually don't deal with things other than personal care products on human beings. I think we can -- just like we don't do environmental accumulation generally.

But how about the former, the bioaccumulation in humans? Is it an additive in the repository in the adipose tissue?

DR. SLAGA: Sebaceous glands.

DR. MARKS: Pardon?

DR. SLAGA: Well, yeah. It can accumulate in different areas.

DR. MARKS: Is it address well enough in here? We actually, in our discussion, in the last meeting said we needed to have a robust discussion about bioaccumulation. Any points to be made?

DR. HILL: I think there's missing science. And I mentioned this the last time. Because if parabens themselves, the estrogenic activity of those, is a red herring, they're not potent estrogenic substances. Were you to form significant amounts of metabolites and it would have to be butyl/isobutyl benzylparaben, but we're not using benzylparaben anymore, so that you could get hydroxylation on both ends. Now you have something that potentially can bind with much higher affinity to estrogen receptors.

But I couldn't find any -- at least when I did a search on the structure bases quickly, I couldn't find anything on that. I think we're actually missing some science. And one thing I also know is, if you have a lipophilic phenolic compound, sometimes you can get sequestration.

If you had a carbon label, for example, you could make a glucuronide and there are places in our body that can sequester those. That doesn't mean it has any deleterious consequence. But as far as I can tell, we don't know anything about that.

But the estrogenic activity of parabens themselves, for me for a long time, has been a complete red herring because they're so weak as to be almost inconsequential even to accumulate.

But the metabolites, the problem there is we don't know, that could vary by genetics. And that could account for a -- it probably does vary by genetics if we're talking about enzymes that do omega and omega -1 hydroxylation, if it's isobutyl and butyl. Again benzyl, then there's probably an array of P450s, but that's not much in use anymore; and there's probably a good reason for that.

I feel like there's missing science; and I can't come to some firm conclusions on some of this because the science hasn't been done. We've been so focused on this red herring that we haven't actually paid attention to the SAR of estrogen receptors and compounds that are active and not active, enough to actually go after what we really need to go after.

DR. MARKS: There's an estimate in refinement of aggregate exposure that's on page 97. That was to address the bioaccumulation issue? I'm on page 97 in the PDF.

DR. HILL: Of the main one?

DR. MARKS: Yes.

DR. HILL: Okay. Not Wave 3 or Wave 2?

DR. MARKS: It's the section right before the summary. Because I think we have to address, straight on, the issue of bioaccumulation, and if we feel it's relevant to cosmetic products.

DR. ZHU: Yes. We'll address that. We will revise it in the next iteration. And I will have some good comments from Dr. Liebler, and those will be incorporated into our discussion.

DR. MARKS: Okay. There's going to be -- and essentially, we're going to address the bioaccumulation issue then in the next rendition?

DR. HELDRETH: Yes. Any additional verbiage that the panel could provide, to renovate the draft discussion section that we provided, so that it's more in line with your thinking, would be greatly appreciated. Certainly, the importance of the aggregate exposure, and looking at all the pieces that go into calculating the margin of safety; and determining if these all fall in line with the panel's consensus.

DR. MARKS: And the margin of safety was right above that section, under risk assessment. You have the calculations of margin of safety. And we felt that was fine in the last meeting, the 160, using that. Is that correct or not?

DR. SLAGA: Yeah.

DR. MARKS: That's page 97. We've seen this before, but I want to confirm that. Ron Shank?

DR. SHANK: In that margin of safety calculation, it's referred to as the dermal absorption rate. And actually, 5 percent ingredient is not a rate, it's an amount. That should be changed throughout the margin of safety discussion. That's not a rate, it's an amount.

DR. MARKS: Thank you.

DR. SHANK: Other than that, the calculation's fine.

DR. MARKS: Good.

MR. GREMILLION: I'd like to ask a question about the bioaccumulation issue. Specifically, what you said, Dr. Heldreth, about the distinction. I wanted to understand that in the context of the study from Wang, that the Women's Voices for the Earth letter cites, and its conclusion. It says a positive correlation between donor's age and the parabens was observed, which suggest bioaccumulation in human adipose fat. Are you saying that that relates to a different kind of bioaccumulation than one that would be concerning? And how does that relate to the conclusion in the draft that says thereby suggest no bioaccumulation? Are we defining bioaccumulation differently from how they're defining it in that study?

DR. HELDRETH: That's for the panel to address. We just provide this and that's their decision, not mine.

DR. HILL: I think the other problem is, as it relates to what I just said, dealing with parabens as a class is part of the problem. Now if it's free radicle formation and reactive oxygen species in something like that sperm study, this is no big shock. Because phenolic hydroxyls do that. In fact, our enzymes use that, that's how our P450s work.

And we also can make catechol metabolites of some of these, although I don't think that's a major metabolite in this case because of the carbonyl on the opposite end. But benzylparaben, if you hydroxylate the other end, you've got something that's guaranteed to be estrogenic in activity, and probably at a fairly robust level. I don't know exactly what the affinity is. It would be the metabolite, not the benzylparaben itself, that will have that activity.

And again, I think it's the use on benzylparabens is furthering down to zero. The accumulation studies tell me which parabens. Don't just deal with them as a class, because I think there's some that are potentially much more concerning than others. All of them are weak as they are, unless they get metabolized on the opposite end. That's estrogenic activity. That's not to say that that's the only activity we deal with.

DR. HELDRETH: Yes, there are no reported uses for the benzyl.

DR. MARKS: Tom, Ron, Ron, anything more you want to add to Bart's response about the bioaccumulation that should be added to the report?

DR. HILL: And I think part of the reason for what I just said, was you have to pay attention to exactly how -- did they measure the accumulation of all parabens as an aggregate? Or are they separating them out in some useful manner and trying to get the correlations?

DR. MARKS: Ron Shank?

DR. SHANK: Nothing to add.

DR. MARKS: Okay. Source of exposure was the next point. And then Bart, once you mention -- your response?

DR. HELDRETH: The point was they wanted to make clear what the major "source" of parabens that could be accumulated in tissue came from. In our report and in this draft, we simply reiterated the conclusion that came from the different articles that we found. We wanted to leave it up to the panel to elaborate, in their discussion, what they felt what the source was.

In many of these studies, like the environmental studies that we were just looking at on accumulation in adipose tissue, we don't know what the source was. It just says environment. And so, it's somewhat hard to relate that to cosmetic use.

DR. HILL: Still, I don't think we can ever ignore how much cosmetic use is potentially contributed to aggregate exposure for things that accumulate. I mean, you don't write it off on that basis. Well, if we're only doing cosmetics and not eating food with parabens in it or other products that might have it, I don't think you write it off.

My big concern right now is that, so you see already a lot of people advertising paraben free, and then if there's no parabens they're preserving somehow else; and is that any safer or not. Because all too often I see, yeah, there's something else in there and there's a whole lot known less about it. This folklore stuff is driving me crazy at this point, because show me the science. But then just writing it off -- we have to respond to these things.

MR. GREMILLION: Yeah. I understood the letter to be asserting that the primary pathway of exposure, for parabens, is cosmetic and personal care products. Not that there weren't other sources of exposure, but that they were insignificant. And it cites some -- you know, once the estimate is less than 4 percent of the total aggregate exposure, you know, that comes from food. I mean, there's a few studies cited.

It seemed important to say, you know, exposure is primarily coming from personal care products. Because otherwise, there's this kind of indeterminacy that can read into it.

DR. HILL: My impression is there probably is a correlation between underarm deodorant or antiperspirants or drugs. There probably is a correlation between that, and what appears in some area of the breast tissue. It would be hard to argue against that now with what we've got out there of what I've seen. And then the question is do you keep using those under deodorants or not.

DR. HELDRETH: I mean, certainly we can't argue that there's not exposure due to cosmetic use. But the studies that we're looking at, where we find accumulation of paraben in tissue, don't tell us what the source was. And so, I think that's what we were trying to get to when we gave those studies and the summaries that came with them. However, I don't disagree that the panel could provide some statement saying, of course, exposure occurs from these materials if they contain the material.

MR. GREMILLION: But it's one thing to tie in -- you know, there's a certain accumulation that's tied to exposure from one thing or another. But it's another thing to say just in general, exposure to parabens is primarily from personal care products. It seemed like there was good support for that assertion. That just in general, a person's exposure to parabens is primarily through personal care products.

DR. HELDRETH: But that's the panel's call to say if they agree with that or not.

DR. HILL: And another way of looking at this is, so what it bioaccumulates, what's the problem? Things that are lipophilic accumulate in fat. Can you show me that there's any problem that comes from that? Because I have yet to see that link.

DR. SLAGA: Well the couple TCDD and compounds like that accumulate in fat throughout the body and it's quite a problem.

DR. HILL: I'm aware of those. Very aware.

DR. SLAGA: That's from agent orange, from mega doses. Humans, you know, once it's in the fat, I'm told, there's some problem with people sometimes when they go on crash diets and lose a lot of fat, that comes back into the circulation. There are problems.

DR. HILL: I won't argue that.

DR. SLAGA: We would need an expert on bioaccumulation to talk and give some examples.

DR. HILL: I'm aware of those, but my contention would be, if these guys come back out of fat, no matter how fast, they'll be glucuronidated so fast, or the ester will be hydrolyzed so fast once it hits the blood stream, either way, that there will be no consequence, systemically, other than they are accumulating in fat. And that's the part of it I feel like we still aren't getting the big picture here. If it's causing DNA mutations in adipose tissue, you would expect maybe cancers in adipose tissue. I haven't seen any such with parabens. Are there? I haven't seen that. I'm not trying to pooh-pooh anything, I'm just saying we might be missing some science. But I doubt it, unless it's benzylparaben that's hydroxylated on the other end, then you can convince me.

DR. MARKS: When I look at the document here on page 83, where it talks about non-cosmetic use, this is -- methylparaben, propylparaben are generally recognized as safe, so they're grass. It's added to food, synthetic flavoring, et cetera, in here. I don't know how much is from cosmetic exposure versus potentially -- in here. I guess if it's grass --

DR. HILL: Well again, like I say, so it accumulates.

DR. MARKS: Yeah.

DR. HILL: Show me the problem that comes from that.

DR. MARKS: Okay. Well, I think we've discussed sources of exposure. How about particles?

DR. BERGFELD: I think I'd like to add something. I think that the documentation, on the fact that when a cosmetic product has a paraben in it, when compared to normal who don't use paraben products, there is a big different, no matter what they measure. They have several studies in here. I don't think you can deny that it's absorbed, and it's excreted.

DR. MARKS: That what?

DR. BERGFELD: Absorbed and excreted.

DR. MARKS: Oh, yeah.

DR. BERGFELD: And it's higher in the user than the nonuser. I think you can clarify, yes, that's true. But in the whole world of affairs, is this the major source? Is that what you're going after?

DR. MARKS: No. I'm just saying --

DR. BERGFELD: Is the environment the major source, or the grass?

DR. MARKS: No. I'm just saying that we know there are other sources, that's all.

DR. BERGFELD: Oh. But I think we have to respond, yes, we agree that it is absorbed.

DR. MARKS: Oh, yeah.

DR. SLAGA: We have to respond.

DR. MARKS: Any other comments about that portion of the letter? How about particle size of parabens?

DR. HELDRETH: The particle sizes of these pure raw materials can be found in the ECHA dossiers, and we don't disagree with that. But how that relates to particle size in the final formulation is a bit of a stretch. At least that's how it seems. But it's the panel who can determine whether they agree with that or not.

In products that could result in incidental inhalation, these materials are used at no more than 0.13 percent in spray formulations. And in powder formulation, no more than 0.3 percent.

DR. MARKS: Okay. And lastly, margin of safety. We said we like it. Their comment was -- and this is yours, Bart. You would modify the calculation of margin of safety?

DR. HELDRETH: We could, if the panel agrees that that's appropriate. We use 0.4 for single paraben use and 0.8 percent for multiple paraben use, as we did in the original paraben's report that came out in 2008. The 0.4 percent is still essentially the maximum use concentration that used in cosmetic products, except for when used in a mascara. That's where the 0.5 percent comes into play.

Whether or not the panel really wants to use the 0.5 percent in a calculation or stay with the 0.4, that's your determination.

DR. MARKS: And you said the impact there would be from 270 to 216 when you calculate it?

DR. HELDRETH: That's correct.

DR. MARKS: Which still at 216 it's still a significant margin of safety. I would think we would want to use the most conservative. Even though it's hard to believe it would be -- even for mascara, that's such a small area of exposure.

DR. EISENMANN: What I would suggest is if you look at this paper, this Cowan-Ellsberry and Robinson paper, where they were refining aggregate exposure; rather than using one concentration, they split the exposure into oral, eye products, non-rinse off and rinse off products. Because then you could use not just one concentration. You could use the .5 concentration for the eye-area products.

The leave-on products really drive it, and I think it's .24 is the maximum concentration in those products. In other words, you wouldn't for the whole expose have one concentration. And that comes out, without any absorption factor, a little bit less than what you already have.

It would be based on what data you received. Because I'm not sure how you'd justify .4 anymore.

Because that's for butylparaben; because that's not the regulation any more for butylparaben in Europe. That would be my suggestion. It's Table 7 of that paper where they give the values that they recommend.

DR. HELDRETH: Right. Those concentrations that are in the MOS calculation of the 0.4, those are in line with the aggregate exposure that is also in that calculation; that 17.76 grams per day, which would be really hard to reach from a mascara, as you were saying. That's sort of what was driving us not making that change, just because the concentration of use in one use type went up by .1 percent. But if the panel wants to make that change, that's their prerogative.

DR. BERGFELD: I think it's a public relations thing that probably you should do the .5. That's the highest you have, and it still you a great margin of safety. The question I have of you, Carol, is if you calculate per site --

DR. EISENMANN: Then they just break it into four different --

DR. BERGFELD: Four sites. Then what do you do, add them together?

DR. EISENMANN: Yes.

DR. BERGFELD: And then you get the maximum for that person? Is that where you got the 17? That was the 17, the daily exposure --

DR. HELDRETH: Right. Across these.

DR. BERGFELD: -- across, you know.

DR. EISENMANN: It comes up -- the total amount of product used is very similar, but they just break it into four different pools.

DR. BERGFELD: It's interesting. Yeah.

MS. KOWCZ: Four different types.

DR. BERGFELD: That's even better.

DR. EISENMANN: Right, four different types. The types are oral hygiene, and includes lipstick, eye products, non-rinse off products, which is face cream, and then the rinse off products. And the most use per day is 13.5, for the non-rinse off products. Because then you could use the actual

highest concentration that was reported in the concentration of use survey, for that set of the product.

You'd use the .5, but you'd only use the .5 for .5 grams of it, because that's about how much eye makeup you would use per day.

DR. HELDRETH: Right. You wouldn't put the .5 in the calculation and multiply it by 17.76.

DR. EISENMANN: Right. Instead of doing that, you would --

DR. HELDRETH: Those numbers don't match. They're not the same exposure.

DR. MARKS: Basically, we would expand the section on margin of safety and make those changes. Do I understand that correctly? We would use the 0.5 percent as the highest concentration, but in a limited area in the calculation. But then would you also go back to wider areas, as you have in that paper? Make a couple margin of safety calculations, one perhaps for the highest concentration of exposure and perhaps a second one with the largest area of exposure. I don't know. Is that what you're aiming at, Carol?

DR. BERGFELD: No. Four different areas.

DR. MARKS: Four different areas?

DR. EISENMANN: Four different areas and then you add it together.

DR. MARKS: Okay.

DR. EISENMANN: Here, I can show you this.

DR. MARKS: No, that's okay, I understand now. Yes, so the four different areas is the largest.

DR. EISENMANN: And then you add it together, so it's just one margin of safety calculation.

DR. MARKS: Okay. Yeah.

DR. HILL: Well, in fact, I don't remember whether it's RIVM or the European -- what is it, SCCS? I mean, they have a set of body areas and surface areas. It's a much larger than four different sets of pools and then add those altogether.

And another thing I was going to say is mascara, you're not even putting it on the skin unless you really mess up. This is mascara, not eyeshadow, right? I mean, you could potentially have a little exposure, but nowhere near how much you're even putting on the eyelashes.

MR. GREMILLION: She had a point about using an 80 percent absorption rate, rather than 50 percent. Is that a change based on an assumption made on a Proctor and Gamble research study?

DR. HELDRETH: Our understanding of it is that 50 percent is already whoppingly conservative, just like over the top. Because we have absorption data that shows that it's at best 3.7 percent.

Therefore, 50 already maybe way, way too conservative. Taking it to 80 -- it's up to the panel to determine whether the data they presented warrants that.

DR. HILL: Well, is that 3.7 percent relative to a mucus membrane in vagina, for example. I don't think it is, actually. It matters where on the body. I don't know if that's on, or sort of half way in, but.

MR. GREMILLION: I also wanted to get back -- I apologize -- to the particle size. Dr. Heldreth, were you saying that the concentrations are so small that the particle size of the raw material isn't relevant?

DR. HELDRETH: There's a number of things that go into when we're looking at the particle size. Our boilerplate right now mostly focuses on particle size, but we're working on that to look at more things. But the particle size, when we look at it, we're looking at it as, what are the particle sizes that could be incidentally inhaled in the final formulation.

And we know -- and we put it in our boilerplate language -- that when you formulate materials together, typically you get things like agglomeration where the particles start sticking together. And you get much bigger particles very quickly. Add to that that these materials are at best no more than 0.3 percent of the final formulation. So, even if they were completely separate, and did not agglomerate, that's still a tiny, tiny fraction of the formulation that was applied. And so, then the number of tiny particles is tiny.

DR. BERGFELD: Tiny, tiny.

DR. EISENMANN: It's not in the formula, the particle. I mean it's dissolved into the solution. So, it's not just a little par -- so, the particle size of the material has really no relevance to what the particle size is in the product.

MR. GREMILLION: What's the relevance of including the particle size of the raw material in the safety assessment?

DR. HELDRETH: None.

MR. GREMILLION: Okay.

DR. HELDRETH: It would be relevant to an occupational exposure for the people manufacturing these raw materials. But from a cosmetic application, there isn't any.

DR. MARKS: Okay. I think that addressed all the points in the letter. Thanks, Bart. Is there anything else that we should discuss? Any other comments?

DR. BERGFELD: I'm sorry. I think that the document has to be modified as to the discussion we've just had.

DR. MARKS: Oh, absolutely.

DR. BERGFELD: And I think that the presentation of different sites and doing that risk calculation is most appropriate in these times. And the fact that instead of using one we're going to look at body parts as well.

And I think that the bioaccumulation has to be discussed fully, as best we can. And of course, the one in question are the vaginal mucosal exposures. So that could be put in our discussion because we have already said it was going insufficient for those kinds of products.

DR. HELDRETH: Any verbiage that the panel would provide to us, in their notes, to lead into redrafting that discussion, would be greatly appreciated.

DR. MARKS: Okay.

MR. GREMILLION: Sorry.

DR. MARKS: No, that's okay.

MR. GREMILLION: I want to make one last point.

DR. MARKS: You can make two last points.

DR. HILL: Ten if you need to.

MR. GREMILLION: There was a point in the letter about just spelling out the decision to go from the NOEL to reject, I guess, the ten or two that was suggested in one of the papers and go with the 160, after Dr. Daston, from Proctor and Gamble, presented. And just having -- I know from my own personal edification, I would like to see that spelled out.

I know he talked a lot about the methodology used in the different research, and different sources of doubt. But it wasn't really clear to me how -- from the conversation, from the transcripts -- why exactly that that 160 ended up being adopted.

DR. HELDRETH: We don't disagree with that at all. And any assistance the panel can provide, in their notes, for better representing that in the discussion section would be appreciated.

DR. MARKS: Okay. One of the other concerns that we spent a lot of time, appropriately so, about the concerns from Women's Voices for the Earth. But also, there was concern by the Belsito team in terms of why the EU has a ban of some parabens, and also concentration limits. And do we have an understanding of that, why that occurred?

Because the next step is obviously, how are we going to move this report forward? Are we going to do a tentative, amended report? Safe for all the ingredients, insufficient for vaginal products. And then in the discussion we'll be doing just what you summarized, Wilma. We'll be talking about the parabens in vaginal products. Asking for margin of safety calculation for DNA sperm damage, about the yeast adherence. We'll recalculate the margin of safety for the four areas of exposure individually, and then together. And then the bioaccumulation discussion.

But, was there anything more about the EU that we should bring up? If not, can we move on to how we want to handle it? Do we want to go with a tentative amended report now, with a safe conclusion?

DR. BERGFELD: With an insufficient portion for vaginal?

DR. MARKS: Yeah. Insufficient for vaginal, safe for all the parabens. We were safe the last time with all the parabens. But now, particular with this issue of the vaginal exposure and toxicity, it would be insufficient for vaginal products. That's what you said -- Ron suggested that.

Tom, Ron Hill, do you like that conclusion? Ingredients are safe other than insufficient for vaginal products.

DR. HILL: Is Benzyl still included in that conclusion?

DR. MARKS: That would be all of them. You have concerns. Was that the one banned by the EU? Refresh my memory.

DR. HILL: I'm pretty confident, yeah.

DR. MARKS: Yeah. And what was the reason?

DR. HILL: I do not know.

DR. MARKS: Well, and we didn't have a reason to not declare it safe.

DR. EISENMANN: As far as I know, it's insufficient data. They can ask for data and they don't get data, they ban it. It is a lack of data rather than anything showing adversely.

DR. HILL: That was kind of my impression, but I wasn't for sure.

DR. MARKS: Okay.

MR. GREMILLION: Sorry. Does that reflect a different approach to read-across with this body? Or is there new data that they didn't consider?

DR. KATZ: Initially, in Europe what they do is they have a call for data and they provide a date by which they expect to have the data. If they don't get the data, or the data they receive isn't sufficient, they can make a cutoff and determine that an ingredient is unsafe.

DR. BERGFELD: We do that too, two years.

MR. GREMILLION: Yeah. I guess, I'm wondering, here it sounds like we're going towards a safe conclusion, not an IDA. Is that because of the data on the other ingredients?

DR. MARKS: Oh, I think if we felt that we had insufficient for the butylparaben, we would say insufficient. Right now, our team feels it's okay except for parabens in vaginal products, if I interpret correct.

DR. HILL: Yeah. And I don't know exactly what to ask you for. I mean, my gut feel is not a basis for asking for something that I'm not sure exactly what that would be. What I really want is people to make those metabolites, and study the estrogenic activity and draw conclusions. My lab, when it was up and running fully, I could make metabolites and we could study them.

DR. MARKS: Does that clarify?

MR. GREMILLION: Yes.

DR. MARKS: With the data we have, we feel we have significant data to say it's safe. We don't feel it should be insufficient.

MR. GREMILLION: I guess, to me, you can't speak for what was going through the minds of the Europeans, so.

DR. MARKS: I'm not even going to go there. I'm more interested in what's going through the minds of the Belsito team.

DR. HELDRETH: If the panel did feel that the data were insufficient for the benzyl.

DR. MARKS: Pardon?

DR. HELDRETH: If the panel did feel that the data were insufficient for the benzyl.

DR. MARKS: Yeah. That's what Ron Hill's saying. What would you ask for?

DR. HELDRETH: The ultimate conclusion would be immediately go to insufficient data, zero use. Because this ingredient currently isn't in use in the US.

DR. HILL: I would struggle right now to put -- I mean, I feel like there's science that's missing. But that's not -- I would still struggle to put what would I be asking for. That's what I would be asking for, but that's pretty extreme, I don't think I can ask for that. You can't have that wish little bear.

MR. GREMILLION: It wasn't the last question last time, sorry. Is the bioaccumulation if -- I guess assuming that the characterization in the Wang Study that was cited in the letter is the correct characterization, that still doesn't affect the safety assessment? Does the safety assessment depend on the characterization of the bioaccumulation?

DR. ZHU: No. The margin of safety calculation is not based on the bioaccumulation. It's based on the observed adverse effect level and the internal exposure dose. It's not related to bioaccumulation.

DR. HILL: I mean, again, show me something that says there's a negative consequence of the bioaccumulation. That's the leap I can't make. It accumulates, so what?

DR. MARKS: Okay. Any other comments? Tom, Ron, Ron? If not, we'll see how tomorrow goes. But our team will support issuing a tentative amended report that all these ingredients are safe except for insufficient for vaginal products, and then I'll go through the needs. Okay. Let me go ahead and close this.

### Full Panel

DR. BELSITO: Okay. Thanks for giving me this one.

DR. MARKS: This and brown algae. Thank you, Don.

DR. BELSITO: We extensively reviewed this; and let me cut to the chase and then there will be a lot of, I think, discussion. Our conclusion is that the entire group, with the exception of benzylparaben, is safe as used in the current concentrations of use. And that benzylparaben is insufficient for DART data.

DR. BERGFELD: And that's a motion?

DR. BELSITO: That's a motion.

DR. BERGFELD: Is there a second?

DR. MARKS: A second with a modification. We were really concerned about parabens in vaginal products, so we felt that we could go forward with a safe conclusion also, Don, as a tentative amended report. We're fine with the insufficient for the benzylparaben, but we really felt unless we had more data, such as a margin of safety calculation for DNA sperm damage and yeast adherence for vaginal products, that we couldn't feel that any of the parabens are safe in vaginal products.

So, that was our take on it, which is not that much different than -- it's a little bit different, but not a huge gap between what your team felt and ours. We felt we could go safe with all of them. We didn't carve out benzylparaben, but we're fine with that carve-out.

DR. BERGFELD: So, you're seconding it, but you've added the vaginal. Comment?

DR. MARKS: Well, it's a different motion.

DR. BELSITO: Paul had specific comments on the DNA damage and the vaginal changes; unfortunately, he's not here and I gave all his comments to the transcriptionist. But I think Dan concurred with those so maybe you can help me out here, Dan.

DR. LIEBLER: If you can just elaborate a little bit on your reasons for concern on vaginal products.

DR. MARKS: I'll let Tom and Ron and Ron comment.

DR. LIEBLER: Just so we're on the same page, so I'm responding to what you're concerned about.

DR. MARKS: We had extensive discussion, as you remember that was brought up by the Women's Voices for the Earth. And then there were two references, and we wanted to delve in more on the references there. And, we didn't feel comfortable that we could say it was safe for vaginal products with the data we had yesterday.

DR. BELSITO: Let me just read Paul's comments. Okay, because Monice provided them. "Exposure from vaginal products, very weak data has not been reproduced. I don't see this as new. We already acknowledged the effect on sperm and our NOAEL is based on the sperm effect."

DR. SLAGA: I wanted to review the papers on the DNA damage, which I haven't done completely yet. I did get the papers, but I didn't have time. That was my initial the same as you. But Women's Voices was so dogmatic about the change in mobility and other aspects of spermatozoa, I just wanted to review that. So, the possibility of tabling just for that, or we can all --

DR. BELSITO: We also wanted to review, that's part of our discussion. But we thought we could -- base upon Paul's comment that we had already looked at the DNA data, he apparently did some work; unfortunately, he's not here to look at this -- looking at these papers, that we could move ahead. We can always change our conclusion at the next meeting.

DR. MARKS: Well, unless Ron and Ron have a comment about it; I would be swayed by Paul's input. He was very definitive in addressing it. We couldn't be as definitive as Paul was in his notes.

DR. BELSITO: I mean, there are numerous aspects of this report that needs to be rewritten and data brought into the text and not just the table. For instance, the Fisher 1999 study is referenced by the SCCS, but it's not in our text. That needs to be brought in and discussed.

We need to bring in the papers that you were talking about that Bart e-mailed us this morning, Samarasinghe and Mundy paper. We need to bring in the Artacho and Wang paper that were referred to by Ms. Scranton. We did not feel we needed to bring in the Xue paper on the parabens and animals. We need to bring in the Boberg paper and discuss it in more detail as to why we dismissed the 10 milligrams, because there was a total lack of effective concentration. It was all over there.

So, there's a lot that needs to be reorganized. There're a lot of data that needs to be brought in and discussed, put into the text, and also discussed in the discussion as to where we're going.

But, having said that, Curt felt very strongly that we need to recalculate the margin of safety, looking at the data we have for dermal absorption and not taking this 50 percent number; because we have very good data showing it's 3.3 percent and not 50. And Curt's point was, 50 is something you use when you don't know dermal absorption and you're just shooting in the air. And we do know it. So, that actually further increases our margin of safety.

There's a lot of work that needs to be done on this report; but the data is there, we're aware of the data, and I think we can get past it at the next meeting.

DR. MARKS: And Don, at the last meeting -- so, I'll second the motion. Further discussion, at the last meeting you mentioned addressing the EU bans and limits; is that all in these articles?

DR. BELSITO: Yes, because the EU ban, as we found out, or the EU restriction on the combination of butyl and propyl, comes from the 1999 Fisher study with a two mg/kg NOAEL that wasn't even discussed in this report. And that needs to be looked at and our NOAEL is going to be higher than that 2 milligrams. And we can look at that paper and describe again why we're discounting the 2 milligrams, just as we're discounting the 10 mg/kg and going at 160 mg/kg for NOAEL for sperm effect.

There're a lot of data that was just missing from this report, that needed to be brought in so that we can discuss why we do not feel that that is scientifically valid.

DR. MARKS: And the only other thing we would add to the discussion, we talked about it yesterday as I'm sure you did, is the bioaccumulation issue, too, needs to be in the discussion.

DR. BELSITO: Right, and that will come from our review of the Artacho paper.

DR. MARKS: Yes.

DR. BERGFELD: Well, this has been a very vigorous discussion. I want to ask, Bart, though how we're going to handle all of these inclusions and the expansion of the discussion. So, if you'll just iterate that for the group.

DR. HELDRETH: Jinqiu will take all of the additions that the Belsito team has mentioned. Dr. Liebler has provided a significant amount of rewrites for how the discussion section is handled; and he will incorporate all those to go out in a tentative report that'll be available for public comment. And if the panel feels that we didn't accurately reflect those pieces of information, or draft the discussion to their liking, when they see the report again in December, they by all means have the prerogative to make changes to it and send it out again.

DR. BERGFELD: And that's satisfactory to everyone? A little nod of the head.

DR. MARKS: Yes.

DR. BERGFELD: Ron Hill, you want to say something?

DR. HILL: Yes, but Dan had something; and I have a clarification question on the 3.7 percent. Is that a human number for human skin, number one? And two, you wouldn't expect that to be the same for every paraben. It should vary methylparaben to ethylparaben to propyl to isobutyl to pentyl. I'm wondering if that number that's being used is as conservative as possible for butylparaben, for example; which those use restrictions are greater and I think that's appropriate, because one thing we know for very sure is human skin does something different than rat skin, completely, in terms of absorption of these parabens.

There's a big difference between rodents and humans in terms of dermal absorption; and, in fact, normally would go the opposite way that rodents would absorb more. It's actually the opposite in this case, human absorbs parabens better than rodents; but it's paraben by paraben dependent. And I think there's information in one of the papers we've gotten in the last couple of years, I think it's the Wong paper but I'm not sure, to give it some information. And I commented at the March meeting that one of those pieces of information, it was probably the most important piece of information in there or in that regard, was buried in Table 10 as opposed to in the main text. And I'm not sure that it's been brought into the main text. I didn't see that it was since then.

DR. BERGFELD: Well, we'll make sure that happens.

DR. HILL: I want to make sure that when we use for the margin of safety, a number like 3.7 percent, that that encompasses every paraben that we're talking about.

DR. BERGFELD: Curt, do you want to respond?

DR. HILL: And also, that we're talking about absorption as far as getting as far as where it can reach the viable epidermis to where we can get paraben in blood, and not some in vitro study that's looking at all the way through the skin into the reservoir on the other side, because that'd be two different numbers.

DR. BERGFELD: Curt?

DR. KLAASSEN: There are a number of studies here. Some are in vitro. And here is one study in human. A dermal was studied in 26 healthy Caucasian male volunteers with butylparaben. And, let's see, what did they find? Well, in this one they only measured the concentrations in the blood and they didn't present it. Where you get the percentages is in the in vitro studies.

DR. HILL: It should be possible to do base on -- they did measure the blood levels in that. I couldn't remember. What stuck out in my mind was that they really misinterpreted what was going on in terms of the dermal absorption, in terms of the lipophilicity. But, if you have blood levels and a pharmacokinetic time course, you can make conclusions as to how much, how fast came in through the skin.

DR. HILL: And I didn't recall that there was enough data to do that thoroughly.

DR. KLAASSEN: No, I don't think there is enough data. In the full thickness of pork skin, they say that methylparaben is absorbed better than ethyl, it's better than propyl, it's better than butyl, with increasing lipophilicity. In fact, they came up with a 2.3 to 3.3 percent absorption.

I guess one could go through there and make sure we have every study and make sure that there isn't anything significantly different than 3.7 percent, but, I don't see it right away. In fact, it's 3.3. I mean, the point is it's not 50 percent.

DR. HILL: I wouldn't expect 50 percent.

DR. KLAASSEN: We have to take the best number we have even though it might not be ideal as every other number we have.

DR. HILL: We also have to pay good attention to delivery vehicles and art of use. But, one of the things that came out is that the SAR for hydrolysis in the skin is different for rodents, actually, drastically different for rodents and humans. So, we got competing effects of, as the chain link goes up the lipophilicity goes up, that actually mass transfer rate up to a point is proportional to lipophilicity -- partition coefficient actually. Direct proportion; if you double the partition coefficient you double the mass transfer rate. Except, you're also putting in long, and especially if they're floppy, chains so when the molecular weight goes up that actually retards the permeability coefficient.

So, anyway there's competing effects there. And then on top of that, the esterase activity in the human skin, what goes on there. And I don't know how it is in the vaginal wall, and how much we apply there. But, we talked about separating the sites of absorption as well in doing these calculations. So there a quite a few things to take into account, but I still feel like there's missing science. Both from that kind of modeling, actually being able to put it on human skin, measure blood levels and time course, and then calculate how much actually made it through. And also, that we're capturing all the metabolites that are formed in humans routinely.

And, I think all of that goes to the bioaccumulation; because what I said twice yesterday, or at least maybe twice maybe three times, is it bio-accumulates -- so what? And then other compounds where we have accumulation being an issue were brought up, such as TCDD; and I said, well this isn't the same because if it's being released from adipose tissue then we should be glucuronidating as fast as it's being released, or esterase cleaving in blood. So, release from adipose tissue shouldn't be a problem. But now I have a question in my mind about a paper I haven't been able to get yet, which is what's actually going on in the adipose tissue. We know there're certain enzymes in there. I don't know what P450 distribution, what metabolites.

And what I know, from some work we actually did is that there are in a group over in Scandinavia somewhere, was certain glucuronides can be sequestered. So, if you're just measuring radioactivity, for example, you might be measuring a glucuronide, and it's releasable. So, anyway, there are a lot of science that people need to be doing, and that's all.

DR. BERGFELD: Thank you. Don't?

DR. BELSITO: Actually, Ron, thank you for bringing up a very important point. Our absorption is based upon cutaneous absorption. And if these are used vaginally, absorption may be very different. In which case we don't know absorption and 50 may be a better number to use.

DR. GREMILLION: And, I believe the Women's Voices letter also brought up another study, from Proctor & Gamble researchers, that used 80 percent as the absorption. So, I mean, there's that precedence for a more conservative number.

DR. MARKS: Yesterday, Carol, you presented an approach of margin of safety using the four individual areas of exposure, and then also altogether. Did you have that discussion in your team yesterday? I mean, like you, we had extensive discussions. Carol did you want to clarify that? I mean, that's another I agree with you one hundred percent about.

DR. EISENMANN: Well, the Women's Voices for the Earth suggested using .5, because that was the maximum use concentration reported, and applying that to the whole 17 point something grams that you're using for exposure. Well, that .5 was for mascara.

In the Proctor & Gamble paper that was published, they suggested splitting the 17 grams into four different categories, eye area, oral care, leave-on, and rinse-off. And they'd have an amount -- and then apply the maximum use concentration for those product categories. So, you'd actually use some of the data that was provided.

So, you put the .5 for the eye area products, which is only a half of gram. And then, I think it was .24 for the leave-on products was the maximum concentration. And then add up those four -- so, instead

of just .5 for the whole 17 grams, it uses the data. It's kind of a compromise. You use the .5, but only for the eye area products.

DR. BELSITO: We had that same discussion.

DR. GREMILLION: But, the Women Voices letter cites a study, Cowan-Ellsberry, Proctor & Gamble researchers that uses an 80 percent absorption rate.

DR. EISENMANN: That's the same study I'm talking about.

DR. GREMILLION: I thought you said -- I heard 50 percent, .5.

DR. EISENMANN: Well, I'm not discussing the absorption rate. I'm discussing the concentration of the ingredient in the product, not the absorption rate. I'm not sure it matters if you use 50 percent or 80 percent. The PBK model by Harvey Clewell's group, they determined it was 16 percent, the model that's they used for dermal penetration.

DR. BERGFELD: Thank you.

DR. BELSITO: The margin of safety for 80 would still be good. Don, do you have any idea why P&G would use 80?

MR. BJERKL: I think the approach, oftentimes, when we're doing risk assessment we use worst-case scenarios. And, if we have an adequate margin of safety using those worst-case scenarios then we're good to go. If you look at the Cowan-Ellsberry, and Robison paper, what I like about that is it goes through the risk assessment and it's very transparent of what assumptions they're using.

And what it demonstrates, is that the risk assessment that we traditionally do is conservative. They went all the way to the biomonitoring data and back calculated to look at that exposure. And what they demonstrated was that the exposure assumptions were conservative and that the traditional risk assessment is protective, even when you bring in biomonitoring data.

DR. BELSITO: Thank you.

DR. BERGFELD: Ron Hill?

DR. HILL: The other reason that I asked for focus on paraben by paraben, as opposed to total paraben load, is we wouldn't expect, for example, methylparaben and ethylparaben to have the same effect that butylparaben has or isobutylparaben, and definitely not benzylparaben. But, it depends on what effect we're talking about. So, same as Tom, I need a better sense of the DNA damaged one; because if it's free radical generation -- I mean, there's no big news that a phenolic hydroxyl can serve to generate free radicals, our human biochemistry uses that. Or are we making catechols that are damaging the DNA -- what's going on?

And so, I think it's very confounding to consider total paraben load, in any event, because you don't know what the concentration response is for the particular effects you're worried about. So, that needs to enter into the calculations.

And then the particular one on sperm, that I also want to know about, is if we're just affecting people's ability to conceive, if we're damaging the sperm beyond repair and they can't fertilize an egg that's one thing. Then the FDA can mandate an advisory to consumers, if you use this stuff there might be a fertility issue. If we're making compromised sperm with DNA that can still fertilize an egg and make a damaged fetus, that'd be a whole other thing.

DR. BERGFELD: All right. Dan?

DR. LIEBLER: I read the papers, and, the Samarasinghe paper, which is the isolated human sperm treated in vitro with paraben, either individually or in a mixture, evaluated several things. The most convincing data are for the effect of the parabens on viability and motility.

They also used some commonly available probes for oxidants generation; and they were able to show sort of inconsistent concentration response for a signal from these probes. I worked in the oxidants and radicals field for a long time, and I know that these assays, when used by inexperienced investigators in poorly controlled studies, can yield misleading data. And that's what this look like to me.

So, then when I went on to the DNA damage. The DNA damage that they measured was primarily by measurement of 8-Oxoguanine, which is one of the most commonly measured oxidized base forms. But it's also subject to high background interferences. And they used an antibody assay. And they even went so far as, in their own paper, to badmouth the assay and said, but it still might be useful. I can't believe it got past reviewers and editors. But the data show no significant effect of 8-Oxoguanine formation.

They also did a TUNEL assay for DNA strand breaks, no significant effect. They also did something called a halo test, which is a morphologic test that looks at the nuclear integrity, no significant effect. So, I think this paper is, you know, kind of a -- as they say -- a nothing burger. It's certainly worth including in our report, but it is I think -- I've had a look, and I encourage my colleagues to take a close look at it. But, that was my take on it.

The other paper, the Mundy paper was a totally different hypothesis and problem. It was the question of whether a yeast pathogen candida could be induced to express elevated levels of adhesion proteins, which would allow the yeast to stick to vaginal epithelial cells, and potentially facilitate infection. And, again, this was a mixture of parabens and other preservatives at high concentrations with the candida.

They were able to show increased expression of the adhesion. And they also did a filter binding assay with the vaginal epithelial cells mixed with yeast with these preservatives. The concentrations of the products that contained preservatives were 15 to 25 percent by weight in the yeast growth medium. So, you can draw your own conclusion from that.

I think these are illustrations of the kinds of papers we often get in our assessment, where it's cells and culture, or some other comparable in vitro system, where a potential hazard is demonstrated, but the risk is probably irrelevant to our consideration in a context of concentration of use. So, it's a relevant point, but since I had actually read the papers, and a lot of people were saying, I don't want to read the papers, I want to give you my take on it, have a look for yourself and then we can come back and discuss it later.

DR. HILL: I did read the first of that before and I remember, now that you ring all the bells, coming to the same conclusion you did. But, I did that months back and I didn't refresh my memory, so I apologize for that.

DR. BERGFELD: All right. We've had a lot of discussion -- Curt?

DR. KLAASSEN: I'd just like to mention one thing. It has been noted that there is a risk assessment done by the people in North Carolina and also by Proctor & Gamble. That's not included in here, to the best of my knowledge. And we definitely need -- those have been published, I assume. We definitely need to get those in here.

DR. BELSITO: Yes, there're a lot of data that's missing that needs to be brought in and discussed. But, overall, I think our team felt we could go safe as used with the exception of benzylparaben, insufficient for DART data.

DR. BERGFELD: That was seconded by the Marks team.

DR. MARKS: That's correct.

DR. BERGFELD: And so, it appears that in the discussion, a lot of this information needs to be discussed, especially regarding the vaginal changes.

DR. MARKS: And margin of safety.

DR. BERGFELD: And margin of safety.

DR. MARKS: And bioaccumulation, so there's a lot.

DR. BERGFELD: Lot to go in.

DR. MARKS: Bring in the articles.

DR. BERGFELD: And, Bart has said that all this can be accomplished even if we send it out. So, having said that, let's call to question. All those in favor of this conclusion, please indicate by raising your hand. Yes, thank you.

So, we should see in December, I believe, a comment period of 60 days and then we'll be able to act on this particular document again. All right, very well done. Moving on to, then, the next ingredient, which is the Xanthine Alkaloids, Dr. Marks?

# Amended Safety Assessment of Parabens as Used in Cosmetics

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Status: Draft Final Amended Report for Panel Review  
Release Date: March 15, 2019  
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The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Jinqiu Zhu, Ph.D., Toxicologist.

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**ABSTRACT:** The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 21 parabens as preservatives in cosmetic products. These ingredients are all reported to function in cosmetics as preservatives; however, five are reported to also function as fragrance ingredients. The Panel reviewed relevant data relating to the safety of these ingredients under the reported conditions of use in cosmetic formulations. The Panel concluded that the 20 of the 21 parabens included in this report are safe in cosmetics in the present practices of use and concentration described in this safety assessment. However, the available data are insufficient to make a determination of safety for Benzylparaben.

## INTRODUCTION

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

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

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

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

This re-review was initiated because 1) some of the ingredients being reviewed for the first time had high frequencies of use (e.g., Sodium Methylparaben was reported to be used in 436 cosmetic formulations at the time of prioritization), and 2) the Panel was concerned that new data from a developmental and reproductive toxicity (DART) study indicated reduced sperm counts and reduced expression of a specific enzyme, and a reduction in a specific cell marker in the testes of offspring of female rats orally dosed with 10 mg/kg/day Butylparaben during 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. After careful consideration of all the new data in the category of endocrine activity and from new DART studies, the Panel determined an adequate NOAEL value of 160 mg/kg/day for Butylparaben, and an MOS was re-calculated accordingly, considering the different use concentrations and exposures of Butylparaben in various cosmetic products category.

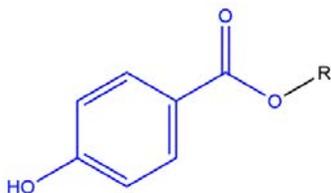
An exhaustive search of the world's literature was conducted for new data on the safety of parabens, as well as 4-Hydroxybenzoic Acid, in preparation of this report. A few short-term, but no new acute, subchronic or chronic toxicity studies, were discovered. This safety assessment includes relevant published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

Pertinent data were discovered in the European Chemicals Agency (ECHA) database.<sup>3-8,8-11</sup> 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 Safety (SCCS).<sup>12-18</sup>

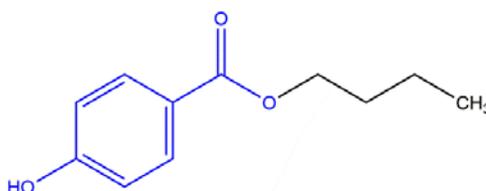
Dermal penetration, toxicokinetic, short-term toxicity, DART, endocrine-activity, genotoxicity, biomonitoring and epidemiology studies are also briefly summarized in the body of the report, and in most cases, details are provided in tables. However, toxicity studies conducted in animals exposed to individual parabens by subcutaneous injection (except the study used by SCCS to derive the MOS of Butylparaben), 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 safety of these ingredients as used in cosmetics.

**CHEMISTRY****Definition and Structure**

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

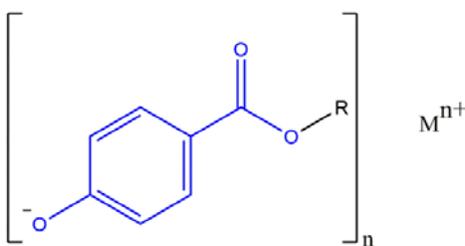


**Figure 1.** Paraben phenolic acids: a generic structure wherein R is an alkyl group from 1 to 4 carbons long, or is benzyl.

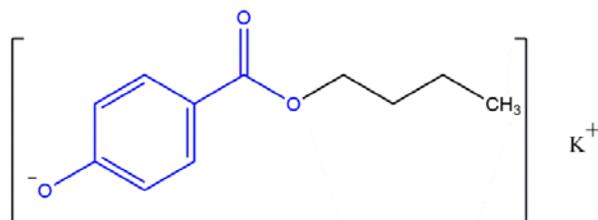


**Figure 2.** Paraben phenolic acids: an example, Butylparaben (wherein R from the generic structure in Figure 1, is an alkyl group 4 carbons long).

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

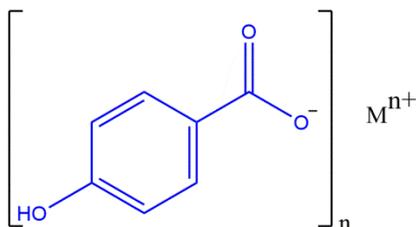


**Figure 3.** Paraben phenolic salts: generic structure wherein R is an alkyl group from 1 to 4 carbons long and M is sodium or potassium.

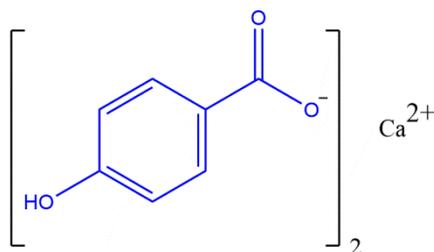


**Figure 4.** Paraben phenolic salts: an example, Potassium Butylparaben (wherein R, from the generic structure in Figure 3, is an alkyl group 4 carbons long and M is potassium).

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



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



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

### Physical and Chemical Properties

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

Parabens form small colorless crystals or white crystalline powders with practically no odor or taste.<sup>2</sup> Parabens are soluble in alcohol, ether, glycerin, and propylene glycol and slightly soluble or almost insoluble in water. As the alkyl chain length increases, water solubility decreases. Parabens are hygroscopic and have a high oil/water partition coefficient. Parabens are relatively stable against hydrolysis during autoclaving and resist saponification.<sup>19</sup>

The particle size distribution of some of the parabens included in the safety assessment is provided in Table 4.<sup>3-6,8,9,11,20-22</sup>

### Method of Manufacture

Paraben phenolic acids (and salts) are prepared by esterifying 4-Hydroxybenzoic Acid with the corresponding alcohol (e.g., butanol to synthesize Butylparaben) in the presence of an acid catalyst, such as sulfuric acid, and an excess of the specific alcohol.<sup>2</sup> The acid is then neutralized with caustic soda, and the product is crystallized by cooling, isolated by centrifugation, washed, dried under vacuum, milled, and blended. Benzylparaben can also be prepared by reacting benzyl chloride with sodium 4-Hydroxybenzoic Acid. Paraben carboxylate salts may be prepared by deprotonating 4-Hydroxybenzoic Acid with an appropriate alkaline salt (e.g., sodium hydroxide could be used to prepare Sodium Paraben).<sup>23</sup>

## USE

### Cosmetic

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

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

According to VCRP survey data received in 2019, Methylparaben was reported to be used in 11,739 formulations (9347 of which are leave-on formulations); this is an increase from the 8786 uses reported in 2006.<sup>2,24,25</sup> Propylparaben had the next highest number of reported uses at 9034 (7520 of which are leave-on formulations); this was an increase from 7118 uses reported in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to none.

The results of the concentration of use survey conducted by the Council in 2016 indicate Methylparaben had the highest reported maximum concentration of use; it is used at up to 0.9% in shampoos.<sup>2,24</sup> The highest maximum concentration of use reported for products resulting in leave-on exposure is 0.8% Methylparaben in a mascara, and for leave-on dermal exposure is 0.65% Ethylparaben in eye shadows. In 2006, Methylparaben had the highest reported maximum concentration of use at 1% in lipsticks. The maximum concentrations of use of the previously reviewed parabens have remained under 1% and the patterns of use are similar to those reported in the previous safety assessment.

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

Several of the parabens are reported to be used in products that can be incidentally ingested, used near the eye, come in contact with mucous membranes, or in baby products.<sup>24,25</sup> For example, Methylparaben is used at concentrations up to 0.35% in lipstick, 0.8% in mascara, 0.5% in bath oils, tablets and salts, and 0.4% in baby lotions, oils and creams.

Some of the parabens were reported to be used in cosmetic sprays (including hair sprays, hair color sprays, skin care products, moisturizing products, suntan products, deodorants, and other propellant and pump spray products)<sup>24,25</sup> and could possibly be inhaled. These ingredients are reportedly used at concentrations up to 0.9 % in spray products (e.g., Methylparaben in the category of other fragrance products). Although there are reported mean diameters as small as 37.8  $\mu\text{m}$  (Sodium Propylparaben) for some of these materials, as pure, raw substances, those diameters are not indicative of particle sizes in final formulations.<sup>6</sup> Accordingly, those raw material mean particle diameters are not relevant to cosmetic safety. In practice, 95% - 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters  $> 10 \mu\text{m}$  with propellant sprays yielding a greater fraction of droplets/particles below  $10 \mu\text{m}$  compared with pump sprays.<sup>26-28</sup> 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.<sup>26,28</sup> 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.<sup>26</sup> The maximum concentration of use recorded for deodorant sprays was 0.00012% (Methylparaben). However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Some of the parabens were reported to be used in dusting powders and face powders (e.g., Ethylparaben in face powders at up to 0.5%), and could possibly be inhaled. Conservative estimates of inhalation exposures to respirable particles during the use of loose-powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.<sup>29-31</sup>

The Scientific Committee on Consumer Safety (SCCS) of the EU has published several opinions on parabens over the last few years (Table 8).<sup>12-17,32</sup> The current SCCS opinion (updated on May 2013) is:

The use of butylparaben and propylparaben as preservatives in finished cosmetic products are safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19%... With regard to methylparaben and ethylparaben, the previous opinion, stating that the use at the maximum authorized concentrations can be considered safe, remains unchanged... Limited to no information was submitted for the safety evaluation of isopropyl-, isobutyl-, phenyl-, benzyl- and -pentylparaben. Therefore, for these compounds, the human risk cannot be evaluated. The same is true for benzylparaben....<sup>16,32</sup>

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

## **Non-Cosmetic**

2008

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

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

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

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

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.<sup>37</sup> There are no established adverse outcome pathways for this weak estrogenic activity.

NICNAS published the following conclusion in 2016:

Current risk management measures are considered adequate to protect public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory.

The main route of public exposure is expected to be through the skin, inhaled from products applied as aerosols, and potential oral exposure from lip and oral hygiene products.

The available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak estrogenic activity, but there are no established adverse outcome pathways for this effect. Should further information on adverse outcome pathways in mammals associated with weak estrogenic activity become available, further assessment of these chemicals at Tier III could be required.<sup>37</sup>

## **TOXICOKINETIC STUDIES**

### **Dermal Penetration**

2008

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

**In Vitro**

In vitro dermal penetration studies are presented in Table 9.

In Franz-type diffusion cells, 2.3% - 3.3% of the applied dose of Methylparaben (0.1% in nine different vehicles) penetrated porcine skin (intact stored frozen) in 4 h.<sup>38</sup> The receptor fluid consisted of phosphate-buffered saline (pH 7.4) and 0.01% of Gentamicin-sulfate. In 24 h, 2.0% - 5.8% and 2.9% - 7.6% of un-metabolized Methylparaben penetrated previously frozen intact and tape-stripped skin, respectively. In full-thickness porcine skin stored frozen, permeability coefficients ranged from  $31.3 \pm 1.6$  to  $214.8 \pm 40$  cm/h x  $10^{-4}$ , decreasing (Methylparaben > Ethylparaben > Propylparaben > Butylparaben) with increasing chain length.<sup>39</sup> Increasing the ethanol concentration in the vehicle or the exposure duration, increased the retention of the parabens in the dermis, relative to the epidermis. Binary combinations of the parabens reduced their permeation rates, which was attributed by the authors to high retention in the epidermis and dermis.

In a different study, the penetration of parabens from 3 commercial facial cream formulations through rabbit ear skin ranged from 20% - 60%, after 8 h in Franz-type diffusion cells, increasing with the water solubility of the paraben (Propylparaben < Ethylparaben < Methylparaben), regardless of the formulation tested.<sup>40</sup> 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%).<sup>41</sup> Residual quantities of parabens remaining in the skin increased as the test concentration increased, with greater amounts in the human epidermis than in mouse skin.

Abdominal skin samples were used to determine the dermal penetration of 0.1% Methylparaben, 0.08% Ethylparaben, 0.2% Propylparaben and 0.15% Butylparaben.<sup>42</sup> Previously frozen skin samples were thawed and mounted on Franz diffusion cells. A dose of 100  $\mu$ L of lotion containing the test substance was applied to the skin once at t=0 or multiple times at t = 0, t = 12 and t = 24. Thirty-six hours after a single application, penetration ranged from  $0.007\% \pm 0.003$  (Butylparaben) to  $0.057\% \pm 0.03$  (Methylparaben). Penetration 12 hours after the t = 24 dosing ranged from  $0.04\% \pm 0.01\%$  (Butylparaben) to  $0.6\% \pm 0.1$  (Methylparaben).

**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 basic cream formulation which also contained 2% diethyl phthalate and 2% dibutyl phthalate.<sup>43</sup> Daily whole-body topical application of 2 mg/cm<sup>2</sup> of the cream formulation without the test substances for 1 week (control week) was followed by daily application of the cream with the test substances for 1 week. Butylparaben serum concentrations in the blood were undetectable in most samples during the control week, with maximum concentrations not exceeding 1.0  $\mu$ g/L. Butylparaben concentrations increased rapidly (mean peak concentration =  $135 \pm 11$   $\mu$ g/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  $18 \pm 3$   $\mu$ g/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.<sup>44</sup> Split thickness (~350  $\mu$ m) samples were thawed and mounted in vertical-flow Neoflon<sup>TM</sup> 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<sup>2</sup>) were maintained at 32°C. Solutions containing one or both compounds were added to the donor chamber at t = 0, and the receptor fluid was sampled hourly for 18 h for analysis by high-performance liquid chromatography (HPLC). Compared with the single-solute solutions, the steady-state flux was more than 5-fold larger for Methylparaben and 2.6-fold larger for CP in the binary solution (i.e., Methylparaben plus CP). The authors noted that the 5-fold increase in Methylparaben flux was consistent with a 6.4-fold increase in uptake of Methylparaben in the stratum corneum (SC), which occurred primarily in the nonlipid regions of the SC. However, the 1.6-fold increase in CP uptake was too small to explain the 2.6-fold increase in the CP flux. The authors concluded that the results above suggested CP enhance skin permeation of Methylparaben primarily by increasing the solubility of Methylparaben in the SC (especially in the nonlipid regions), and Methylparaben increase skin permeation of CP by enhancing both the solubility and diffusivity of CP in the SC.

**Absorption, Distribution, Metabolism, and Excretion (ADME)**1984

*Parabens are quickly absorbed from the blood and gastrointestinal tract, hydrolyzed to 4-Hydroxybenzoic Acid, conjugated, and the conjugate excreted in the urine.<sup>45</sup> Data obtained from chronic administration studies indicate that parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Varying amounts of parabens are passed in the feces depending upon which paraben is administered and the size of the dose. Little or no unchanged paraben is excreted in the urine. Most of an administered dose can be recovered within 5 to 72 hours as 4-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.<sup>46</sup> Excretion is in the urine. Small amounts of the ester are excreted unmetabolized or hydrolyzed to the benzyl alcohol and 4-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 4-Hydroxybenzoic Acid; 20% was not recovered.<sup>47</sup>*

2008

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

The ADME studies summarized below are presented in Table 10.

**In Vitro**

Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for  $\alpha$ -fetoprotein (AFP).<sup>48</sup> On the other hand, the 50% inhibitory concentration (IC<sub>50</sub>) of Benzylparaben was 0.012  $\mu$ M. Butylparaben was de-esterified to 4-Hydroxybenzoic Acid in the S9 fraction of skin obtained from 5-week old male rats, with a maximum rate at saturating concentration (V<sub>max</sub>) of 8.8 nmol/min/mg protein.<sup>49</sup>

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

Methylparaben, Ethylparaben, Propylparaben and Butylparaben were hydrolyzed by rat liver microsomes (RLM) and HLM in in vitro tests.<sup>51</sup> Butylparaben was most effectively hydrolyzed by the RLM, which showed relatively low hydrolytic activity towards parabens with shorter and longer alkyl side chains. In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. Rat small-intestinal microsomes exhibited relatively higher activity toward longer-side-chain parabens. Human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes.

Metabolism rates of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben by HLM were inversely proportional to chain length (overall rate dominated by esterase-catalyzed hydrolysis, where the longer the alcohol moiety, the slower the hydrolysis).<sup>52</sup> This trend was also observed for human skin microsomes (HSM), but at much lower rates. Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the paraben. In contrast to human tissue fractions, the rat tissue fractions tested, including skin and liver fractions, hydrolyzed the parabens at rates that increased as the ester chain length increased. Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin.

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference.<sup>53</sup> Butylparaben was extensively hydrolyzed to 4-Hydroxybenzoic Acid as the major metabolite for both sexes and species. The other metabolite observed in the human hepatocytes was 4-hydroxyhippuric acid, which is the glycine conjugate (i.e., a Phase II metabolite) of 4-Hydroxybenzoic Acid.

**Animal****Dermal**

Nine rats were given a single dermal dose of 100 mg/kg bw 4-hydroxy [ring-U-<sup>14</sup>C]-labeled Methylparaben, Propylparaben, or Butylparaben in 60% aqueous ethanol vehicle.  $C_{max}$  ( $\geq 693$  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.<sup>54</sup> Most of the dosage ( $\geq 46.4\%$ ) was not absorbed, and less than 25.8% was found in the urine. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of [<sup>14</sup>C]-Butylparaben was absorbed 72 h following application to the skin in rats.<sup>53</sup> Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in the liver.

**Oral**

In rats exposed to a single oral dosage of 100 mg/kg bw [ring-U-<sup>14</sup>C]-labeled Methylparaben, Propylparaben, or Butylparaben,  $C_{max}$  ( $\geq 11,432$  and  $\geq 21,040$  ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h.<sup>54</sup> 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 1000 mg/kg bw/day dosage of [<sup>14</sup>C]-Butylparaben.<sup>53</sup> The rate of urinary excretion was similar across all dosages, with  $\geq 66\%$  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.

**Human****Dermal**

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

**Oral**

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

**Physiologically Based Pharmacokinetic (PBPK) Modelling**

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

**TOXICOLOGICAL STUDIES****Acute Dose Toxicity**

No new published acute toxicity studies were discovered in the published literature, and no unpublished data were submitted.

**1984**

*Acute toxicity studies in animals indicate that parabens are practically nontoxic by various routes of administration.*<sup>45</sup>

**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.*<sup>46</sup>

1995

*Isobutylparaben had a subcutaneous LD<sub>50</sub> of 2600 mg/kg in mice.*<sup>47</sup>

### **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.*<sup>47</sup>

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

The short-term toxicity studies that are summarized below are presented in Table 11.

#### Dermal

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

#### Oral

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

### **Subchronic Toxicity Studies**

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

1984

*Subchronic... oral studies indicate that parabens are practically nontoxic.*<sup>45</sup> *A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses up to 2 g/kg/day.*

### **Chronic Toxicity Studies**

No new published chronic toxicity studies were discovered in the published literature, and no unpublished data were submitted, since the 2008 CIR report.

1984

*Chronic oral studies indicate that parabens are practically nontoxic.<sup>45</sup> A 60:40 mixture of the sodium salts of Propylparaben and Ethylparaben did not induce significant pathologic changes in rats treated at 1.4 g/kg bw/day for 18 months. At 2 percent of the diet, Methylparaben and Propylparaben exerted no toxic effect in rats after 96 weeks exposure. Weanling dogs treated by Methylparaben or Propylparaben at 1 g/kg bw/day for 378 to 422 days were in excellent condition throughout the experiment.*

1995

*Mice were orally dosed with 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks.<sup>47</sup> Upon necropsy, the only effect noted was amyloidosis in 58% of dosed males and 33% of dosed females surviving past 78 weeks, as compared with 25% of control males and 10% of control females.*

### **DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES**

1984

*Methylparaben was nonteratogenic in rabbits, rats, mice and hamsters, and Ethylparaben was nonteratogenic in rats.<sup>45</sup> Pregnant animals were given orally 5.0 to 550 mg/kg bw/day (rats, mice) or 3.0 to 300 mg/kg bw/day (hamsters) Methylparaben from Day 6 of gestation to Day 10 (hamsters) or 15 (rats, mice). Pregnant rabbits were orally administered 3.0 to 300 mg/kg bw/day Methylparaben daily from Day 6 of gestation to Day 18. Pregnant rats were dosed in diet of Ethylparaben at concentrations of 0.1, 1, or 10 % between gestation Days 8 and 15. On day 21 of pregnancy, rats were killed, and the number of fetal implantations, status of maternal visceral organs, fetal body weights, and numbers of skeletal, visceral, and external defects in fetuses were recorded. No apparent teratogenesis or toxicity was observed in 363 fetuses from rats fed up to 10% Ethylparaben.*

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

2008

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

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

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

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

## Dermal

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

## Oral

The oral DART studies summarized below are described in Table 12.

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

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

Estradiol (E<sub>2</sub>) level was significantly elevated in weanling male rats orally exposed to Butylparaben at 50 mg/kg for 8 consecutive weeks, whereas serum levels of the hormones T, LH, and follicle-stimulating hormone (FSH), as well as ratios of T/E<sub>2</sub> and T/LH was decreased, compared to control groups.<sup>64</sup> Butylparaben treatment elevated markers of testicular DNA damage in a comet assay, such as the increase in the tail DNA%, tail length of DNA, and tail moment. In addition, the testicular malondialdehyde level was significantly elevated, along with a significant decrease in catalase enzyme activity. Histopathological examination showed a reduction in Leydig cells population along with pathological alternations of dilated congested subcapsular blood vessels and the dilation and congestion of interstitial vasculature.

The increase of CYP19 and estrogen receptor (ER) $\alpha$  expression; the reduction of steroidogenic acute regulatory protein (StAR), cytochrome cholesterol side-chain cleavage enzyme (P450scc), estrogen sulfotransferase (SULT1E1), and testes androgen receptor (AR) expression; and the reduced methylation rate of the ER $\alpha$  promoter, were statistically significant in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD 7 to GD 21.<sup>65</sup> 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.<sup>66</sup> Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

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

F2 pups exhibited a statistically-significant greater mortality at PND 7 and thereafter, compared with controls, in a DART study in which F0 females and their F1 offspring were exposed to 0.105 mg/kg bw/day Methylparaben by gavage.<sup>69</sup> 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.<sup>70</sup>

Methylparaben was associated with a statistically-significant higher incidence of abnormal sperm in rats exposed to 1000-ppm or 10,000-ppm in the diet for 8 weeks, mostly sperm with no head in 4% to 5% of sperm, compared with 2.3% in 100-

ppm and control groups.<sup>49</sup> 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 no-observed-adverse-effect-concentration (NOAEC) was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of about 1140 and 1100 mg/kg/day for Methylparaben and Butylparaben, respectively.

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

#### Aquatic

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

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

## GENOTOXICITY STUDIES

### 1984

*Numerous mutagenicity studies, including the Ames test, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the Methylparaben, Ethylparaben and Propylparaben are non-mutagenic.*<sup>45</sup>

### 1995

*Chinese hamster fibroblast cell lines treated with 0.03% Isobutylparaben had no chromosomal aberrations after 48 h.*<sup>47</sup>

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

### 2008

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

## **In Vitro**

### Methylparaben

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

### Propylparaben

Vero cells (derived from African green monkey kidney) were grown and incubated for 24 h with 0, 50, 200, 300, 400, or 500  $\mu\text{M}$  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.<sup>75</sup> A statistically-significant, dose-dependent decrease in percentage of mitotic cells was observed across the concentrations tested (4-fold decrease at 500  $\mu\text{M}$ , compared with control). Flow-cytometric analysis of DNA content revealed that the decline was attributable mainly to cell-cycle arrest at the G0/G1 phase. Immuno-detection techniques revealed statistically-significant induction of DNA DSBs (2-fold compared to control) verified by 8-OHdG staining at all concentrations tested (maximum intensity at 500  $\mu\text{M}$ ).

CHO cells were grown, and incubated for 1 or 3 h with 0, 0.5, 1, 1.5, 2, or 2.5  $\mu\text{M}$  Propylparaben.<sup>76</sup> Sister chromatid exchange (SCE), chromosome aberration (CA), and DNA strand break (comet) assays were performed. Statistically-significantly elevated SCEs/cell and CAs/cell were observed in cells incubated with Propylparaben ( $\geq 1.5$   $\mu\text{M}$ ) and Propylparaben ( $\geq 1.0$   $\mu\text{M}$ ) for 3 h, respectively.

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

### Butylparaben

CHO cells were grown, and incubated for 1 or 3 h with 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mM or 0, 0.1, 0.25, 0.5, or 0.75  $\mu\text{M}$ , respectively Butylparaben.<sup>76</sup> SCE, CA, and DNA strand break (comet) assays were performed. Statistically-significantly elevated indices of DNA fragmentation were observed in cells incubated for 1 h with  $\geq 0.4$   $\mu\text{M}$  Butylparaben. Comparatively high incidences of fragmentation were observed. Statistically-significant, elevated SCEs/cell and CAs/cell were observed in cells incubated with 0.75  $\mu\text{M}$  Butylparaben for 3 h.

### Methylparaben, Ethylparaben, Propylparaben, and Butylparaben

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

## **In Vivo**

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

## **CARCINOGENICITY STUDIES**

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

### 1984

*Methylparaben was non-carcinogenic when administered intravaginally in rats and was not co-carcinogenic when injected with dibenzo[a,i]pyrene (DBP) subcutaneously in mice.<sup>45</sup> 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.<sup>47</sup>*

### 2008

*Isobutylparaben and Butylparaben were noncarcinogenic when given to mice in diet at levels of 0.15%, 0.3%, and 0.6% for 102 weeks, respectively*<sup>2</sup>

## **OTHER RELEVANT STUDIES**

### **Endocrine Activity**

**2008**

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

*Methylparaben failed to produce any effect in uterotrophic assays in two laboratories, but did produce an effect in other studies from another laboratory. The potency of Methylparaben was 1000 to 20,000 less when compared to natural estradiol. The same pattern was reported for Ethylparaben, Propylparaben, and Butylparaben when potency was compared to natural estradiol; in positive studies the potency of Ethylparaben was 346 to 25,000 less; the potency of Propylparaben was 1612 to 20,000 less; and the potency of Butylparaben was 436 to 16,666 less. In two studies, Isobutylparaben did produce an estrogenic response in the uterotrophic assay, but the potency was 240,000 to 4,000,000 less than estradiol. In one study, Benzylparaben produced an estrogenic response in the uterotrophic assay, but the potency was 330,000 to 3,300,000 less than estradiol.*

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

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

The endocrine activity studies summarized below are described in Table 13.

### **In Vitro**

Weak activation of murine peroxisome proliferator-activated receptor (mPPAR) $\alpha$  was seen in murine NIH-3T3-L1 cells at the highest concentrations of Butylparaben tested (100  $\mu$ M).<sup>77</sup> Butylparaben activated mPPAR $\gamma$  with a lowest observed effect concentration (LOEC) of 30  $\mu$ M and a maximal (4-fold) induction at 100  $\mu$ M. The human data for Butylparaben (hPPAR $\alpha$  and hPPAR $\gamma$ ) were comparable to those obtained with mPPAR $\alpha$  and mPPAR $\gamma$ , indicating a similar responsiveness.

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

Butylparaben exhibited estrogen agonism at all concentrations tested in T47D-KBluc cells.<sup>82</sup> The maximum effect was observed at 10  $\mu$ M.

The EC<sub>50</sub>s for stimulating proliferation of MCF-7 cells ranged from 0.4-40  $\mu$ M, LOECs from 0.1-20  $\mu$ M, and no observed effects levels (NOECs) from 0.05 - 8  $\mu$ M for the parabens tested.<sup>83</sup> The parabens tested, in descending order of these values, were Isobutylparaben > Butylparaben > Propylparaben > Ethylparaben > Methylparaben. In comparison, corresponding values for E2 were EC<sub>50</sub> =  $2 \times 10^{-6}$   $\mu$ M, LOEC =  $10^{-6}$   $\mu$ M, and  $1 \times 10^{-7}$   $\mu$ M. Propylparaben at 10  $\mu$ M resulted in deformed acini and filling of the acinar lumen in non-transformed MCF-12A and MCF-10A cells.<sup>84</sup> MCF-7 and HCl-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.<sup>85</sup> 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.<sup>86</sup> In the presence of differentiation media, 50 µM Butylparaben or Benzylparaben promoted lipid accumulation in human adipose-derived stem cells (hADSCs) as early as day 3 and throughout the differentiation process. Butylparaben had the strongest adipogenic effects of the parabens tested, whereas other parabens had no effect at 1 or 10 µM.

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

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

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

### **Animal**

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

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

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

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

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

### **Human**

In 26 healthy Caucasian males, minor differences in inhibin B, LH, estradiol, total thyroxine (T4), free thyroxine (FT4), and TSH concentrations were observed after daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben as well as 2% diethyl phthalate and 2% dibutyl phthalate, compared to the concentrations measured before the treatment.<sup>43</sup> The differences could not be attributed to the treatment.

## Effects on Human Breast Cells

### Methylparaben, Propylparaben, Butylparaben

MCF-10A non-transformed, immortalized human breast epithelial cells were exposed to 500  $\mu\text{M}$  Methylparaben, 10  $\mu\text{M}$  Propylparaben or Butylparaben in semi-solid 2% methylcellulose suspension culture, or 1  $\mu\text{M}$  Methylparaben or 0.1  $\mu\text{M}$  Propylparaben or Butylparaben in monolayer culture.<sup>96</sup> 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  $\mu\text{M}$  Methylparaben or 10  $\mu\text{M}$  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 17 $\beta$ -estradiol (70 nM). Concentration-response experiments showed that maximal numbers of colonies were formed at 100  $\mu\text{M}$  Methylparaben or 1  $\mu\text{M}$  Propylparaben or Butylparaben. Control experiments showed that the parabens did not influence the growth of MCF-10A cells under adherent conditions (i.e., monolayer cultures).

Human high-risk donor breast epithelial cells (HRBECs) were collected from the unaffected contralateral breasts of women undergoing breast surgery with a personal or family history of breast cancer, atypical neoplastic histopathology, and/or high mammographic density.<sup>97</sup> The cells were incubated for 7 days with 10 nM to 1  $\mu\text{M}$  (vehicle not specified) Methylparaben in phenol red-free medium supplemented with 0.2% charcoal-stripped fetal bovine serums.<sup>97</sup> Some cells were exposed to 10  $\mu\text{M}$  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%  $\pm$  6.77% at 1  $\mu\text{M}$ , 55.93%  $\pm$  10.54% at 100 nM, and 28.14%  $\pm$  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 ( $p < 0.001$ ).

### Biomonitoring

The biomonitoring studies summarized below are described in Table 14.

Biomonitoring is the direct measurement of human exposure by measuring the parabens or their metabolites in human biological fluids (e.g., urine, blood), which account for both dietary intake (e.g. from foods and medicinal products with paraben preservatives) and dermal application of products with parabens. However, the presence of a substance in the blood or urine does not mean that it will cause effects or disease.<sup>98</sup> Chemical toxicity is related to its dose or concentration, in addition to a person's individual susceptibility. Small amounts may be of no health consequence, whereas larger amounts may cause adverse health effects.

The US NHANES program (the Fourth National Report) provides a large dataset for human spot urine levels of parabens, collected from 2005 to 2014, with 2013 - 2014 being the most recent collection period.<sup>98</sup> A total of 2686 urine specimens from a representative sample of persons  $\geq 6$  years of age in the US general population, was analyzed for the exposure level to Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. For the 2013 - 2014 sampling period, the median concentration of Methylparaben in urine was 48.1  $\mu\text{g/L}$  (95<sup>th</sup> percentile: 819  $\mu\text{g/L}$ ), and Propylparaben in urine was 5.74  $\mu\text{g/L}$  (95<sup>th</sup> percentile: 224  $\mu\text{g/L}$ ). For Butylparaben, the median concentration in urine was below the limit of detection (LOD, 0.1  $\mu\text{g/L}$ ) for all groups (age, gender, and race/ethnicity) in the 2011 - 2014 reporting period. In females, the median concentration of Ethylparaben in the 2013 - 2014 reporting period was 1.6  $\mu\text{g/L}$  (95<sup>th</sup> percentile: 145  $\mu\text{g/L}$ ) while concentrations in males were below the LOD (1  $\mu\text{g/L}$ ).

Data from the US NHANES program were also used to analyze the exposure to parabens through oral hygiene products and sunscreen use.<sup>99</sup> Compared to individuals who reported "never" using mouthwash, individuals who reported daily use had significantly elevated urinary concentrations of Methylparaben and Propylparaben (30 and 39% higher, respectively). Individuals who reported "always" using sunscreen had significantly higher urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben (92, 102, and 151% higher, respectively) compared to "never" users of sunscreen. Associations between exposure biomarkers and sunscreen use were stronger in women compared to men, and associations with mouthwash use were generally stronger in men compared to women.

A community-based intervention study indicated that using personal care products (PCPs) that are labeled to be free of parabens, for 3 days, lowered urinary concentrations of Methylparaben and Propylparaben in 100 girls: Methylparaben and Propylparaben concentrations decreased by 43.9% (95% CI: -61.3, -18.8) and 45.4% (95% CI: -63.7, -17.9), respectively.<sup>100</sup> The GM concentration of Methylparaben decreased from 77.4 to 43.2  $\mu\text{g/L}$ , and Propylparaben decreased from 22.6 to 12.3  $\mu\text{g/L}$ . In contrast, the GM concentration of Ethylparaben increased from 2.9 to 4.2  $\mu\text{g/mL}$ , and Butylparaben increased from 0.8 to 1.7  $\mu\text{g/mL}$ . Concentrations of both Ethylparaben and Butylparaben were low overall and not detected in almost half the samples. In the same study population of 100 adolescent girls, participants who reported using "makeup" every day vs.

rarely/never, had higher urinary concentrations of Methylparaben (120.5 ng/mL vs. 13.4 ng/mL,  $p < 0.01$ ) and Propylparaben (60.4 ng/mL vs. 2.9 ng/mL,  $p < 0.01$ ).<sup>101</sup> However, ingredients (including Methylparaben and Propylparaben) in “makeup” products used by the girls were not disclosed. Other sources of parabens (food, pharmaceuticals, endogenous, etc.) were not considered.

A statistically significant difference was observed between serum parabens in 18 women who used lipstick containing Methylparaben and Propylparaben for 5 days compared with those not using this cosmetic ( $p = 0.0005$  and  $0.0016$ , respectively), and a strong association was observed between serum parabens and lipstick use (Spearman correlation =  $0.7202$ ).<sup>102</sup>

One study reported the free and total paraben concentrations in 16 human serum samples in the US.<sup>103</sup> The mean total paraben concentrations in serum are  $42.6 \mu\text{g/L}$  and  $7.4 \mu\text{g/L}$  for Methylparaben and Propylparaben, respectively; whereas the free concentration of Methylparaben and Propylparaben in the serum is  $2.2 \mu\text{g/L}$  and  $0.5 \mu\text{g/L}$ , respectively, indicating that parabens that are not hydrolyzed to 4-Hydroxybenzoic Acid are rapidly conjugated.

One or more of 5 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isobutylparaben) was detected in 99% of breast tissue samples collected from women with breast cancer, and all 5 were detected in 60% of the samples.<sup>104</sup> Median concentrations were highest for Propylparaben ( $16.8 \text{ ng/g tissue}$ ) and Methylparaben ( $16.6 \text{ ng/g tissue}$ ). Propylparaben concentrations were statistically significantly higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts.

Ethylparaben, Butylparaben, and Benzylparaben were detected in all placenta samples collected from healthy mothers.<sup>105</sup> The highest measured concentration was  $11.77 \text{ ng Methylparaben/g tissue}$ . The amount of Butylparaben, Ethylparaben, Methylparaben and Propylparaben was studied in human ovarian tumor samples.<sup>106</sup> The tissue mass fractions of the four parabens in the malignant tissues were at least twice as much as those present in the benign tissues. The tissue mass fractions of Methylparaben and Ethylparaben were higher than Propylparaben and Butylparaben.

Thirty-one pregnant women who provided multiple spot urine samples ( $n = 542$ ) collected over two 24-h periods had their samples analyzed for Methylparaben, Propylparaben, Ethylparaben, Butylparaben, Isobutylparaben, and Benzylparaben.<sup>107</sup> These parabens were also measured in breast milk samples collected at approximately 3 months postpartum ( $n = 56$  women). Women who used body and face lotions in the past 24 h had significantly higher geometric mean (GM) paraben concentrations (80 - 110%) in their urine than women who reported no use in the past 24 h. There was 100%, 72%, 96%, and 90% detection of Methylparaben, Butylparaben, Propylparaben, and Ethylparaben in urine, respectively. Lower detection rates were seen for Isobutylparaben (39%) and Benzylparaben (41%). Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben, respectively.

The conjugated or free species of six parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben), or their metabolite, 4-Hydroxybenzoic Acid, were measured in human adipose fat samples collected from 20 donors who underwent liposuction surgery.<sup>108</sup> Ethylparaben and Propylparaben were more frequently detected than the other parabens, at a detection frequency of 60% and 50%, and a GM concentration of  $0.90$  and  $0.49 \text{ ng/g}$ , respectively. GM concentrations of other parabens were not calculated due to their detection of lower than 50%. The GM concentration of the sum of six parabens and 4-Hydroxybenzoic Acid ( $C_{\Sigma\text{parabens}}$ ) in adipose fat was  $3420 \text{ ng/g}$ . While a positive correlation between donor's age and  $C_{\Sigma\text{parabens}}$  (75<sup>th</sup> percentile of adipose concentrations;  $n = 15$ ) was observed, no significant difference in concentrations of  $C_{\Sigma\text{parabens}}$  between the two age groups were found (18 - 33 yr and 34 - 58 yr). However, the authors noted that total paraben measurements may have been compromised by alkaline hydrolysis in the tissue due to the use of alkali in the liposuction procedure.

The conjugated or free species of six parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and heptylparaben (not a cosmetic ingredient)), or their metabolite, 4-Hydroxybenzoic Acid, were measured in urine samples collected from 40 US children, 70 Chinese children, and 26 Chinese adults.<sup>109</sup> Parabens were present predominantly (> 90%) as conjugated species in urine. Among the six parabens analyzed, Methylparaben and Propylparaben were the predominant compounds, which accounted for 57 - 98% and 1.4 - 12%, respectively, of the total concentrations. The median concentrations of Methylparaben and Propylparaben in US adults were  $43.9$  and  $9.1 \text{ ng/mL}$ , respectively. The median concentrations of the sum of six parabens in urine from US children were  $54.6 \text{ ng/mL}$ . The GM concentrations of 4-Hydroxybenzoic Acid in urine from US children were  $752 \text{ ng/mL}$  for girls and  $628 \text{ ng/mL}$  for boys, which were 2 - 3 times lower than the concentrations determined for Chinese children.

One or more of 7 parabens (Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben and Benzylparaben) were measured in 144 human adipose tissue samples collected from patients > 16 years old, who were undergoing non-cancer-related surgery and absent of absence of diagnosed hormone-related disease or cancer.<sup>110</sup> Detection frequencies and median concentrations were: Methylparaben (100.0%,  $0.40 \text{ ng/g tissue}$ ), Ethylparaben (20.1%, < LOD), Propylparaben (54.2%,  $0.06 \text{ ng/g tissue}$ ), Butylparaben (5.6%, < LOD), and Isobutylparaben (2.1%, < LOD). Isopropylparaben and Benzylparaben were not detected in any of the samples, while Butylparaben and Isobutylparaben concentrations above LOD were only recorded in 8 and 3 of the 144 samples. Methylparaben, Ethylparaben, and Propylparaben levels were significantly and positively correlated. No statistically significant relationship between age and

paraben concentrations in human adipose tissue was identified. Of the seven parabens measured, only a positive association between age and Methylparaben concentrations was found (close to, but not statistical significance,  $p = 0.06$ ).

The Environment and Reproductive Health (EARTH) study examined the association between the use of 14 PCPs and the urinary concentrations of parabens in 400 men (18 - 55 year of age).<sup>111</sup> The largest percent increase for parabens was associated with the use of suntan/sunblock lotion (66 - 156%) and hand/body lotion (79 - 147%). A subset of 10 PCPs that were used within 6 h of urine collection contributed to at least 70% of the weighted score and predicted elevated urinary concentrations of Methylparaben, Propylparaben, and Butylparaben (788%, 1333%, and 254% higher, respectively). GM concentrations of Methylparaben, Propylparaben, and Butylparaben in urine were 28, 2.86, and 0.26  $\mu\text{g/L}$ , respectively.

The EARTH study also showed that, among 346 infants, none of the maternal preconception paraben concentrations were associated with birth weight.<sup>112</sup> Maternal preconception Methylparaben concentration was associated with a decreased head circumference of 0.27 cm (95% CI:  $-0.54, 0$ ), while no associations were observed between Ethylparaben, Propylparaben, and Butylparaben concentrations and head circumference.

### **Effects on Adhesin Genes in *Candida glabrata***

Culture of *Candida glabrata* (a yeast pathogen) in Synthetic Complete (SC) medium containing 1.5 mM Methylparaben and 165  $\mu\text{M}$  Propylparaben induced expression of EPA6 adhesin gene, leading to increased adherence to cultured human Lec2 epithelial cells as well as primary human vaginal epithelial cells.<sup>112</sup> Culture of *Candida glabrata* in a variety of over-the-counter (OTC) vaginal products (concentrations ranged from 15% to 25%) also induced expression of EPA6.<sup>113-115</sup>

## **DERMAL IRRITATION AND SENSITIZATION STUDIES**

1984

*Methylparaben (10% and 100%), Propylparaben (10%), and Ethylparaben (10% and 100%) were, at most, mildly irritating when applied to rabbit skin.*<sup>45</sup>

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

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

1986

*Benzylparaben was not a skin irritant when tested in rabbits.*<sup>46</sup>

*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.*<sup>2</sup>

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

### **In Vitro**

The parabens were tested individually for irritancy and sensitization potential in co-cultured human keratinocyte and peripheral blood mononuclear cells (PBMCs).<sup>115</sup> 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  $\mu\text{M}$ , 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<sub>50</sub>s calculated from concentration-response data for cell death (irritancy) and CD86-expression (sensitization), compared with vehicle controls. Substances with EC<sub>50</sub> for cell death  $\leq 50 \mu\text{M}$  were considered to be irritating, with EC<sub>50</sub> ranging from 50 - 1000  $\mu\text{M}$  were weakly irritating, and substances that did not reach the 50% threshold for cytotoxicity, or for which EC<sub>50</sub>  $> 1000 \mu\text{M}$ , were considered non-irritating. Substances with EC<sub>50</sub> for CD86-expression  $\leq 12.5 \mu\text{M}$  were categorized as extreme sensitizers,  $> 12.5 \mu\text{M} < 50 \mu\text{M}$  as strong sensitizers,  $> 50 \mu\text{M} < 100 \mu\text{M}$  as moderate sensitizers, and  $> 100 \text{EC}_{50}$  as non-sensitizers. Methylparaben and Ethylparaben showed no potential for irritation in this test. Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben appeared to be weak irritants. The sensitization potential of the parabens tested was correlated with side-chain length: Methylparaben, Ethylparaben, Propylparaben, and Isopropylparaben were classified as weak sensitizers; and Butylparaben, Isobutylparaben, and Benzylparaben were strong sensitizers in this study.

### **Photosensitization/Phototoxicity**

1984

*Photo-contact sensitization and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methylparaben, Propylparaben, and/or Butylparaben gave no evidence for significant photoreactivity.*<sup>45</sup>

### **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.<sup>113</sup> The cells were grown and incubated, with or without Methylparaben, for 6 or 24 h in DMEM supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine, and 100 U/mL penicillin/streptomycin at 37°C. Methylparaben-treated and -untreated cells were exposed to medium-wavelength ultraviolet light (UVB; 15 or 30 mJ/cm<sup>2</sup>) 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 long-wavelength ultraviolet light (UVA) and short-wavelength ultraviolet light (UVC). After irradiation, the cells were incubated in culture medium without Methylparaben for various durations. Methylparaben reduced cell viability in a statistically significant manner within 6 h at 0.3% and within 24 h at 0.03%. Fluorescent microscopy using a fluorescent micro-plate reader revealed little evidence of reactive oxygen species (ROS) or nitric oxide (NO) production after Methylparaben exposure. UVB irradiation at 30 mJ/cm<sup>2</sup> (but not at 15 mJ/cm<sup>2</sup>) induced small amounts of late apoptosis and necrosis. Methylparaben induced statistically significant elevation of ( $p < 0.5$ ) UVB-induced cell death, as evaluated by immunocytochemistry and flow cytometry; the propidium iodide (PI) index increased 3- and 7-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 15 mJ/cm<sup>2</sup>, and 2- and 3-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 30 mJ/cm<sup>2</sup>. Methylparaben at both concentrations elevated ( $p < 0.05$ ) measurements of ROS and NO production and lipid peroxidation, and activated NF $\kappa$ B and AP-1 in UVB-irradiated cells.

### **OCULAR IRRITATION STUDIES**

1984

*Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits.*<sup>45</sup> *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.*<sup>46</sup>

2008

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

### **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.<sup>116</sup> 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 tetrazolium 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.<sup>45</sup>*

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

Parabens were designated “non-allergen” of the year (2019) by the American Contact Dermatitis Society.<sup>117,118</sup> Monitoring for paraben allergy followed with studies reporting paraben testing in standard screening fashion since 1940. The frequency of its sensitivity has remained low and remarkably stable for many decades despite wide use. Parabens have been considered relatively nonirritating at levels used in current formulations, as verified in extensive experience with the mix at current applied patch test concentrations.

### **Retrospective and Multicenter Studies**

In one retrospective analysis, 1363 cumulative irritation test studies in more than 45,000 subjects, who use-tested 151 different paraben-containing formulations (along with other ingredients), did not demonstrate parabens to be irritating in typical in-use conditions and irritation scores did not correlate with preservative concentrations.<sup>119</sup>

Allergic contact dermatitis caused by paraben mixture was analyzed on the basis of data collected by the European Surveillance System on Contact Allergies (ESSCA) network between 2009 and 2012 from 12 European countries (Table 15).<sup>120</sup> Of the 52,586 tests during the study period, parabens yielded less than 1% positive reactions. Of the results obtained from 2362 TRUE-test<sup>®</sup>, the paraben mixture yielded only 0.4% positive reactions. The allergic contact dermatitis data are summarized in

## **EPIDEMIOLOGICAL STUDIES**

The epidemiological studies summarized below are described in Table 16.

### **Prospective Studies**

In vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility.<sup>121</sup> No significant associations were observed between current exposure levels of Methylparaben, Ethylparaben, and Propylparaben in Chinese pregnant women and size of infants at birth.<sup>122</sup>

Urinary Methylparaben and Propylparaben concentrations were associated with an increase in gestational age in northern Puerto Rico.<sup>123</sup> Methylparaben, Butylparaben, and Propylparaben were associated with a 34 - 50% decrease in the odds of Small for Gestational Age (SGA).

Among 501 male partners of couples planning to become pregnant, urinary concentrations of Methylparaben, Ethylparaben, and Butylparaben were negatively associated with diminished sperm count and several sperm motility parameters.<sup>124</sup> In contrast, hydroxylated paraben metabolites (methyl-protocatechuic acid and ethyl-protocatechuic

acid) were positively associated with select semen quality parameters. The median urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben among 419 participants who provided both urine and semen samples are 6.51, 0.36, 1.39, 0.03, and 0.02 ng/mL, respectively. In the same study population, no associations were observed between paraben concentration in seminal plasma and 35 semen quality parameters among 339 male partners after false discovery rate (FDR) adjustment.<sup>125</sup> In addition, seminal plasma concentrations of Ethylparaben and Benzylparaben were associated with an increased percentage of sperm motility.

Among 936 men of couples seeking infertility treatment, urinary concentrations of Methylparaben and Propylparaben remained stable over the study period between 2000 and 2017.<sup>126</sup> The downward trends in sperm concentration and normal morphology were not affected when including urinary paraben concentrations in linear regression models; i.e., parabens did not substantially change the downward trends in semen parameters (volume, sperm concentration, count, motility, and morphology).

Among 482 pregnant women, an interquartile range increase of urinary Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in pro-inflammatory marker interleukin-1 $\beta$  (95% CI: -14.1, -0.86).<sup>127</sup> However, the association between Ethylparaben and interleukin-1 $\beta$  differed across pregnancy, becoming positive at the end of study visit.

In Latino girls at age 9, earlier thelarche, pubarche, and menarche were associated with urinary Methylparaben concentrations, and earlier pubarche was associated with urinary Propylparaben concentrations.<sup>128</sup> In boys, no prenatal parabens were associated with pubertal timing, while one association of earlier gonadarche with urinary Propylparaben concentrations was observed. However, associations of peripubertal measurements with parabens may reflect reverse causality: children going through puberty early may be more likely to use products that expose them to parabens.

### **Retrospective Studies**

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).<sup>129</sup> The authors stated that the OR of 0.03 for Benzylparaben indicated a “protective effect” of Benzylparaben for preterm birth. Linear regression analysis indicated an association between maternal urinary concentrations and decreased gestational age and body length in newborns. No statistically-significant associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated (i.e., body length, gestational age at birth, birth weight, head circumference). No statistically-significant associations were found between prenatal or postnatal growth of male newborns and maternal urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben.<sup>130</sup>

The incidence of cryptorchidism and/or hypospadias, combined, was associated with placental concentrations of Methylparaben  $\geq$  1.96 ng/g (OR = 3.18; CI = 0.88 - 11.48) and Propylparaben concentrations  $\geq$  1.16 ng/g (OR = 4.72; CI = 1.08 - 20.65).<sup>131</sup> Of 436 children at 3 years of age, the median values of estimated daily intake of Methylparaben, Ethylparaben, Propylparaben, Butylparaben and Benzylparaben were 12.10, 5.68, 4.50, 0.06 and 0.17  $\mu$ g/kg bw/day, respectively.<sup>132</sup> Urinary Ethylparaben concentrations of boys were positively associated with weight z scores ( $\beta$  = 0.16, 95% CI: 0.04, 0.29,  $p$  = 0.01) and height z scores ( $\beta$  = 0.15, 95% CI: 0.03, 0.27;  $p$  = 0.01). Positive associations were found between the sum of molar concentrations of all five parabens and height z scores among all children ( $\beta$  = 0.24, 95% CI: 0.04, 0.45;  $p$  = 0.02). All regression coefficients calculated for girls and all other coefficients for boys were not statistically significant.

Mean percent change (MPC) and the results of statistical tests for trends were not statistically significant in a study of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben in women undergoing infertility evaluation and ovarian volume (OV) or antral follicle count (AFC) measurements.<sup>133</sup>

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.<sup>134</sup> The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

### **Cross-Sectional Studies**

Among 315 men under 45 years of age who attended an infertility clinic for diagnostic purposes in Poland, urinary concentrations of Ethylparaben and Butylparaben were associated with an increase in the percentage of sperm with abnormal morphology.<sup>135</sup> Urinary Isobutylparaben concentrations were significantly associated with an increase in the percentage of sperm with high DNA stainability. Urinary concentrations of parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben and Isobutylparaben) were not associated with the level of reproductive hormones, including FSH, T and E<sub>2</sub>. In addition, urinary concentrations of Methylparaben and Propylparaben were not related to any of the examined semen quality parameters, sperm DNA damage, or the level of reproductive hormones. The unadjusted GM urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Isobutylparaben were 14.7, 1, 4.3, 0.3, and 0.4  $\mu$ g/L, respectively.

In cord plasma of 27 healthy pregnant women (37<sup>th</sup> week of pregnancy), Methylparaben, Propylparaben and the sum of all measured parabens (Methylparaben + Ethylparaben + Propylparaben + Butylparaben) were inversely associated with T levels.<sup>136</sup>

Urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were measured in 215 young healthy men (18 - 23 years old), 94% of whom had detectable urinary concentrations of parabens.<sup>137</sup> Urinary concentrations of parabens were not significantly associated with any semen parameters or any of the reproductive hormone levels, including FSH, LH, T, inhibin B and E<sub>2</sub>. The unadjusted GM urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben were 11.2, 1.1, and 0.64 ng/mL, respectively.

Among 42 partners (36.8 ± 5.4 years old) of couples who visited a gynecology clinic in Tokyo for infertility consultation, no significant association was found between semen parameters (sperm volume, concentration and motility) and urinary paraben concentrations in regression analyses.<sup>138</sup> The GM urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were 48.2, 1.88, 1.13, and 0.184 ng/mL, respectively.

Linear regression analyses of data from the US NHANES program indicated an association between reduced serum thyroxine (T4) concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben.<sup>139</sup>

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).<sup>140</sup> The results also indicated an association between urinary concentrations of Methylparaben and nonatopic asthma (OR = 0.025; CI = 0.07 - 0.90) and nonatopic wheeze (OR = 0.23; CI = 0.05 - 0.99).

## **RISK ASSESSMENT**

### **Margin of Safety**

For the purpose of this risk assessment, the Panel determined an adequate NOAEL value of 160 mg/kg/day for Butylparaben in consideration of the new data in the category of endocrine activity and from DART studies.<sup>62,63,69,70,141-143</sup> Specifically, the NOAEL has been derived from a study where pregnant rats were orally exposed to Butylparaben by gavage from gestation day 7 through postnatal day 21.<sup>63</sup> Above a dose of 160 mg/kg/day, Butylparaben exerted adverse effects on the reproductive system in male offspring, including delayed preputial separation, reduced reproductive organ weights at several ages, reduced luteinizing hormone level, and elevated estradiol and progesterone levels in serum from prepubertal male rats. Importantly, Butylparaben exposure in utero and during lactation significantly reduced epididymal cauda sperm counts, daily sperm production, and serum testosterone in a dose-dependent manner.

In comparison, the SCCS chose an NOEL of 2 mg/kg bw/day for the calculation of the MOS of Butylparaben. The NOEL was derived from a study in which three neonatal male rats were exposed subcutaneously to 2 mg/kg bw/day Butylparaben from PND 2 to PND 18 (Table 12).<sup>141</sup> No effects on any of the measured reproductive parameters were documented, compared with the control group. DART parameters examined in this study included testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts, and immunoexpression of the APQ-1. However, the Panel considered that such study suffers from several critical limitations: it involves a route of subcutaneous exposure; it is not an OECD TG study; and only one postpartum dose was tested.

For the purposes of an MOS calculation, the Panel considered a scenario wherein a consumer would use a set of cosmetic products containing Butylparaben; aggregate exposure to seventeen cosmetic products is calculated to be 17.4 g/day based on addition of deterministic values for a range of products (Table 17).<sup>144</sup> These seventeen cosmetic products are divided into four main categories:<sup>145</sup> (1) oral products, (2) eye products, (3) non rinse-off products and (4) rinse-off product; the global daily exposure of products for each category was estimated using the data summarized in Table 17.

The Panel also considered the different use concentrations and exposures of Butylparaben in each main cosmetic product category. For purposes of worst case assumption, the maximum use concentration of Butylparaben was set to represent the concentrations of use across the products in that category. The Council's concentration of use survey indicates that the maximum use concentration of Butylparaben in the category of (1) oral products, (2) eye products, (3) leave-on products, and (4) rinse-off product is 0.2%, 0.5%, 0.24%, and 0.33%, respectively.<sup>24</sup> (Table 17)

The Panel noted that the measured extent of dermal penetration rates of parabens is variable ranging from 1% to 75%, probably due to differences in animal species used, matrix effects, and other experimental conditions.<sup>145,146</sup> For purposes of calculating an MOS, the systemic availability of un-metabolized Butylparaben after topical application to human skin is of the primary concern. A human toxicokinetic study has been conducted in 26 young adult males with dermal repeated exposure to Butylparaben at a daily dose of 10 mg/kg bw/day for five days.<sup>43,55</sup> No effects of Butylparaben on serum hormonal levels were observed during the exposure time of 5 days; and, about 2.1% un-metabolized Butylparaben was detected in the urine of the participants. Note that Butylparaben was applied to the whole-body in this human study (10 mg/kg bw/day), while a conservative estimation indicates that daily exposure of consumers to Butylparaben is much lower (0.66 mg/kg bw/day, as shown in Table 17). In addition, the available in vitro percutaneous absorption studies using human

split- or full thickness skin suggest a conservative assumption of human dermal penetration rate of un-metabolized Butylparaben at 3.7% (which was used by SCCS to calculate the MOS of Butylparaben and then to derive the recommended maximum use concentration of Butylparaben in the EU).<sup>14,16</sup> Taking into account this weight-of-evidence, the Panel selected an estimate of a 50% dermal absorption rate of un-metabolized parabens in the calculation of the MOS, which thereof represents a very conservative assumption.

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

$$\text{Global daily exposure (GDE, Butylparaben)} = (2.36 \text{ g/day of oral products} \times 0.2 \% \text{ maximum use concentration}) + (0.05 \text{ g/day of eye products} \times 0.5 \% \text{ maximum use concentration}) + (13.93 \text{ g/day of non-rinse-off products} \times 0.24 \% \text{ maximum use concentration}) + (1.04 \text{ g/day of rinse-off products} \times 0.33 \% \text{ maximum use concentration}) = 0.042 \text{ g/day}$$

$$\text{Systemic exposure dose (SED, Butylparaben)} = \text{GDE} \div 60 \text{ kg body weight} \times 50 \% \text{ dermal absorption} \times 1000 \text{ mg/g conversion factor} = 0.35 \text{ mg/kg/day}$$

$$\text{MOS (adult, Butylparaben)} = \text{NOAEL/SED} = 160 \text{ mg/kg/day} / 0.35 \text{ mg/kg/day} = 457$$

The Panel considered exposures to cosmetic products containing multiple parabens at use level of 0.8%.

$$\text{Systemic exposure dose (SED, multiple parabens)} = 17.4 \text{ g/day of product} \times 0.8 \% \text{ use concentration} \div 60 \text{ kg body weight} \times 50 \% \text{ absorption} \times 1000 \text{ mg/g conversion factor} = 1.16 \text{ mg/kg/day}$$

$$\text{MOS (adult, multiple paraben)} = \text{NOAEL/SED} = 160 \text{ mg/kg/day} / 1.16 \text{ mg/kg/day} = 138$$

This conservative MOS of Butylparaben for adults could then be inferred to other single parabens.

### **Estimate and Refinement of Aggregate Exposure**

#### Estimate of Aggregate Exposure

In addition to cosmetic and personal care products, parabens are also widely used in drugs and foods. According to one study, considering aggregate exposure to parabens from various sources, the total combined exposure was 76 mg/day: with cosmetics and personal care products accounting for 50 mg/day; drugs, 25 mg/day; and foods, 1 mg/day.<sup>147</sup>

The Dutch National Institute for Public Health and the Environment (RIVM) conducted an exposure assessment in consideration of the aggregated exposure to parabens via three major sources: PCPs, foods, and medicinal products.<sup>146</sup> For Methylparaben, adding exposures results in an aggregate exposure estimate of 3.0 mg/kg/day for both adults and children. The estimate for medicinal products contributes 70 - 74% of this value, while the contribution of food is less than 1%. For Propylparaben, adding the exposures results in an aggregate exposure estimate of 1.2 mg/kg/day for both children and adults; 64 - 72% of the exposure is from medicinal products, and less than 1% from food. For Ethylparaben, due to the lack of use information on medicinal products, the summation of exposure via PCPs and exposure via foods will result in an aggregate exposure of 0.2 mg/kg/day for adults and children and, as with Methylparaben and Propylparaben, the contribution of foods is less than 1%. However, the authors noted that such an aggregation estimate was based on a series of studies with varying levels of information and uncertainties.

#### Refinement of Aggregate Exposure

In current risk assessments, aggregate exposure of parabens is commonly estimated by using a simplistic approach of summing the exposures from all the individual product types in which parabens are used. However, this summation will result in an unrealistic estimation because 1) the use frequency of products and the amount of product applied are over-estimated, 2) parabens may not be used in all products of a given type (e.g., all make-up products), 3) the extent of use factors for parabens in products is not considered, 4) individuals in the population vary in their patterns of product use including co-use and non-use, and 5) the extent to which parabens are absorbed from the skin into the internal system warrants further studies. Use of multiple exposure models help provide realistic estimates in comparison with observational biomonitoring data.<sup>148</sup> A recent study indicated that approximately 60 - 90% of the model predictions from five implemented models were within a factor of 10 of the observed paraben exposures, while 30 - 40% of the predictions were within a factor of 3 (i.e., a factor of 3 or 10, above or below the minimum observed absorbed doses). These models included three of the screening models (i.e., RIVM ConsExpo, SCCS notes of guidance algorithms, and the Risk Assessment Identification and Ranking-Indoor and Consumer Exposure (RAIDAR-ICE)) and two higher tier probabilistic models (US EPA's Stochastic Human Exposure and Dose Simulation – High Throughput (SHEDS-HT), and Creme Care & Cosmetics). A number of uncertainties affect interpretation of the modeled vs. measured exposures, such as parabens in preservative product concentrations, dermal absorption parameters, and degree of metabolism following dermal absorption.

An approach has been developed to refine the aggregate exposure estimates using four of the more commonly used parabens (i.e., Methylparaben, Ethylparaben, Propylparaben, and Butylparaben).<sup>145</sup> The relative refinement allowed co-use and non-use data, as well as the extent of parabens use data, to be developed for nine cosmetic and skin care products, including body lotion, body cream, facial mask, hand lotion, foundation/liquid make-up, facial moisturizer, lipcolor, night cream and facial cleanser. Simple summed aggregate exposure from these nine cosmetic and skin care products was 1.61, 0.80, 1.70, and 0.016 mg/kg/day for Methylparaben, Propylparaben, Ethylparaben, and Butylparaben, respectively. When the refining factors were applied, and a conservative dermal penetration rate of 80% was chosen, the aggregate exposure compared to the simple addition approach was reduced by 51%, 58%, 90%, and 92% for Methylparaben, Propylparaben, Butylparaben, and Ethylparaben, respectively. In comparison, estimated internal exposure based on the 95<sup>th</sup> percentile values of parabens concentration in human urine was 19.9, 8.2, 1.39, and 0.86 µg/kg/day for Methylparaben, Propylparaben, Ethylparaben, and Butylparaben, respectively. This means that in all cases the aggregate exposure estimates are significantly greater than the exposures derived from the biomonitoring data.<sup>145</sup> If exposure via food was included, the aggregate exposure for Methylparaben and Propylparaben, which are used extensively in foods, would only increase by 1% and 4%, respectively. That is, estimates for exposure to Methylparaben and Propylparaben via food are at least 25-fold lower than the estimates for aggregate exposure resulting from dermal exposure to cosmetic products.<sup>147, 145</sup>

Another study takes population variability of individual characteristics and behavior within the female US population into account.<sup>149</sup> Daily parabens intake was estimated based on skin permeation coefficient models, product use characteristics, and multi-pathway exposure model, i.e., aqueous dermal uptake, gaseous dermal uptake, inhalation intake, and environmentally mediated intake due to disposal after parabens use. The mean (2.5<sup>th</sup> - 97.5<sup>th</sup> percentiles) modeled population intakes were 0.2 (0.003 - 0.8), 0.03 (0 - 0.2), 0.06 (0 - 0.3), and 0.02 (0 - 0.1) mg/kg/day for Methylparaben, Ethylparaben, Propylparaben, and Butylparaben, respectively. This intake estimate represents a consumer who uses the following eleven PCPs which all contain parabens: shampoo, conditioner, body lotion, facial cream, night cream, facial cleanser, deodorant, body wash, foundation, eye shadow, and lipstick. The environmentally mediated parabens intake from disposal stage was three to four orders of magnitude lower than use stage.<sup>149</sup>

## SUMMARY

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

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

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

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

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

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

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

Methylparaben and Ethylparaben were stable in human plasma, but Propylparaben, Butylparaben and Benzylparaben concentrations decreased by 50% within 24 h. All parabens tested were rapidly hydrolyzed when incubated with HLM,

depending on the alkyl chain length. Parabens, but not 4-Hydroxybenzoic Acid, were actively glucuronidated by liver microsomes and human recombinant UGTs.

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

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

Nine rats were given a single dermal dose of 100 mg/kg bw [ring-U-<sup>14</sup>C]-labeled 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. Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in liver.

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

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

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

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

Elevations of serum markers of lipid-peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically significant in rats exposed to 250 mg/kg bw/day Methylparaben. Malondialdehyde levels were elevated in the liver in a statistically-significant, dose-dependent manner, among other effects, in mice orally exposed to 1.33 - 40 mg/kg bw/day Butylparaben for 30 days.

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

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

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

CYP19 and ER $\alpha$  expression were significantly increased, and the expression of StAR, P450<sub>scc</sub>, SULT1E1, and AR in the testes and methylation rate of the ER $\alpha$  promoter were significantly reduced, in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD7 to GD21.

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

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

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

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

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

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

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

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

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

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

Human spermatozoa were exposed to a paraben mixture containing equal concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben. Significantly reduced motility was observed immediately after the treatment and was further exacerbated after 24 h at doses of 1, 2 and 4 mM. Caspase activation was observed following exposure to parabens concentrations above 1 mM and increased still further after 24 h.

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

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

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

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

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

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

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

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

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

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

The analysis of a total of 2686 urine specimens from the NHANES program showed that, for the 2013 - 2014 sampling period, the median concentration of Methylparaben in urine was 48.1  $\mu$ g/L (95<sup>th</sup> percentile: 819  $\mu$ g/L), and Propylparaben in urine was 5.74  $\mu$ g/L (95<sup>th</sup> percentile: 224  $\mu$ g/L). For Butylparaben, the median concentration in urine was below the LOD (0.1  $\mu$ g/L). In females, the median concentration of Ethylparaben was 1.6  $\mu$ g/L (95<sup>th</sup> percentile: 145  $\mu$ g/L) while males were below the LOD (1  $\mu$ g/L).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Among 346 infants, none of the maternal preconception paraben concentrations were associated with birth weight.<sup>112</sup> Maternal preconception Methylparaben concentration was associated with a decreased head circumference of 0.27 cm (95% CI: -0.54, 0).

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

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

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

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

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

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

Analysis of data from the US NHANES program indicated an association between aeroallergen and food sensitization, combined, and urinary concentrations of Methylparaben (OR = 1.74; CI = 1.02 - 3.22), Propylparaben (OR = 2.04; CI = 1.12 - 3.74), and Butylparaben (OR = 1.55; CI = 1.02 - 2.33). The results also indicated 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 (for all but one indicator). The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

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

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

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

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

A conservative risk assessment was performed. Therein, an NOAEL value of 160 mg/kg/day for Butylparaben was determined to be adequate in consideration of the new data in the category of endocrine activity and from DART studies. For the purposes of an MOS calculation, the Panel considered a scenario wherein a consumer would use a set of cosmetic products containing Butylparaben. Therein, an aggregate exposure to four main categories of products was considered: (1) oral products, (2) eye products, (3) leave-on products and (4) rinse-off product; the global daily exposure of products for each category was estimated using the maximum use concentration of Butylparaben in each category, 0.2%, 0.5%, 0.24%, and 0.33%, respectively. The Panel noted that the measured extent of dermal penetration rates of parabens is variable ranging from 1% to 75%, probably due to differences in animal species used, matrix effects, and other experimental conditions. Considering the weight-of-evidence, however, a 50% dermal absorption rate of un-metabolized parabens, was determined to be adequately conservative for the calculation of the MOS. The MOS for adults was 457 and 138 for Butylparaben and multiple paraben, respectively.

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

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

## DISCUSSION

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

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

The Panel noted that the principle study chosen by SCCS for the calculation of the MOS of Butylparaben was the one examined DART endpoints in male rats, which involved subcutaneous instead of oral administration of Butylparaben during lactation period. SCCS acknowledged an NOEL of 2 mg/kg bw/day, instead of an NOAEL, for deriving the MOS of Butylparaben. In order to obtain an acceptable MOS  $\geq 100$ , SCCS recommended the maximum use concentration of Butylparaben in the finished cosmetic products set to be 0.19% (0.14% as acid). The calculation is based on the assumptions of the maximum exposure to preservatives of an adult (60 kg body weight) at 17.4 g/day and a human dermal penetration rate of un-metabolized Butylparaben at 3.7%. However, the Panel considered that the study with an NOEL of 2 mg/kg bw/day suffers from several critical limitations: 1) this study involves route of subcutaneous exposure which may result in chemicals circumventing the physiological barriers and bypassing the portal of entry metabolism, and therefore not considered suitable for quantitative risk assessment in the context of cosmetic usage; 2) this study is not an OECD TG study (e.g., the Butylparaben treated group contained only 3 rats and the control group contained only 5 rats); and 3) only one postpartum dose at 2 mg/kg bw/day was tested.

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

The Panel noted that both in vitro and in vivo studies indicate a rapid and effective metabolism of parabens by carboxylesterases after oral or dermal exposure. Parabens are further metabolized by conjugation with glucuronide, sulfate, or glycine prior to excretion. When applied to human skin, parabens are metabolized to 4-Hydroxybenzoic Acid. Whereas older studies suggested that un-metabolized parabens are not excreted, recent studies with more sensitive analytical methods have measured un-metabolized parabens and their metabolites following dermal exposures.

The Panel discussed concerns about the relevance of the oral animal studies to human risk assessment in that the rapid and effective metabolism of parabens in rodents does not occur in humans. Species differences in the esterase affinities and activities must be carefully taken account for deriving a safe level of exposure in humans. The Panel noted that uncertainties relate to data gaps on dermal absorption of un-metabolized parabens by human skin in vivo and in vitro. One human toxicokinetic study indicates after dermal repeated exposure to Butylparaben at a daily dose of 10 mg/kg bw/day for five days, about 2.1% un-metabolized Butylparaben was detected in the urine of the participants. However, the Panel noted that a conservative estimation shows that daily exposure of consumers to Butylparaben is much lower (0.66 mg/kg bw/day). While SCCS derived the value of 3.7%, based on in vitro studies using human split- or full thickness skin, as a worst case assumption for the dermal absorption of un-metabolized Butylparaben, uncertainties need to be addressed considering that absorption may be variable between different parabens, especially when parabens are used in vaginally applied cosmetic

products. In light of these facts, the Panel selected an estimate of a 50% dermal absorption rate of un-metabolized parabens in the calculation of MOS, which thereof represents a very conservative assumption.

The Panel discussed the bioaccumulation potential of parabens. The Panel noted that, as lipid-soluble chemicals, parabens may distribute to tissues despite metabolism. Recent studies have demonstrated the presence of parabens in various human tissues. However, the data are equivocal regarding cumulative storage in such tissues; and importantly, the available evidence suggests no significant association of cosmetic parabens exposure with diseases or other adverse health conditions.

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

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

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

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

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

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

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

**CONCLUSION**

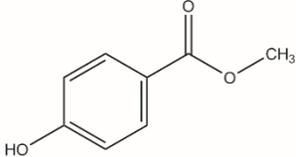
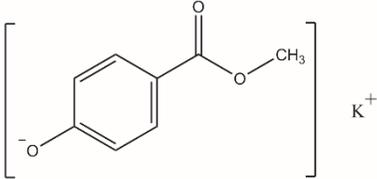
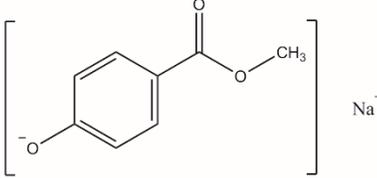
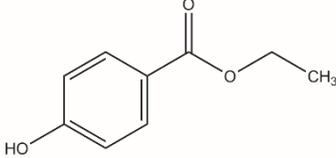
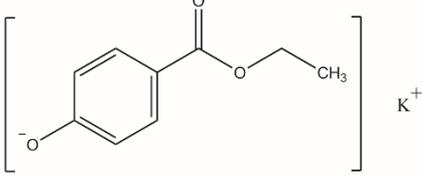
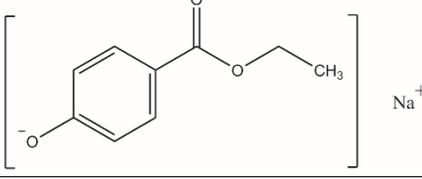
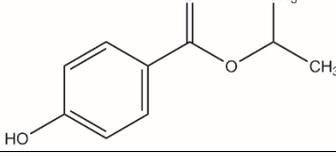
The CIR Expert Panel concluded that the following 20 parabens are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

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

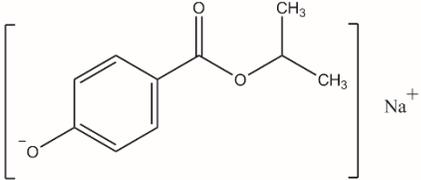
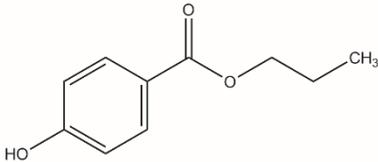
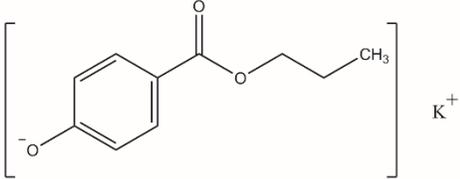
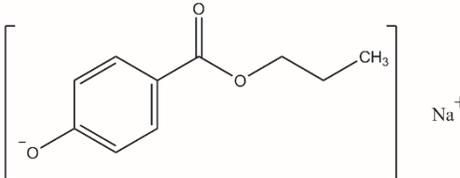
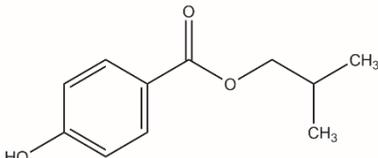
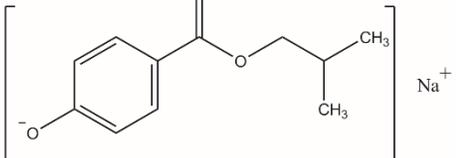
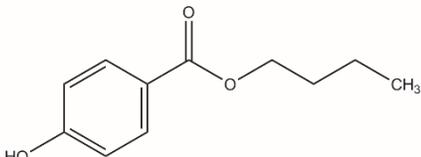
*\*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.*

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

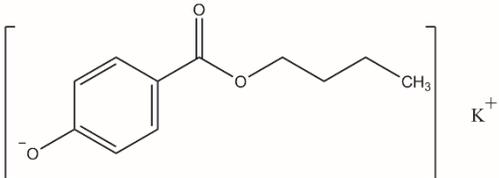
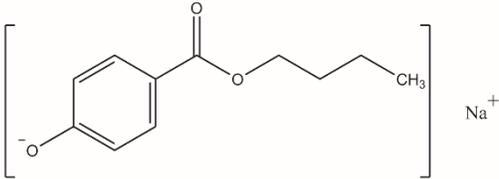
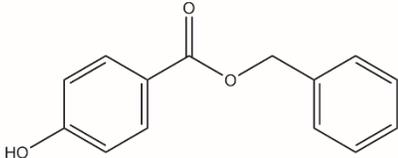
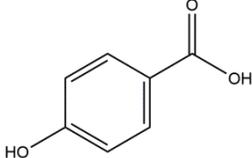
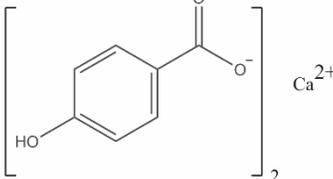
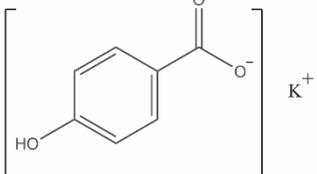
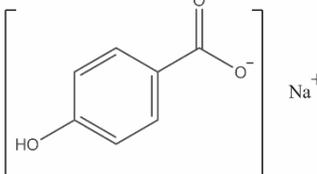
**TABLES****Table 1.** Definitions, structures, and functions of parabens in this safety assessment. <sup>1: CIR Staff</sup>

Ingredient CAS No.	Definition & Structure	Function
<i>Parabens and Paraben Salts</i>		
Methylparaben 99-76-3	Methylparaben is the ester of methyl alcohol and 4-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 4-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 4-Hydroxybenzoic Acid. It conforms to the formula: 	Preservative

**Table 1.** Definitions, structures, and functions of parabens in this safety assessment. <sup>1</sup>: CIR Staff

Ingredient CAS No.	Definition & Structure	Function
Sodium Isopropylparaben	Sodium Isopropylparaben is the sodium salt of Isopropylparaben: 	Preservative
Propylparaben 94-13-3	Propylparaben is the ester of n-propyl alcohol and 4-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 4-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 4-Hydroxybenzoic Acid. It conforms to the formula: 	Fragrance ingredient, preservative

**Table 1.** Definitions, structures, and functions of parabens in this safety assessment. <sup>1</sup>: CIR Staff

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

**Table 2.** Previous CIR safety assessments of parabens

Parabens	Conclusion	Reference
Methylparaben, Ethylparaben, Propylparaben, and Butylparaben	Safe as cosmetic ingredients in the present practices of use	1984 <sup>45</sup>
Benzylparaben	Available data are insufficient to support the safety	1986 <sup>46</sup>
Isobutylparaben and Isopropylparaben	Safe as cosmetic ingredients in the present practices of use	1995 <sup>47</sup>
Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, Isopropylparaben, and Isobutylparaben	Safe in the present practices and concentrations	2008 <sup>2</sup>

**Table 3.** Chemical and physical properties of parabens.

Property	Value	Reference
<b>Benzylparaben</b>		
Physical Form	Solid, crystalline	7
Color	White	7
Odor	Odorless	7
Molecular Weight g/mol	228.25	2
Molecular Volume m <sup>3</sup> /kmol		
Density g/cm <sup>3</sup> @ 20°C	1.224±0.06 est.	150
Vapor Density mmHg	0 est.	7
Melting Point °C	110-112	2
Boiling Point °C	389.8±17.0 est.	150
Water Solubility g/L @ 25°C	1.08	7
	10	2
Other Solubility g/L		
Propylene glycol	130	2
log P <sub>ow</sub>	3.97	7
Disassociation constants (pKa, pKb)		
pK <sub>a</sub>	8.18±0.15 est.	150
<b>Butylparaben</b>		
Physical Form	Crystals or powder	151
Color	White	151
Odor	Odorless	151
Molecular Weight g/mol	194.23	151
Vapor pressure mmHg @ 25°C	1.86x10 <sup>-4</sup>	151
Melting Point °C	68-69	2
	68-72	2
Boiling Point °C	309.2±15.0	150
Water Solubility g/L @ 20°C	0.0027x10 <sup>2</sup>	151
	Insoluble	2
Other Solubility g/L		
Alcohol	Soluble	2
Ether	Soluble	2
Glycerin	Slightly soluble	2
Disassociation constants (pKa, pKb)		
pK <sub>a</sub>	8.37	2
	8.47	151
<b>Ethylparaben</b>		
Physical Form	Crystals or powder	152
Color	Colorless or white	152
Molecular Weight g/mol	166.18	2
Density @ 20°C	1.291	4
Vapor pressure mmHg @ 25°C	9.29x10 <sup>-5</sup>	152
Melting Point °C	116-118	2
	115-118	2
Boiling Point °C	297-298	2
Water Solubility g/L @ 25°C	0.885	152
Other Solubility		
Alcohol	Very soluble	2
Ether	Very soluble	2
Glycerin	Slightly soluble	2
log K <sub>ow</sub>	2.47	4,152
	2.27	40

**Table 3.** Chemical and physical properties of parabens.

Property	Value	Reference
Disassociation constants (pKa, pKb)		
pK <sub>a</sub>	8.22	2
	8.34	152
<b>Isobutylparaben</b>		
Physical Form	Solid, powder	21
Color	White	21
Molecular Weight g/mol	194.25	2
Density g/cm <sup>3</sup> @ 20°C	1.105±0.06	150
Vapor pressure mmHg @ 25°C	0.000381	21
Melting Point °C	72.95 est.	21
Boiling Point °C	302.3±15.0	150
Water Solubility g/L @ 25°C	2.24	21
log P <sub>ow</sub>	3.04	21
<b>Isopropylparaben</b>		
Molecular Weight g/mol	180.22	2
Melting Point °C	96-97	153
Boiling Point °C	294	154
<b>Methylparaben</b>		
Physical Form	Powder	19
	Liquid	19
Color	White or colorless	19
Odor	Characteristic	19
Molecular Weight g/mol	152.16	2
Density g/cm <sup>3</sup> @ 137.2°C	1.1208	155
@ 20°C	1.209±0.06 est.	150
Vapor pressure mmHg @ 25°C	2.37x10 <sup>-4</sup>	19
Melting Point °C	131	2
	125-128	2
Boiling Point °C	270-280	2
	265	154
	140-141	156
Water Solubility g/L @ 25°C	2.50x10 <sup>3</sup>	19
	Slightly soluble	2
Other Solubility		
Alcohol	Very soluble	2
Benzene	Slightly soluble	2
Ether	Very soluble	2
Glycerin	Slightly soluble	2
log K <sub>ow</sub>	1.93	40
Disassociation constants (pKa, pKb)		
pK <sub>a</sub>	8.17	2
<b>Propylparaben</b>		
Physical Form	Crystal or powder	157
Color	Colorless or white	157
Odor	Odorless or faint	157
Molecular Weight g/mol	180.21	2
Density	1.0630	2
	1.28	157
Vapor pressure mmHg @ 25°C	5.55x10 <sup>-4</sup> est.	157
Melting Point °C	96.2-98	2
	95-98	2
Boiling Point °C	294	154
	271	157
Water Solubility g/L	0.0500	157
	Insoluble	2
Other Solubility		
Alcohol	Soluble	2
Ether	Soluble	2
log K <sub>ow</sub>	2.34	5
	2.81	40
Disassociation constants (pKa, pKb)		
pK <sub>a</sub>	8.35	2
<b>Calcium Paraben</b>		
Formula Weight g/mol	314.306	158
<b>Potassium Butylparaben</b>		
Formula Weight g/mol	232.32	159

**Table 3.** Chemical and physical properties of parabens.

Property	Value	Reference
<b>Potassium Ethylparaben</b>		
Formula Weight g/mol	204.266	160
<b>Potassium Methylparaben</b>		
Formula Weight g/mol	190.239	161
<b>Potassium Paraben</b>		
Formula Weight g/mol	176.212	162
<b>Potassium Propylparaben</b>		
Formula Weight g/mol	218.293	163
<b>Sodium Butylparaben</b>		
Formula Weight g/mol	216.212	164
<b>Sodium Ethylparaben</b>		
Physical Form	Solid, powder	22
Color	White	22
Formula Weight g/mol	188.157	37
Density g/cm <sup>3</sup> @ 20°C	1.34	22
Melting Point °C	268	22
Water Solubility g/L @ 23°C & pH 10.4	> 1000	22
log K <sub>ow</sub>	-0.14	22
<b>Sodium Isobutylparaben</b>		
Formula Weight g/mol	216.212	165
<b>Sodium Methylparaben</b>		
Physical Form	Crystalline solid	3
Color	White	3
Formula Weight g/mol	174.131	166
Density g/ml @ 20°C	1.42	3
Melting Point °C	313	3
Water Solubility g/L @ 20°C & pH 11.4	> 10.0	3
log P <sub>ow</sub>	-0.63	3
Disassociation constants pKa @ 23°C	8.4	3
<b>Sodium Paraben</b>		
Formula Weight g/mol	160.104	167
<b>Sodium Propylparaben</b>		
Physical Form	Solid, powder	6
Color	White	6
Formula Weight g/mol	202.185	168
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 <sub>ow</sub>	0.27	6
<b>4-Hydroxybenzoic Acid</b>		
Molecular Weight g/mol	138.12	169
Melting Point °C	214.5	170
Boiling Point °C	336.2 est.	169
log K <sub>ow</sub>	1.39 est.	171
Disassociation constants (pKa, pKb) pKa	4.57±0.10 est	172

Decomp=decomposes on melting

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

<b>Ingredient</b>	<b>D<sub>10</sub> (μm)</b>	<b>D<sub>50</sub> (μm)</b>	<b>D<sub>90</sub>/D<sub>100</sub> (μm)</b>	<b>Fraction &lt;10 μm diameter (vol %)</b>	<b>Reference</b>
Butylparaben	28.5 ± 0.9	114.8 ± 2.4	332.9 ± 16.4	2.1 ± 0.2	<sup>9</sup>
Isobutylparaben	3.1 ± 0.2	25.4 ± 1.5	80.5 ± 4.1	--	<sup>21</sup>
Isopropylparaben	--	150 (6.82%) 106 (35.38%) 75 (27.51 %) 53 (3.15 %)	--	--	<sup>20</sup>
Methylparaben	22.0 ± 0.9	141.7 ± 18.4	426.7 ± 82.6	3.7 ± 0.2	<sup>8</sup>
Sodium Methylparaben	7.9 ± 3	117.1 ± 17.5	693.5 ± 96.8	11.6 ± 2.2	<sup>3</sup>
Ethylparaben	50 ± 4.3	307.5 ± 21.9	770.6	3.0 ± 0.2	<sup>4</sup>
Propylparaben	2.6 ± 0.1	16.2 ± 0.7	113 ± 5	37.8 ± 1.0	<sup>5</sup>
Sodium Ethylparaben	6.5 ± 0.3	49.5 ± 6.4	147.1 ± 28.3	--	<sup>22</sup>
Sodium Propylparaben	6.7 ± 0.3	37.8 ± 4.9	164.5 ± 36.7	--	<sup>6</sup>
4-Hydroxybenzoic acid	--	≥ 59.5 - < 85.5	--	No detection	<sup>11</sup>

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

	# of Uses				Max Conc of Use (%)			
	Benzylparaben				Butylparaben			
	2019 <sup>25</sup>	2006 <sup>2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>	2019 <sup>25</sup>	2006 <sup>2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>
<b>Totals*</b>	NR	1	NR	NR	3884	3001	0.00000006-0.5	0.00002-0.54
<b>Duration of Use</b>								
Leave-On	NR	1	NR	NR	3127	2409	0.00000006-0.5	0.00002-0.4
Rinse-Off	NR	NR	NR	NR	734	551	0.0000004-0.33	0.00004-0.54
Diluted for (Bath) Use	NR	NR	NR	NR	23	41	0.00002-0.1	0.00004-0.07
<b>Exposure Type</b>								
Eye Area	NR	NR	NR	NR	777	812	0.000002-0.5	0.00002-0.3
Incidental Ingestion	NR	NR	NR	NR	273	219	0.0000026-0.2	0.0008-0.1
Incidental Inhalation-Spray	NR	NR	NR	NR	13; 709 <sup>a</sup> ; 671 <sup>b</sup>	27; 453 <sup>a</sup> ; 320 <sup>c</sup>	0.00000011-0.1; 0.00059-0.22 <sup>a</sup>	0.0004-0.2; 0.03-0.4 <sup>a</sup> ; 0.0004-0.4 <sup>c</sup>
Incidental Inhalation-Powder	NR	NR	NR	NR	119; 671 <sup>b</sup> ; 6 <sup>c</sup>	88; 21 <sup>b</sup> ; 320 <sup>c</sup>	0.0057-0.3; 0.0001-0.24 <sup>c</sup>	0.07-0.14; 0.05 <sup>b</sup> ; 0.0004-0.4 <sup>c</sup>
Dermal Contact	NR	1	NR	NR	3156	2406	0.0000004-0.4	0.00004-0.54
Deodorant (underarm)	NR	1 <sup>a</sup>	NR	NR	8 <sup>a</sup>	10 <sup>a</sup>	0.000025 <sup>d</sup>	0.002 <sup>a</sup>
Hair - Non-Coloring	NR	NR	NR	NR	283	246	0.00000011-0.22	0.0004-0.25
Hair-Coloring	NR	NR	NR	NR	33	28	0.0000005-0.05	0.03
Nail	NR	NR	NR	NR	43	21	0.00000006-0.07	0.003-0.2
Mucous Membrane	NR	NR	NR	NR	517	312	0.0000026-0.2	0.00004-0.11
Baby Products	NR	NR	NR	NR	11	28	NR	0.05
<b>Ethylparaben</b>								
	2019 <sup>25</sup>	2005 <sup>**2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>	2019 <sup>25</sup>	2006 <sup>2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>
<b>Totals*</b>	3802	2679	0.00000032-0.65	0.00002-0.98	1918	642	0.00000006-0.3	0.000007-0.5
<b>Duration of Use</b>								
Leave-On	2878	2066	0.00000032-0.65	0.00002-0.6	1447	435	0.00000006-0.3	0.000007-0.5
Rinse-Off	893	562	0.0000008-0.5	0.0001-0.98	446	178	0.0000004-0.23	0.0001-0.4
Diluted for (Bath) Use	31	51	0.005-0.1	0.00004-0.15	25	29	0.000012-0.005	0.00002-0.2
<b>Exposure Type</b>								
Eye Area	545	543	0.000002-0.65	0.00002-0.49	213	59	0.00000006-0.14	0.000007-0.5
Incidental Ingestion	64	72	0.000008-0.3	0.0002-0.2	63	11	0.000004-0.09	0.0001-0.4
Incidental Inhalation-Spray	13; 786 <sup>a</sup> ; 370 <sup>b</sup>	23; 431 <sup>a</sup> ; 330 <sup>c</sup>	0.000031-0.22; 0.00059-0.2 <sup>a</sup> ; 0.06-0.15 <sup>b</sup>	0.02-0.2; 0.0001-0.6 <sup>a</sup> ; 0.0004-0.4 <sup>c</sup>	7; 383 <sup>a</sup> ; 444 <sup>b</sup>	7; 109 <sup>a</sup> ; 129 <sup>c</sup>	0.000004-0.023; 0.00002-0.18 <sup>a</sup>	0.01-0.2; 0.0002-0.3 <sup>a</sup> ; 0.02-0.4 <sup>c</sup>
Incidental Inhalation-Powder	64; 370 <sup>b</sup> ; 12 <sup>c</sup>	122; 12 <sup>b</sup> ; 330 <sup>c</sup>	0.0057-0.5; 0.06-0.15 <sup>b</sup> ; 0.0002-0.48 <sup>c</sup>	0.04-0.5; 0.0004-0.4 <sup>c</sup>	21; 444 <sup>b</sup> ; 2 <sup>c</sup>	8; 5 <sup>b</sup> ; 129 <sup>c</sup>	0.0029-0.0086; 0.0000007-0.24 <sup>b</sup>	0.00001-0.04; 0.02-0.4 <sup>c</sup>
Dermal Contact	2988	2147	0.000002-0.65	0.00004-0.98	1577	525	0.0000006-0.3	0.00001-0.5
Deodorant (underarm)	10 <sup>a</sup>	10 <sup>a</sup>	not spray: 0.5 spray: 0.00005 <sup>d</sup>	0.002-0.1 <sup>a</sup>	5 <sup>a</sup>	3 <sup>a</sup>	NR	0.002 <sup>a</sup>
Hair - Non-Coloring	102	229	0.0000008-0.3	0.001-0.6	141	83	0.0000004-0.17	0.01-0.3
Hair-Coloring	115	92	0.000004-0.2	0.2	30	1	0.000036-0.00008	NR
Nail	15	10	0.00000032-0.2	0.01-0.2	37	3	NR	0.006
Mucous Membrane	310	170	0.000008-0.3	0.00004-0.2	265	63	0.000004-0.09	0.00002-0.4
Baby Products	15	15	0.032	NR	5	7	NR	NR

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

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	Isopropylparaben				Methylparaben			
	2019 <sup>25</sup>	2006 <sup>2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>	2019 <sup>25</sup>	2006 <sup>2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>
<b>Totals*</b>	<b>274</b>	<b>48</b>	<b>0.000005-0.32</b>	<b>0.00001-0.3</b>	<b>11,739</b>	<b>8786</b>	<b>0.000001-0.9</b>	<b>0.0003-1</b>
<b>Duration of Use</b>								
Leave-On	231	39	0.00004-0.32	0.00001-0.3	9347	6468	0.0000043-0.8	0.0008-1
Rinse-Off	42	8	0.000005-0.22	0.03-0.2	2333	2105	0.000001-0.9	0.001-0.46
Diluted for (Bath) Use	1	1	NR	0.005	59	213	0.21-0.5	0.0003-0.5
<b>Exposure Type</b>								
Eye Area	45	10	0.19	0.06-0.2	1797	1610	0.000002-0.8	0.07-0.6
Incidental Ingestion	31	1	0.12	0.2	305	301	0.000032-0.35	0.07-1
Incidental Inhalation-Spray	2; 88 <sup>a</sup> ; 21 <sup>b</sup>	2; 6 <sup>a</sup> ; 6 <sup>c</sup>	0.00004; 0.00004 <sup>a</sup>	0.0005-0.3 <sup>a</sup> ; 0.1-0.2 <sup>c</sup>	86; 3299 <sup>a</sup> ; 1851 <sup>b</sup>	111; 1382 <sup>a</sup> ; 968 <sup>c</sup>	0.0000043-0.41 0.0024-0.5 <sup>a</sup> ; 0.25-0.6 <sup>b</sup>	0.1-0.35; 0.07- 0.5 <sup>a</sup> ; 0.15-0.44 <sup>c</sup>
Incidental Inhalation-Powder	6; 21 <sup>b</sup>	5; 6 <sup>c</sup>	NR	0.00001- 0.00002; 0.1-0.2 <sup>c</sup>	346; 1851 <sup>b</sup> ; 20 <sup>c</sup>	376; 33 <sup>b</sup> ; 968 <sup>c</sup>	0.004-0.4; 0.0024-0.6 <sup>b</sup> 0.001-0.8 <sup>c</sup>	0.1-0.5; 0.2-0.4 <sup>b</sup> ; 0.15- 0.44 <sup>c</sup>
Dermal Contact	197	39	0.031-0.32	0.00001-0.3	9310	6898	0.000001-0.6	0.0003-0.7
Deodorant (underarm)	NR	NR	NR	NR	20 <sup>a</sup>	35 <sup>a</sup>	not spray: 0.15-0.4 spray: 0.000075- 0.00012	0.0008-0.3 <sup>a</sup>
Hair - Non-Coloring	23	6	0.000005-0.22	0.001	1500	1137	0.0002-0.9	0.1-0.4
Hair-Coloring	NR	NR	NR	NR	237	197	0.0000016-0.4	0.05-0.35
Nail	6	NR	0.00012	0.1	68	37	0.0000012-0.41	0.002-0.4
Mucous Membrane	52	2	0.12	0.005-0.2	833	751	0.000001-0.5	0.0003-1
Baby Products	NR	NR	NR	NR	36	60	0.13-0.4	0.2-0.4
<b>Propylparaben</b>								
	2019 <sup>25</sup>	2006 <sup>2</sup>	2016 <sup>24</sup>	2003 <sup>2</sup>	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 <sup>a</sup> It is possible these products <u>may</u> be sprays, but it is not specified whether the reported uses are sprays. <sup>b</sup> 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 <sup>c</sup> It is possible these products <u>may</u> be powders, but it is not specified whether the reported uses are powders.			
<b>Totals*</b>	<b>9034</b>	<b>7118</b>	<b>0.00000014-0.7</b>	<b>0.00002-0.7</b>				
<b>Duration of Use</b>								
Leave-On	7520	5585	0.00000014-0.7	0.00002-0.7				
Rinse-Off	1465	1422	0.00000026-0.3	0.01-0.5				
Diluted for (Bath) Use	49	140	0.0001-0.3	0.04-0.3				
<b>Exposure Type</b>								
Eye Area	1564	1477	0.00000014-0.7	0.02-0.5				
Incidental Ingestion	586	527	0.000004-0.3	0.03-0.62				
Incidental Inhalation-Spray	35; 2532 <sup>a</sup> ; 1349 <sup>b</sup>	62; 996 <sup>a</sup> ; 706 <sup>c</sup>	0.00000014- 0.31; 0.0003-0.25 <sup>a</sup> ; 0.02-0.25 <sup>b</sup> <sup>c</sup>	0.1-0.3; 0.001-0.5 <sup>a</sup> ; 0.03-0.4 <sup>c</sup>				
Incidental Inhalation-Powder	272; 1349 <sup>b</sup> ; 21 <sup>c</sup>	308; 31 <sup>b</sup> ; 706 <sup>c</sup>	0.0018-0.3; 0.02-0.25 <sup>b</sup> ; 0.0001-0.3 <sup>c</sup>	0.1-0.7; 0.2 <sup>b</sup> ; 0.03-0.4 <sup>c</sup>				
Dermal Contact	7232	5598	0.00000014-0.4	0.00002-0.7				
Deodorant (underarm)	13 <sup>a</sup>	29	not spray: 0.025-0.15 spray: 0.000025- 0.000058	0.002-0.2 <sup>a</sup>				
Hair - Non-Coloring	749	623	0.0000055-0.4	0.03-0.5				
Hair-Coloring	168	150	0.00000026- 0.25	0.04-0.5				
Nail	58	27	0.0000003-0.2	0.002-0.4				
Mucous Membrane	983	832	0.000004-0.3	0.02-0.62				
Baby Products	35	56	0.15	0.05-0.2				

**Table 6.** Frequency (2019)<sup>25</sup> and concentration (2016)<sup>24</sup> of use according to duration and exposure of parabens.

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Sodium Butylparaben		Sodium Ethylparaben		Sodium Isobutylparaben	
<b>Totals*</b>	<b>2</b>	<b>NR</b>	<b>27</b>	<b>0.000012-0.062</b>	<b>2</b>	<b>NR</b>
<b>Duration of Use</b>						
Leave-On	2	NR	25	0.000012-0.062	2	NR
Rinse-Off	NR	NR	2	0.0036	NR	NR
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR
<b>Exposure Type</b>						
Eye Area	NR	NR	10	0.0036	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2 <sup>a</sup>	NR	5 <sup>a</sup> ; 4 <sup>b</sup>	NR	2 <sup>a</sup>	NR
Incidental Inhalation-Powder	NR	NR	4 <sup>b</sup>	0.0036 <sup>c</sup>	NR	NR
Dermal Contact	2	NR	24	0.0036-0.062	2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	0.0036	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.000012	NR	NR
Mucous Membrane	NR	NR	2	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
<b>Duration of Use</b>						
	Sodium Methylparaben		Sodium Paraben		Sodium Propylparaben	
<b>Totals*</b>	<b>414</b>	<b>0.000005-0.4</b>	<b>NR</b>	<b>0.008</b>	<b>134</b>	<b>0.000015-0.28</b>
<b>Duration of Use</b>						
Leave-On	216	0.00001-0.4	NR	0.008	100	0.000017-0.28
Rinse Off	189	0.000005-0.4	NR	NR	30	0.000015-0.1
Diluted for (Bath) Use	9	NR	NR	NR	4	NR
<b>Exposure Type</b>						
Eye Area	46	0.000012-0.4	NR	NR	18	0.004-0.28
Incidental Ingestion	NR	NR	NR	NR	NR	0.1
Incidental Inhalation-Spray	2; 46 <sup>a</sup> ; 79 <sup>b</sup>	0.00002; 0.00022-0.3 <sup>b</sup>	NR	NR	15 <sup>a</sup> ; 16 <sup>b</sup>	NR
Incidental Inhalation-Powder	79 <sup>b</sup>	0.00013; 0.00016-0.3 <sup>c</sup>	NR	NR	16 <sup>b</sup>	0.0051 <sup>c</sup>
Dermal Contact	257	0.000005-0.4	NR	0.008	124	0.0004-0.28
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	72	0.00002-0.4	NR	NR	3	0.000015
Hair-Coloring	75	0.3-0.4	NR	NR	1	0.0051
Nail	NR	0.000046	NR	NR	NR	0.000017
Mucous Membrane	23	0.25	NR	NR	10	0.1
Baby Products	NR	NR	NR	NR	1	NR

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

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

NR=Not Reported

<sup>a</sup> It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

<sup>b</sup> Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

<sup>c</sup> It is possible these products may be powders, but it is not specified whether the reported uses are powders.

**Table 7.** Parabens with no current reported use according to VCRP data (2019) and the Council survey (2016).<sup>2,24,173</sup>

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

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

Year	Conclusion	Reference
2005	It is the opinion of the SCCP that, viewing the current knowledge, there is no evidence of demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics containing parabens.	<sup>12</sup>
2005	Methylparaben and Ethylparaben can be safely used up to the maximum authorized concentration as actually established (0.4%).  The available data do not enable a decisive response to the question of whether propyl, butyl and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%.  More information is needed in order to formulate a final statement on the maximum concentration of propyl, isopropyl, butyl and isobutyl paraben allowed in cosmetic products.	<sup>13</sup>
2006	The conclusion of opinion SCCP/0873/05 (i.e., ref.16) remains unchanged.	<sup>14</sup>
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.	<sup>14,15</sup>
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.	<sup>16</sup>
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.	<sup>17</sup>
2013	The concerns of the SCCP/SCCS expressed previously and reiterated in recent Opinions remain unchanged and reinforced after the evaluation of both the reproductive toxicity and the toxicokinetic studies on Propylparaben recently submitted to the SCCS. The same data were extrapolated for the evaluation of the risk by Butylparaben exposure.  The additional submitted data does not remove the concern expressed in the previous opinions on the relevance of the rat model for the risk assessment of parabens. Although much toxicological data on parabens in rodents exists, adequate evidence has not been provided for the safe use of propyl- or Butylparaben in cosmetics. For these reasons, the 22 SCCS reiterates its previous conclusions and requests regarding an improvement of the data, in particular  a) on the exposure of humans including children to Propyl- and Butylparaben in cosmetic products and  b) the toxicokinetics of Propylparaben and Butylparaben in humans.	<sup>17,32</sup>

**Table 9.** In vitro dermal penetration studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
Methylparaben	Pig	Skin from the upper half of the ears of 6-month-old pigs	0.1% in aqueous, or hydrogel or emulsion oil-in-water formulations with and without a penetration enhancer (urea, Transcutol or propylene glycol), 0.1%, pH=5.5	Porcine skin used fresh or after storage at 4°C for 18 h or frozen, clamped between donor and receptor chambers of Franz-type diffusion cells	Receptor fluid (phosphate-buffered saline and 0.01% of Gentamicin-sulphate) and skin samples (~3.3 cm <sup>2</sup> discs, intact or tape-stripped 20 times; diffusion area 2 cm <sup>2</sup> ) maintained at 32°C; nine formulations, representing the most frequently types of MP-containing topical leave-on products, were prepared with a combination of difference concentrations of the following chemicals: aqua, urea, ethoxydiglycol, propylene glycol, olea europaea oil, glyceryl stearate, C12-14 Pareth-3, cetyl alcohol, carbomer, sodium hydroxide, and lactic acid; 20 µL aqueous solution was added to the donor chamber or ~20 mg of hydrogel or emulsion was applied to the skin sample at t=0; 50 µL samples removed from the receptor chamber at intervals for up to 4 h or 24 h (depending on the experiment) for analysis by HPLC and replaced by fresh receptor medium	For freshly excised intact skin and previously frozen intact skin, concentrations of unmetabolized Methylparaben in receptor fluid <LOD-2.3% and 2.3%-3.3% of applied dose, respectively, after 4-h exposure; for previously frozen intact and tape-stripped skin, concentrations of unmetabolized Methylparaben in receptor fluid were 2.0%-5.8% and 2.9%-7.6% respectively, after 24-h exposure; absorption rate was higher from emulsions vs. hydrogels, enhancer-containing formulations vs. enhancer-free formulations, and when skin was tape stripped	<sup>38</sup>
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 <sup>2</sup> ); 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 <sup>-4</sup> ), in descending order: Methylparaben, 214.8 ± 40, Ethylparaben, 197.5 ± 10; Propylparaben, 101.9 ± 15; Butylparaben 31.3 ± 1.6; skin penetration was inversely proportional to lipophilicity; Increasing ethanol concentration in the vehicle and exposure duration increased parabens retention in dermis compared epidermis; Binary combinations of the parabens reduced their permeation rates, attributed by the authors to high retention in the epidermis and dermis	<sup>39</sup>
Methylparaben Ethylparaben Propylparaben	Rabbit (mixed breed)	Skin excised from ears of 6-month-old animals	3 commercial facial moisturizing creams containing 0.23%-0.32% (w/w) Methylparaben, 0%-0.1% Ethylparaben, and 0.04%-0.19% Propylparaben.	Full-thickness skin, stored frozen, thawed and mounted on Franz-type diffusion cells	Receptor fluid (saline) and skin samples (diffusion area 0.6 cm <sup>2</sup> ); Donor chamber filled with 2 mg/cm <sup>2</sup> cream at t=0; 300 µL samples removed from the receptor chamber at intervals for up to 8 h for analysis by HPLC and replaced by fresh receptor medium	Percentage of applied dose in receptor fluid after 8 h exposure, in descending order: Methylparaben, 60%; Ethylparaben, 40%; Propylparaben, 20% of PP – penetration decreased with decreasing water solubility, regardless of the formulation tested; Retention varied widely in the epidermis (14.0-253.0 µg/g) and dermis (0-19.3 µg/g), depending on the formulation	<sup>40</sup>
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	Receptor fluid (1:1 ethanol/water, v/v) and skin samples (diffusion area 0.785 cm <sup>2</sup> ) 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	Permeability coefficients (K <sub>ps</sub> ; cm/h x 10 <sup>-4</sup> ) were similar regardless of concentration tested; K <sub>ps</sub> were directly related to paraben concentration  K <sub>ps</sub> for human skin ranged from 0.74 ± 0.19 to 0.91 ± 0.44 for Methylparaben, 0.54 ± 0.14 to	<sup>41</sup>

**Table 9.** In vitro dermal penetration studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
				diffusion cells	medium	<p>0.91 ± 0.22 for Propylparaben, and 0.37 ± 0.15 to 0.56 ± 0.32 for Butylparaben</p> <p><math>K_{ps}</math> 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</p> <p>Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin;</p> <p>Residual quantities in human epidermis (<math>\mu\text{g/ml} \times 10^{-4}</math>): Methylparaben, 235 ± 132 to 7198 ± 4662; Propylparaben, 375 ± 212 to 4120 ± 2344; Butyl paraben, 436 ± 226 to 5480 ± 2593;</p> <p>Residual quantities in mouse skin: Methylparaben, 14 ± 5 to 286 ± 104; Propylparaben, 21 ± 9 to 410 ± 112; Butyl paraben, 15 ± 2 to 358 ± 118</p> <p>Authors state results show that parabens may be classified as moderate penetrants</p>	
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 <sup>2</sup> ) maintained at 32°C; single 100 $\mu\text{L}$ (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	<p>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;</p> <p>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</p>	42

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
<b>In Vitro</b>						
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 $1.5 \times 10^{-6}$ 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 $\mu$ M; AFP did not exhibit binding affinity for Methylparaben, Ethylparaben, and Propylparaben	48
Butylparaben	Rat (Wistar)	S9 fraction of 5-week old males (n not specified)	Twelve concentrations between about 5 $\mu$ M and 90 $\mu$ M	Reactions performed in PBS, pH 7.4, at 37°C in shaking water bath and stopped by adding ice-cold methanol; supernatant was separated by HPLC and formation of 4-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 4-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 $V_{max}$ ( $K_m$ )=28.6 mM	49
Butylparaben	Human  Rat (Harlan Sprague-Dawley)	Hepatocytes from human subjects (59-year-old woman and 45-year-old man, both non-smokers) and 8 to 12 week old male and female rats	1 $\mu$ M radiolabeled Butylparaben (phenyl ring- $^{14}$ C(U) – 53.1 mCi/mmol); 10 $\mu$ M 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 $\mu$ L 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 $\pm$ 0.3 min and 3.3 $\pm$ 0.1 min for hepatocytes from males and females, respectively, corresponding to $Cl_{int}$ =811 $\pm$ 53 and 903 $\pm$ 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 $\pm$ 1.3 min and 29.6 $\pm$ 5.2 min, respectively, corresponding to $Cl_{int}$ =92 $\pm$ 5 and 111 $\pm$ 22 mL/min/kg); Butylparaben was extensively hydrolyzed to 4-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%)	53
Methylparaben Ethylparaben Propylparaben Butylparaben	Human  Rat (Sprague-Dawley)  Monkey (African green)	Pooled human liver and small intestine microsomes  Rat liver, skin, kidney, pancreas, and small intestine microsomes and blood plasma  S9 from COS cells (Monkey-kidney derived, fibroblast like)	100 nmol paraben and tissue microsomes or plasma in final volume of 1 mL 0.1 M K, Na-phosphate buffer (pH 7.4)	Incubation was for 7 min at 37°C, then 10 mg 2,4-dihydroxybenzophenone (internal standard) and 1 mL acetonitrile added; aliquot of the supernatant was collected for analysis of paraben hydrolase activity by HPLC  Carboxylesterase activity was determined by measuring deacetylase activities toward 4-nitrophenol acetate and 4-methylumbelliferyl acetate: 4-nitrophenol acetate deacetylase activity measured by spectrophotometry at 405 nm; 4-methylumbelliferyl acetate deacetylase activity measured by fluorophotometry at 329 nm (excitation) and 448 nm (emission)	Rat liver microsomes (RLM) showed the highest activity toward parabens, followed by small-intestinal and lung microsomes; Butylparaben was most effectively hydrolyzed by the RLM, which showed relatively low hydrolytic activity towards parabens with shorter and longer alkyl side chains; In contrast, rat small-intestinal microsomes exhibited relatively higher activity toward longer-side-chain parabens; Rat lung and skin microsomes showed liver-type substrate specificity; Kidney and pancreas microsomes and plasma of rats showed small-intestinal-type substrate specificity; Rat small-intestinal microsomes exhibited higher activity toward longer-side-chain parabens – carboxylase 2 showed a similar activity pattern;	51

**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 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-glucuronosyltransferases (UGT) was performed by a modified of the method of Bansal and Gessner (1980)	In contrast, human liver microsomes showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length; human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes  Methylparaben 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 <sub>1/2</sub> =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	50
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (strain not specified)	HLM, HSM, HLC, and HSC  RLM, RSM, RLC, and RSC	100 µM in 50 mM potassium phosphate, pH 7.4	Reactions were initiated with the addition of 100 µM paraben; mixture incubated for 30 min at 37°C; 4-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 contrast to human tissue fractions, hydrolysis rates of the parabens increased as the ester chain length increased in rat tissue. Methylparaben and Propylparaben was the preferred substrate for human tissue fractions and rat tissue fractions, respectively; Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin	52
<b>ANIMAL</b>						
<b>Dermal</b>						
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- <sup>14</sup> 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	For all 3 parabens, C <sub>max</sub> (≥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	54

**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
				<p>exposure period</p> <p>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;</p> <p>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</p> <p>Organs were collected, weighed, and analyzed for radioactivity..</p>	<p>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;</p> <p>≤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;</p> <p>Metabolic profiling of pooled plasma collected 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of 4-<i>Hydroxybenzoic Acid</i></p>	
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- <sup>14</sup> C(U) – 53.1 mCi/mmol; 50 µCi dose/animal) in 95% ethanol, applied to the skin	<p>Single dermal dosages (0.5 mL/kg bw) were applied onto a 4 cm<sup>2</sup> (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</p> <p>Urine and feces of rats were collected separately for up to 72 h post-exposure; the animals were then killed, blood was collected and the tissues were excised and weighed. The protective appliance was removed, dose-site skin was excised and washed with a series of water-wetted gauzes and appliance.</p>	<p>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;</p> <p>About 21% of the 10 mg/kg dosage remained unabsorbed; about 16% was recovered in the dose-site skin;</p> <p>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;</p> <p>Urine was the primary route of elimination, with about 46% of 10 mg/kg recovered in urine and in cage</p> <p>rinse at 72 h; fecal elimination of radioactivity accounted for 1.7%;</p> <p>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</p>	53
<i>Oral</i>						
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- <sup>14</sup> C) paraben, in 60% aqueous ethanol vehicle, administered by gavage	<p>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;</p> <p>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</p>	<p>For all 3 parabens, C<sub>max</sub> (≥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;</p> <p>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</p>	54

**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
				dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection.  Organs were collected, weighed, and analyzed for radioactivity.	dose ( $\geq 71\%$ ) was eliminated in urine, while $\leq 3.3\%$ was eliminated in the feces; radioactivity was eliminated rapidly with averages $\geq 69.6\%$ 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 <i>4-Hydroxybenzoic Acid</i>	
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- $^{14}\text{C}$ (U) – 53.1 mCi/mmol; 50 $\mu\text{Ci}$ 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 $\geq 66\%$ 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	53
<b>HUMAN</b>						
<i>Dermal</i>						
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean=26 years old), n=26	2% (w/w) Butylparaben in cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate	In a 2-week single-blinded study, male subjects were given a whole body topical application of basic cream 2 mg/cm <sup>2</sup> (control week) and then a cream containing 2% (w/w) of diethyl phthalate (DEP), dibutyl phthalate (DBP) and Butylparaben each (treatment week) daily; 24-h urine samples were collected and analyzed for total	All 26 subjects showed increased excretion of Butylparaben following topical application; Mean total Butylparaben excreted in urine during treatment was $2.6 \pm 0.1$ mg/24 h; on average, 0.32% of the applied dose was recovered in urine as Butylparaben; the concentration peaked in urine 8-12	55

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				and unconjugated Butylparaben by LC-MS/MS	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	
<i>Oral</i>						
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, <i>4-Hydroxybenzoic Acid</i> and <i>p</i> -hydroxyhippuric acid, were detected in the urine samples; new oxidized metabolites with hydroxy groups on the alkyl side chain (3OH-n-butylparaben and 2OH-iso-butylparaben) and species with oxidative modifications on the aromatic ring were discovered; 17.4 %, 6.8 %, 5.6% of the doses of Methylparaben, Isobutylparaben and Butylparaben, respectively, were excreted in the urine; about 16% and 6% of Isobutylparaben and Butylparaben were excreted as 2OH-iso-butylparaben and 3OH-n-butylparaben, respectively; less than 1% was excreted as ring- hydroxylated metabolites; For all parabens tested, 4-Hydroxybenzoic Acid was the major metabolite (57.2% - 63.8%) and urinary <i>p</i> -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	<sup>56</sup>

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

Table 11. Short-Term Toxicity Studies

Test Substance(s)	Species/ Strain	Test Group	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
<b>Animal</b>							
<b>Dermal</b>							
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	<p>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;</p> <p>The relative weight of heart and kidneys increased in a dose dependent manner in male rats treated by paraben mixture; The relative weight of testes showed significant increase in males treated by Isobutylparaben and Isopropylparaben at 600 mg/kg bw/day;</p> <p>Analysis of serum concentrations showed that FSH was dose-dependently decreased in animals treated with <math>\geq 200</math> mg/kg bw/day of the mixture (i.e. <math>\geq 100</math> mg/kg bw/day each of Isopropylparaben and Isobutylparaben combined);</p> <p>No significant change of serum T3, TSH, insulin, E2, or testosterone concentrations in female rats treated by parabens</p>	58
<b>Oral</b>							
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	<p>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;</p> <p>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);</p> <p>Statistically-significant decrease in GSH, CAT and SOD activities, and increase of lipid peroxidation and NO generation (at both dosage rates);</p> <p>Statistically-significant dose-dependent reduction in serum testosterone concentration and T/E2 ratio, and elevation in serum E2;</p>	59

**Table 11.** Short-Term Toxicity Studies

Test Substance(s)	Species/ Strain	Test Group	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
						<p>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);</p> <p>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</p>	
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 10 <sup>th</sup> 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)	<sup>60</sup>
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	<p>All three dosage rates elevated MDA levels in the liver in a statistically-significant (p &lt; 0.05), dose-dependent manner</p> <p>TAA levels were reduced by 11.34%, 27.03%, and 41.02% at 13.33, 20 and 40 mg/kg bw/day (p &lt; 0.05), respectively; GSH levels were reduced by 22.22%, 44.53% and 55.74% at 13.33, 20 and 40 mg/kg bw/day (p &lt; 0.05), respectively;</p> <p>Statistically-significant (p &lt; 0.05), dose-dependent reductions in SOD, CAT, GPx, GR, and GST levels were noted in Butylparaben treated mice at all doses</p>	<sup>61</sup>

2,3-DHBA=2,3-dihydroxybenzoic acid; Alb=Albumin; ALP=Alkaline phosphatase; ALT=Serum alanine aminotransferase; AST=Aspartate aminotransferase; BSP=Bromosulphophthalein; ELISA=Enzyme-linked immunosorbent assay; CAT=Catalase; E2=17-β 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.** Developmental and reproduction toxicity (DART) studies

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

**Table 12.** Developmental and reproduction toxicity (DART) studies

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

**Table 12.** Developmental and reproduction toxicity (DART) studies

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

**Table 12.** Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben	Rat (Sprague- Dawley)	“Nulliparous”/virgin (n=10/group) and “parous” (n=10/group) females	0, 0.105 mg/kg bw/day in olive oil (vehicle), by gavage	Parturition marked LD0 for the F0 females and PND0 for the offspring; F0 females were dosed orally and, thereby, F1 offspring were exposed through lactation After weaning on LD 28, F1 offspring were separated from the F0 females were divided into two groups, “nulliparous” and “parous,” and exposed orally PND 181. “Parous” F1 females were mated on PND 97 and exposure continued through pregnancy and delivery of F2 pups and lactation, ending on LD 28; after LD 28, the animals (F1) were separated from their mothers (F0), divided into two groups, “nulliparous” and “parous”, and exposed through gavage until the final sacrifice at PND 181	Number of pups born to treated F1 females was statistically-significantly greater than that of controls; F2 pups exhibited statistically-significantly greater mortality at PND 7 and thereafter, compared with controls; All “nonparous” F1 females (treated and controls) exhibited normal mammary-tissue morphology; In treated “parous” F1 females, during lactation, mammary alveoli were not always milk- filled, increase in adipose tissue was noted, and collapsed alveolar and duct structures showed residual secretory content. Whole-mount preparations showed differences in lobular development among control and treated animals, including marked decrease in the size of the lobular structures in all treated F1 females; In treated “parous” F1 females, at PND 181, there were no histopathological differences among treated and control groups	<sup>69</sup>
Propylparaben	Rat (Wistar- Crl:WI [Han])	Lactating females (n=36), each with a litter ≥5 male pups supplied on PND14, n=20 pups/group (10/subgroup)	0, 10, 100, 1000 mg/kg bw/day, 2% suspended in a 1% aqueous hydroxycellulose, by gavage	Juvenile male rats were dosed for 8 weeks starting on PND21; all animals were sacrificed after the treatment	There was no evidence of an effect on the weight of the male reproductive organs, epididymal sperm parameters, hormone concentrations, or histopathology; The highest dosage rate tested (1000 mg/kg/day) was the NOAEL	<sup>70</sup>
Butylparaben	Rat (Sprague- Dawley)	Males, 7-week-old, n=5/group, 4 groups	0, 10, 100 and 1000 mg/kg in corn oil (vehicle), by gavage	Performed in accordance with OECD TG 407 for repeated 28-day oral toxicity studies; 24 h after the last dose, testes, tails and epididymal spermatozoa samples were collected, DNA was extracted, and the DNA samples from each group were pooled, digested (methylation-specific restricted restriction digestion), and analyzed by differential display random amplification of polymorphic DNA (RAPD)	Among 57 RAPD amplicons, six were methylation specific. Densitometric analysis of stained agarose gels revealed that five of these amplicons were elevated 1.4- to 3.8-fold in epididymal sperm DNA in treated vs. control animals, indicating an epigenetic effect on spermatogenic germ cells in adult rats	<sup>174</sup>
Methylparaben Butylparaben	Rat (Wistar -Crl:WI [BR])	Males, 22 days of age, n=16/group, 4 groups	0, 100, 1000 or 10,000 ppm in the diet	Rats were 22 days of age at the start of exposure, which was continued for 56 days; parameters evaluated included organ weights, histopathology of reproductive tissues, sperm production, motility, and morphology; reproductive hormone concentrations (LH, FSH, and T) were measured in blood samples; animals were sacrificed on study days 32, 44 and after final	Methylparaben exposure resulted in a statistically-significantly higher incidence of abnormal sperm in the 1000-ppm (p≤0.01) and 10,000-ppm (p≤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%,	<sup>49</sup>

**Table 12.** Developmental and reproduction toxicity (DART) studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
				treatment	respectively) in the 10,000-ppm Butylparaben-treated group, compared with the control group; LH concentrations were statistically-significantly lower ( $p \leq 0.01$ ) in the 1000-ppm (by 35%) and 10,000-ppm (by 30%) exposure groups, compared with controls, but only at the 5-week exposure point The authors concluded that none of the parameters evaluated for either paraben showed compound- or dosage-dependent adverse effects, and the NOAEC was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of $1141.1 \pm 58.9$ and $1087.6 \pm 67.8$ mg/kg/day for Methylparaben and Butylparaben, respectively	
<b>Subcutaneous</b>						
Butylparaben	Rat (Wistar)	Male, 2 days of age, N=8, n= 3 or 5/ group, 2 groups	2 mg/kg bw /day in corn oil (vehicle), by subcutaneous injection	Male rats were dosed subcutaneously for 17 days starting on PND2; control group contained 5 rats, and Butylparaben treated group contained 3 rats; parameters evaluated included testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts, and immunoexpression of the water AQP-1 (The epithelial cells of the efferent ducts decrease in height coincident with reduced expression of the water channel protein AQP-1; animals that were sampled on day 18 were killed 4 h after injection	No detectable effect on any of the measured reproductive parameters after subcutaneous administration of Butylparaben for 17 days (PND 2-18); the NOEL was 2 mg/kg bw/day.	<sup>141</sup>

AQP-1=channel aquaporin-1; AR=Androgen receptor; CYP19=Aromatase; E2=17 $\beta$ -estradiol; EE=17 $\alpha$ -ethynylestradiol; ER $\alpha$ =Estrogen receptor  $\alpha$ ; 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 for Economic Co-operation and Development Test Guideline; P450<sub>scc</sub>=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; TNF $\alpha$ =Tumor necrosis factor  $\alpha$

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/Dosage (Vehicle)	Procedure	Results	Reference
<b>In Vitro</b>						
Butylparaben	Mouse (strain not specified)	Murine NIH-3T3-L1 fibroblasts	0, 1, 3, 10, 30, and 100 µM in DMSO (<0.3%)	<p>For the mPPAR<math>\alpha</math>/<math>\gamma</math> transactivation assay, cells were transfected with the luciferase reporter plasmid 4xUAS-TK and either gal4-DBD_mPPAR<math>\alpha</math>LBD or gal4-DBD_mPPAR<math>\gamma</math>LBD expression vectors; media containing Butylparaben was added and cells incubated for 22 h at 37°C;</p> <p>For analysis of the human PPAR, cells were transfected with expression plasmid for the ligand binding domain of the hPPAR<math>\alpha</math> or hPPAR<math>\gamma</math> coupled to Gal4 and a plasmid containing an UAS linked luciferase reporter gene (UAS-TK-luc);</p> <p>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;</p> <p>Cytotoxicity was evaluated in parallel experiments not used for Oil Red staining, with resazurin for 3 h followed by measuring fluorescence;</p> <p>To quantify the concentrations of resistin, leptin, and adiponectin in the supernatant from the adipocyte differentiation assay using commercially-available assay kits</p>	<p>Weak activation of mPPAR<math>\alpha</math> was seen with the highest concentrations of Butylparaben;</p> <p>Butylparaben activated mPPAR<math>\gamma</math> with a LOEC of 30 µM and a maximal 4-fold induction at 100 µM;</p> <p>The human data for Butylparaben (hPPAR<math>\alpha</math> and hPPAR<math>\gamma</math>) were comparable to those obtained with mPPAR<math>\alpha</math> and mPPAR<math>\gamma</math>;</p> <p>Butylparaben showed induction of lipid accumulation at 20 µM, and increased leptin, resistin and adiponectin release</p>	77
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Chinese hamster	CHO cells, AR-transfected	0, 12 concentrations within the range of 0.025 - 50 µM	<p>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;</p> <p>The principle of concentration addition was applied to predict the effects caused by an equimolar (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</p>	<p>Only Isobutylparaben antagonized the AR; the effect was statistically significant at <math>\geq 25</math> µM;</p> <p>Butylparaben and Propylparaben inhibited the R1881-induced response, but only at cytotoxic concentrations;</p> <p>The mixture was predicted to antagonize the AR at concentrations <math>\geq 2</math> µM</p>	78
Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0-200 µM (stock and working solutions in DMSO)	Cells were incubated for 24 h, with or without DHT (1000 pM) in phenol red-free culture medium at 37°C	Butylparaben, tested individually, had no statistically-significant androgen agonistic activity, but exhibited concentration-dependent anti-androgenic activity at $>10$ µM	175
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 ER $\alpha$ -positive; MCF-7 cells are ER $\alpha$ -positive and HER2-negative; SKBR3 cells are HER2-positive and ER $\alpha$ -negative;	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;	79

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/Dosage (Vehicle)	Procedure	Results	Reference
				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	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 <sub>50</sub> =0.551 µM); the effect was enhanced in the presence of HRG (EC <sub>50</sub> =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	
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 <sub>50</sub> for glucocorticoid-like activity was 1.75 mM for Butylparaben and 13.01 mM for Propylparaben; Butylparaben and Propylparaben tested separately induced glucocorticoid-like activity at 1 µM, but only Butylparaben induced activity (44% higher than control) at 10 nM	80
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, and 25 µM in DMSO (vehicle)	MDA-kb2 cells are stably transformed with the MMTV luciferase neo reporter gene construct, and express high levels of functional endogenous AR and GR, which can both act through the MMTV promoter; cells were cultured and then incubated for 24 h, in the presence or absence of paraben, with and without the AR antagonist flutamide (5 µM), in Leibovitz's L-15 medium supplemented with 10% FBS, with 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C	Butylparaben statistically-significantly enhanced the hydrocortisone-induced GR signal by 85%; Methylparaben, Ethylparaben, and Propylparaben did not; Without hydrocortisone but with flutamide, Ethylparaben, Propylparaben, and Butylparaben increased GR activity by more than 50%, and Methylparaben by more than 20%	81
Butylparaben	Human	T47D-KBluc human breast carcinoma cells (ERα and ERβ positive)	0, 3, 10, 30, 60, and 100 µM in DMSO vehicle	Cells were incubated in phenol red-free Dulbecco's Modified Eagle's F-12 containing 10% charcoal stripped FBS, with and without Butylparaben, in the presence or absence of E2 (20 pM), for 24 h at 37°C	Butylparaben exhibited estrogen agonism at all concentrations tested; maximum effect (24% greater than that of E2) was observed at 10 µM; Butylparaben exhibited estrogen antagonism at all concentrations tested in the presence of 30 pM E2; maximum effects at 10 and 30 µM; calculated IC <sub>50</sub> =59.82 µM	82
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 <sup>-8</sup> 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 <sub>100</sub> , EC <sub>50</sub> , LOEC, and lowest concentration which gave an increase in cell number statistically different (P<0.05) from the LOEC were	After 14 days of exposure, the EC <sub>50</sub> 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 <sub>50</sub> =2 x 10 <sup>-6</sup> µM, LOEC=10 <sup>-6</sup> µM, and 1 x10 <sup>-7</sup> µM;	83

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/Dosage (Vehicle)	Procedure	Results	Reference
				reported	A mixture of all 5 parabens, each at its 7-day NOEC, increased the number of cell doublings above that with any of the parabens tested individually, but lower than with E2	
Propylparaben	Human	MCF-12A and MCF-10A non-transformed, immortalized breast epithelial cells (3D cultures)	10 $\mu$ M 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 (ER $\alpha$ and ER $\beta$ ) and GPER competent; cells were cultured, with or without Propylparaben, for 16 days in Matrigel at 37°C	ER $\alpha$ and ER $\beta$ were expressed at relatively high levels in MCF-12A cells; MCF-10A cells express no measurable levels of ER $\alpha$ and very low levels of ER $\beta$ ; Both cell lines expressed the transmembrane GPER MCF-12A cells formed organized acini, with deposition of basement membrane and hollow lumen; treatment with E2 or Propylparaben resulted in deformed acini and filling of the acinar lumen; the ER-inhibitor (ICI 182,780) and/or GPER-inhibitor (G-15) inhibited the Propylparaben-induced effects on acini	84
Methylparaben	Human Mouse (FVB)	MCF-7 and MDA-MB-231 human breast adenocarcinoma cells; HCl-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 $\mu$ M 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; HCl-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 HCl-7-Luc2 mammospheres; Methylparaben also upregulated NANOG protein expression in MCF-7 mammospheres; none of these effects were seen in MDA-MB-231 mammospheres; Neither tamoxifen nor fulvestrant inhibited effects of Methylparaben on MCF-7 mammospheres	85
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben 4-Hydroxybenzoic Acid	Mouse (strain not specified) Human	Murine 3T3-L1 fibroblasts Differentiated hADSCs	0, 1, 10, 100 $\mu$ M 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, methylisobutylxanthine, and insulin)  For the studies of the antagonists of GR or PPAR $\gamma$ , cells were pretreated with the antagonists of PPAR $\gamma$ (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 PPAR $\gamma$ ; no direct binding to, or modulation of, the ligand binding domain of GR was detected in competitor assays; 50 $\mu$ M Butylparaben or Benzylparaben, in the presence of	86

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/Dosage (Vehicle)	Procedure	Results	Reference
					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 $\mu$ M Butylparaben had the strongest adipogenic effects of the parabens tested, whereas Ethylparaben, Propylparaben, and Benzylparaben had no effect at 1 or 10 $\mu$ M)	
Butylparaben	Mouse (F1 hybrid (C57BL/6j $\times$ CBA/Caj)	Ovaries from immature 13-day-old female mice were used for follicle isolation;	10 nM, 100 nM, 1 $\mu$ M and 10 $\mu$ M (1.9 ng/ml to 1.9 $\mu$ g/ml) in DMSO vehicle	After 24 h of incubation to allow cell attachment, the medium was replaced by fresh equilibrated medium containing different concentrations of Butylparaben, DEHP or a mixture of both; The cells were treated with Butylparaben at different concentrations, for 24, 48, 72, or 96 h; Two control groups (control and DMSO) were included in each experiment which consisted of three independent cultures; Progesterone output was measured using commercial progesterone enzyme immunoassay kit	In follicle culture, DEHP and Butylparaben attenuate estradiol output but only when present together; Butylparaben attenuated DEHP induced reduction of progesterone concentrations in the spent media of hGC cultures; No effects on follicular development or survival were noted in the culture systems; DEHP and Butylparaben adversely affect steroidogenesis from the preantral stage onward and the effects of these chemicals are both stage-dependent and modified by co-exposure	89
	Human	Human granulosa cell (hGC) were isolated from blood cells and follicular fluid				
Butylparaben Isobutylparaben	Human	MCF-7 and T47D human breast cancer cells	10 $\mu$ M in ethanol or DMSO vehicle	MCF-7 and T47D cells were treated at 10 $\mu$ M with Butylparaben, Isobutylparaben, 3-hydroxy n-butyl 4-hydroxybenzoate (3OH), and 2-hydroxy iso-butyl 4-hydroxybenzoate (2OH) for 2, 4, 6, or 18 h; Cell viability was measured by PrestoBlue assay; GREB1 expression was evaluated by Real-time PCR; ERE-luciferase reporter assay was performed to determine whether the estrogenic activity of the paraben metabolites is mediated by classical estrogen receptor mediated signaling; Computational docking studies were conducted to examine the ligand-binding domain interactions between paraben compounds and human ER $\alpha$	The 3OH metabolite induced cellular proliferation with EC <sub>50</sub> of 8.2 $\mu$ M in MCF-7 cells; The EC <sub>50</sub> for 3OH in T47D cells could not be reached; The 2OH metabolite induced proliferation with EC <sub>50</sub> of 2.2 $\mu$ M and 43.0 $\mu$ M in MCF-7 and T47D cells, respectively; The EC <sub>50</sub> for the parental Isobutylparaben and Butylparaben was 0.30 and 1.2 $\mu$ M in MCF-7 cells, respectively; The expression of GREB1 was induced by these compounds and blocked by co-administration of an ER antagonist (ICI 182, 780), confirming the ER-dependence of these effects; The metabolites promoted significant ER dependent transcriptional activity of an ERE-luciferase reporter construct at 10 and 20 $\mu$ M for 2OH and 10 $\mu$ M for 3OH; The expression of GREB1 was significantly induced in MCF-7 cells treated by 10 $\mu$ M Butylparaben, Isobutylparaben, 3OH, and 2OH for 2, 4, and 6h; Molecular docking prediction studies showed that the paraben compounds exhibited the potential for favorable ligand-binding domain interactions with human ER $\alpha$ in a manner similar to known x-ray crystal structures of E2 in complex with ER $\alpha$	88
<b>In Vivo</b>						
Methylparaben	Zebrafish	Embryos, n=10/well	0.1, 1, 10, and 100 ppb in egg water	The collected embryos were segregated for each exposure group in 6-well plates; Exposure groups were maintained in egg water with varying concentrations of Methylparaben, following the guidelines of OECD fish embryo acute toxicity assay; The hatching and heart rate were observed at 48 hpf	Alterations in heart rate and hatching percentage were observed in embryos exposed to 10 ppb and 100 ppb of Methylparaben; Novel tank diving test indicated that anxiety-like behavior is induced in larvae exposed to 0.1 ppb and 1 ppb of Methylparaben;	72

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				using a microscope: percentage of hatched embryos was calculated as number of embryos hatched divided by the number of incubated embryos; heart rate was recorded for 30 sec in the embryos at 48 hpf; Novel tank diving and Light-dark preference test: novel tank diving was then assayed in 6-day old larvae using a trapezoid tank; behavioral parameters observed, including latency to reach upper half of the tank, total number of transitions to the upper half, total time spent in the upper half, total erratic movements and total freezing bouts, and natural preference of zebrafish to light or dark compartment; Cortisol assay: a set of 20 larvae per group were taken at 6 dpf, the samples were homogenized and cortisol was estimated using commercially available ELISA kit.	Methylparaben exposure in zebrafish at sub-lethal concentration inhibited AChE activity and increased cortisol levels	
Methylparaben	Zebrafish	Embryos, n=30 -50 /group	100, 200, 400, 800 and 1000 µM in fish water	Malformations such as coagulation of embryo, lack of somite formation, tail detachment and heart beat were monitored at 24, 48, 72, and 96 hpf; Embryo toxicity assay were carried out in triplicates: in a 24 well plate, 30 embryos were exposed to Methylparaben for 8hpf; Non-lethal malformations like heartbeat, hatching rate, pericardial edema and bent spine were observed under the microscope and vitellogenin I gene expression was analyzed by qRT-PCR	With increasing concentrations of Methylparaben 200 µM, 400 µM and 800 µM, the heart rate decreased to 36, 33 and 22 beats per 20s respectively, while Control larvae showed an average heart rate of 42 beats; A deceleration in the hatching rate was observed with increasing concentration of Methylparaben, with 80% of embryos hatching in 100 µM, 55% in 200 µM, 40% in 400 µM and 10% in 800 µM; Defects including pericardial edema blood cell accumulation and bent spine were observed in all the treated concentration, except at 100 µM; The 96 hpf LC <sub>50</sub> of Methylparaben was calculated to be 428 µM (0.065 mg/L); In larval zebrafish exposed to 100 µM (0.015 mg/L) for 96 hpf, expression of vitellogenin I was significantly upregulated	<sup>73</sup>
<b>ANIMAL</b>						
<i>Oral</i>						
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	<sup>93</sup>

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben	Rat (Sprague-Dawley)	Immature females (PND 20); n=6 - 9/ group (n=17 in one of the control groups)	0, 0.8, 4, and 20 mg/kg bw/day (20 mg/kg bw/day when tested with 10 mg/kg bw/day fulvestrant) in peanut oil, by gavage	Rats were exposed to a paraben for 3 days, beginning on PND 21; rats were then weighed and sacrificed, and uteri dissected and weighed, and relative uterine weights calculated, except for 1 group that was transferred on PND 23 to individual metabolic cages in which only pure water was available, ad libitum, and from which urine was collected for 24 h and analyzed for Methylparaben and Ethylparaben concentrations; Relative expressions of estrogen-responsive genes in the uteri were evaluated by quantitative real-time RT-PCR	LOELs for increased relative uterine weight after treatment with Methylparaben and Ethylparaben were 20 and 4 mg/kg bw/day, respectively; NOELs for Methylparaben and Ethylparaben were 4 and 0.8 mg/kg bw/day, respectively; The uterotrophic effects of 25 µg/kg bw/day E2 or 20 mg/kg bw/day Methylparaben or Ethylparaben were antagonized by 10 mg/kg bw/day fulvestrant; Expression of icabp, itmap1, CaBP-9k, and/or Pgr biomarker genes were elevated in a concentration-dependent manner after treatment with 4 or 20 mg/kg bw/day Methylparaben or Ethylparaben; Mean urinary concentrations of the Methylparaben and Ethylparaben increased in a dose-dependent manner, from 491 to 17,635 ng/mL for Methylparaben and 376 to 11,906 ng/mL for Ethylparaben in rats that received 0.8 to 20 mg/kg/day Methylparaben or Ethylparaben	94
Ethylparaben Propylparaben	Mouse (C57BL/6J)	Ovariectomized females, 8 weeks of age, n=6/group, 11 groups	0, 1000 mg/kg bw/day in corn oil, by gavage	Study was performed in compliance with OECD TG 440 (Uterotrophic Bioassay in Rodents); mice were dosed daily for 7 consecutive days; 6 µg/kg bw/day E2 was given orally as the positive control in the test for agonism, and subcutaneously 15 min after administration of the test compound in the test for antagonism; 24 h after the last treatment, the animals were killed, and uteri were excised and weighed	Ethylparaben and Propylparaben were negative for estrogen agonism and antagonism	95
Butylparaben	Rat (Sprague-Dawley)	3-week old males, n=8	0, 1000 mg/kg, single oral dosage in 5% ethanol/95% corn oil vehicle	Rats were killed 3, 6, or 24 h after administration of Butylparaben; testes were collected for histopathological examination, in situ terminal deoxynucleotidyl transferase-mediated TUNEL assay, and analysis using transmission electron microscopy	Histopathologic examination revealed progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules and reduction and/or disappearance of tubular lumen 3 h after Butylparaben treatment; Sertoli cells and spermatogonia with few spermatocytes remained within the seminiferous tubules were observed at 6 h; thin seminiferous epithelia and wide tubular lumen were found at 24 h; TUNEL assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h, and declined at 24 h, though still substantially greater than in the controls; Apoptotic spermatogenic cells were found in semi-thin sections of the testes to be more frequently in treated rats, compared with controls; Apoptotic cells were rounded-up and sur-rounded by empty space, sometimes appearing to be separate from neighboring cells; transmission electron microscopy revealed condensed chromatin and shrinkage of cytoplasm and nucleus of apoptotic spermatocytes.	71
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	Female rats (8-week old), n=6/group, 8 groups	100 mg/kg/day in the diet	Rats were orally exposed to 100 mg/kg bw/day for 5 weeks; Ovarian follicle development and steroid synthesis were investigated through real-time PCR and histological analyses; A disruptor of ovarian small pre-antral follicle 4-vinylcyclohexene diepoxide (VCD, 40 mg/kg bw/day), was used to induce premature ovarian failure (POF)	Propylparaben and Butylparaben treatment prolonged diestrus phases and shortened the interval of the estrous cycle, whereas Methylparaben treatment did not; No effect on number of primary follicles, and secondary follicles showed a decrease in total number in all treated groups; Propylparaben and Butylparaben decreased mRNA level of	90

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population/Sex	Concentration/Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben	Rat (Sprague-Dawley)	Female rats (n= 3-10/group, 12 groups)	0.105 mg/kg /day, by gavage	Rats were orally exposed across several key developmental stages including perinatal (GD1–GD20, n=10 or PND1–PND21, n=10), prepubertal (PND21–PND42, n=5) and pubertal (PND42–PND63, n=5) windows as well as long-term exposures from birth to lactation (PND1–PND146, n=3)	folliculogenesis-related genes ( <i>Foxl2</i> , <i>Kitl</i> and <i>Amh</i> ); All three Parabens induced an increase in FSH levels in serum, which implied impairment of ovarian function Perinatal Methylparaben exposure decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad; Pubertal Methylparaben exposure elevated the amounts of glandular tissue, visible as a higher degree of branching relative to the total gland area; Long-term Methylparaben treatment from birth to lactation did not result in significant histological changes; In the pubertal window, expression alterations in 993 genes enriched in pathways including cholesterol synthesis and adipogenesis were observed	91
Methylparaben	Gerbils	Male and female adults (3-month old) n=16/group, 4 groups	500 mg/kg/day in 0.2 mL of 1% hydroxyethyl-cellulose, orally	8 control males and 8 control females received daily oral doses of 1% hydroxyethyl-cellulose for 21 days; 24 males and 24 females were randomly distributed in three groups that received daily oral doses of Methylparaben at 500 mg/kg (in 0.2 mL of 1% hydroxyethyl-cellulose) for 3, 7, and 21 days; After treatment, the body, ovary, testis, and prostatic complex (urethral segment, ventral, dorsolateral, and dorsal prostate lobes in males, and urethral segment plus prostatic tissue in females) were weighed;  Various biometrical, morphological, and immunohistochemical analyses were performed	Methylparaben caused morphological changes in gerbil prostates in all experimental groups; Animals displayed similar alterations such as prostate epithelial hyperplasia, increased cell proliferation, and a higher frequency of AR-positive cells; The Skene's paraurethral glands of the female gerbil showed additional changes such as stromal inflammatory infiltration, intraepithelial neoplasia foci, and an increase in AR-positive frequency	92
<b>HUMAN</b>						
<b>Dermal</b>						
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean= 26 years old), n=26	2% (w/w) Butylparaben in cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate	Daily whole-body topical application of 2 mg/cm <sup>2</sup> of the cream formulation without the test substances for 1 week, followed by daily application of cream with test substances for 1 week; concentrations of the following hormones were measured in blood serum (as well as the serum concentrations of Butylparaben): FSH, LH, T, estradiol, inhibin B, TSH, FT4, T3, and T4; Application of cream and blood sampling were done at same time every day at 0, 24, 96 and 120 h	Minor differences in serum inhibin B, LH, E2, T4, FT4, and TSH concentrations were observed during the treatment week, compared with the control week; the differences could not be attributed to the treatment because they were also seen at t=0, when treatment had not yet started	43

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

Table 14. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	US NHANES, 2686 urine samples, male and female participants ≥ 6 years of age	Aggregate exposures (undefined sources)	-Annual survey conducted by CDC between 2005 and 2014; -Three age groups (6-11years, 12-19 years, 20 years and older), total 13,076 subjects: 2005-2006, n= 2448; 2007-2008, n= 2604; 2009-2010, n= 2749; 2011-2012, n= 2489; 2013-2014, n= 2686; -NHANES includes household interviews, standardized physical examinations, and collection of urine specimens for parabens exposure examination via HPLC-MS/MS analysis; -Urine samples were treated to free conjugated paraben in urine, thus representing a total concentration	- The median urine concentration was similar across the two sampling periods of 2011-2012 and 2013-2014 for the three parabens with Methylparaben at much higher concentrations than Propylparaben and Butylparaben; - The median urine concentration of the three parabens was decreased in the 2011-2014 sampling period comparing to the 2005-2010 sampling period; - For the 2013–2014 sampling period, Methylparaben in urine was 48.1 µg/L (95th percentile: 819 µg/L), and Propylparaben in urine was 5.74 µg/L (95th percentile: 224 µg/L); - For Butylparaben, the median concentration in urine was below the limit of detection (0.1 µg/L) for all groups in the 2011–2014 reporting period; - In females, the median concentration of Ethylparaben in the 2013–2014 reporting period was 1.6 µg/L (95th percentile: 145 µg/L) while males were below the limit of detection (95th percentile: 34 µg/L); - The reported median concentration in male urine for Methylparaben (24.4 µg/L) and Propylparaben (1.7 µg/L) was lower than that for females (Methylparaben: 73.9 µg/L; Propylparaben: 13.5 µg/L)	<sup>98</sup>
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	US NHANES, 3529 adults	Aggregate exposures (undefined sources)	- Mouthwash use was estimated from the Oral Health questionnaire; responses were recoded as follows: “Always” (reported use 7 out of the last 7 days); “Sometimes” (reported use 1–6 out of the last 7 days); or “Never” (reported use 0 out of the last 7 days); - Sunscreen use was estimated from the Dermatology questionnaire, with a subset of participants ages 20–59; responses were coded as “Always”; “Sometimes” (reported use Most of the time, Sometimes, or Rarely); and “Never”; - A panel of phthalate metabolites and environmental phenols were measured in urine samples using HPLC-MS/MS and on-line solid phase extraction (SPE) coupled to HPLC-isotope dilution MS/MS; - For phthalate analysis, urine samples first underwent enzymatic deconjugation from glucuronidated forms; - Levels below LOD were replaced with the LOD divided by the square root of 2; - Urinary creatinine concentrations, indicative of urine dilution, were assessed using an enzymatic reaction and measurement with a Hitachi Modular P Chemistry Analyzer	Mouthwash use: -The distribution of use was: “Always” use (n=973, 34.3%); “Sometimes” use (n=654, 23.1%); and “Never” use (n=1209, 42.6%); - Compared to “Never” use, individuals with daily use had significantly elevated urinary concentrations of Methylparaben and Propylparaben (30 and 39%, respectively); - Associations with mouthwash use were generally stronger in men compared to women  Sunscreen use: - The distribution of use was: “Always” use (n=296, 12.1%); “Sometimes” use (n=1051, 42.9%); “Never” use (n=1101, 45.0%); - Compared to “Never” use, individuals who reported “Always” had significantly higher urinary concentrations of Methylparaben, Ethylparaben, and Propylparaben, (92, 102, and 151% higher, respectively); - Associations between exposure biomarkers and sunscreen use were stronger in women compared to men	<sup>99</sup>
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Human	80 pregnant women (age 18 years or older) ) at the Ottawa Hospital, Canada	Aggregate exposures (undefined sources)	- Prior to 20 weeks of pregnancy, 80 women collected all their urine from two 24 h periods on a weekday and/or a weekend day as multiple spot urine samples; a subset of women (n = 31) who provided multiple spot urine samples (n = 542) collected over two 24-h periods;	- Women who used lotions in the past 24 h had significantly higher geometric mean paraben concentrations (80 - 110%) in their urine than women who reported no use in the past 24 h; -Women who used shampoo, conditioner, and cosmetics	<sup>107</sup>

Table 14. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Benzylparaben				<ul style="list-style-type: none"> <li>- Women were instructed to keep the urine cool at all times and samples were delivered to hospital within 36 h;</li> <li>- Breast milk samples were collected at the woman's home 2-3 months after delivery (n =56);</li> <li>- Women recorded the date and time of the sample collection, which breast they collected it from, the time since the last feed from that breast and the name of any creams, lotions, or cleansers used on their breast;</li> <li>- At the same time as the urine collection, women were asked to record their activities, food consumption, and PCP use throughout the day; the PCP content of the diaries were manually categorized into the 16 mutually exclusive categories;</li> <li>- Five parabens were measured in urine and breast milk samples by HPLC-MS/MS analysis</li> </ul>	<ul style="list-style-type: none"> <li>also showed 70.80% higher Butylparaben concentrations in their urine;</li> <li>- There was 100%, 72%, 96%, and 90% detection of Methylparaben, Butylparaben, Propylparaben, and Ethylparaben in urine respectively; Lower detection rates were seen for Isobutylparaben (39%) and Benzylparaben (41%);</li> <li>- All parabens with &gt;70% detection (Methylparaben, Ethylparaben, Butylparaben, and Propylparaben) were significantly and strongly correlated with each other with Spearman correlation coefficients ranging from 0.48 (Methylparaben and Ethylparaben) to 0.86 (Propylparaben and Methylparaben);</li> <li>-Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben;</li> <li>- There was &lt;1% detection for Butylparaben, Benzylparaben and Isobutylparaben</li> <li>- GM concentrations of parabens in urine samples: Methylparaben 30.02 µg/L (95th percentile: 403.26 µg/L), Ethylparaben 2.43 µg/L (95th percentile: 84.16 µg/L), Propylparaben 4.6 µg/L (95th percentile: 111.18 µg/L), Butylparaben 0.15 µg/L (95th percentile: 12.20µg/L), No GM concentrations for Isobutylparaben and Benzylparaben ;</li> <li>- GM concentrations of parabens in breast milk samples: Methylparaben 0.0672 µg/L (95th percentile: 6.792 µg/L), Ethylparaben 0.0023 µg/L (95th percentile: 0.614 µg/L), Propylparaben 0.0277 µg/L (95th percentile: 1.32 µg/L); No GM concentrations for Butylparaben, Isobutylparaben and Benzylparaben</li> </ul>	
Methylparaben Ethyl paraben Propylparaben Butylparaben	Human	100 Latina girls (14-18 years old) living in Salinas, California	Each girl was provided with small (2–4 oz) containers of shampoo, conditioner, body wash, and moisturizing lotion; a bar of hand soap; a container of liquid; and roll-on deodorant	<ul style="list-style-type: none"> <li>- Participants enrolled in the Health and Environmental Research on Makeup of Salinas Adolescents (HERMOSA) Study which was a youth empowerment intervention study examining strategies to reduce PCP chemical exposure to adolescent girls;</li> <li>- Girls participating in the study were provided with low-chemical PCPs and asked to refrain from using their regular products for 3 days;</li> <li>- Each girl was allowed to choose four items from among liquid or powder foundation, mascara, eyeliner, lipstick/lip gloss/lip balm, and sunscreen;</li> <li>- Participants were asked to avoid using any personal care products or cosmetics other than those provided by the study; the replacement personal care products provided to participants were selected to be free of parabens;</li> <li>- Pre- and post-intervention urine samples were analyzed for parabens using HPLC-MS/MS</li> </ul>	<ul style="list-style-type: none"> <li>- Methylparaben and Propylparaben concentrations decreased by 43.9% (95% CI: –61.3, –18.8) and 45.4% (95% CI: –63.7, –17.9, respectively);</li> <li>- The GM concentration of Methylparaben decreased from 77.4 to 43.2 µg/L;</li> <li>- The proportion of girls with detectable concentrations of Methylparaben decreased non significantly from 93% to 87%, and decreases in concentrations were observed in 61% of girls;</li> <li>- The GM concentration of Propylparaben decreased from 22.6 to 12.3 µg/L, with decreases observed in 63% of girls; - The proportion of girls with detectable concentrations of Propylparaben also decreased between pre- and post-intervention (90% vs 87%), but not significantly;</li> <li>- Unexpectedly, Ethylparaben and Butylparaben concentrations both increased over the course of the intervention period, with Butyl paraben increasing by 101.7% (95% CI: 35.5, 203.2) and Ethylparaben increasing by a nonsignificant 47.3% (95% CI: –0.7,</li> </ul>	100

Table 14. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	100 Latina girls (14-18 years old) living in Salinas, California	Aggregate exposures; participants reported using specific makeup, including foundation, blush, and mascara every day	<ul style="list-style-type: none"> <li>- Participants enrolled in the HERMOSA Study;</li> <li>- Evaluated the relationship between recent self-reported PCPs use and concentrations for urinary metabolites of parabens and other endocrine disruptors in 100 Latina adolescents;</li> <li>- The analysis focused on use of a comprehensive list of personal care products, including face products, oral hygiene, soap, nail and hair products, and feminine care products;</li> <li>- Urine samples were subjected to HPLC-MS/MS analysis;</li> <li>- GMs were compared across categories and calculated a P value for trend using one-way ANOVA and linear regression;</li> <li>- Urinary concentrations of Methylparaben and Propylparaben were compared in girls who used products every day, 2-6 times per week, once a week, and rarely/never;</li> <li>- GM urinary concentrations of Methylparaben and Propylparaben metabolites were compared by frequency of use of make-up, fragrance, and moisturizer</li> </ul>	<p>118.4); however, concentrations of both Ethylparaben and Butylparaben were low overall and not detected in almost half the samples;</p> <ul style="list-style-type: none"> <li>- The absolute changes in concentrations were small for both Butylparaben (preintervention GM = 0.8 µg/L vs. postintervention GM = 1.7 µg/L) and Ethyl paraben (preintervention GM = 2.9 µg/L vs. postintervention GM = 4.2 µg/L)</li> </ul>	101
Methylparaben Propylparaben	Human	18 females (21-25 years old) from the Federal University of Alfenas-MG located in Minas Gerais, Brazil	Using lipstick containing parabens for 5 days, lipstick used/day was 0.001 mg/kg/day ± 0.05	<ul style="list-style-type: none"> <li>- In phase 1, the women used paraben-containing products according to their routine</li> <li>- In phase 2, the women used donated lipstick containing Methylparaben and Propylparaben for 5 days in conjunction with the routine use of paraben-containing products</li> <li>- In phase 3, the women routinely used paraben-containing products while abstaining from lipstick for five days, and blood (15mL) was collected for HPLC-MS/MS analysis</li> </ul>	<ul style="list-style-type: none"> <li>- Urinary concentrations of Methylparaben and Propylparaben were detected in over 90% of participants;</li> <li>- Detection frequencies were below 49% for Butylparaben and 55% for Ethylparaben, so these two analytes were not included in final statistical analyses;</li> <li>- Girls who reported using makeup every day vs. rarely/never had higher urinary concentrations of Methylparaben (120.5 ng/ mL vs. 13.4 ng/mL, P &lt; 0.01), and Propylparaben (60.4 ng/mL vs. 2.9 ng/mL, P &lt; 0.01);</li> <li>- Girls who reported recent use of specific makeup products, including foundation, blush, and mascara, had higher urinary concentrations of Methylparaben, and Propylparaben;</li> <li>- Both Methylparaben and Propylparaben urinary concentrations were positively associated with use of foundation (Methylparaben: 52.1%, Propylparaben: 69.3%), blush (Methylparaben: 34.0%, Propylparaben: 44.9%), mascara (Methylparaben: 64.3%, Propylparaben: 76.3%), any eye makeup (Methylparaben: 58.0%, Propylparaben : 84.3%), and any makeup (Methylparaben: 77.9%, Propylparaben:75.7%);</li> <li>- Propylparaben urinary concentrations were negatively associated with lip gloss use (-51.1%);</li> <li>- Concentrations also varied by frequency of fragrance use for Methylparaben (112.1 ng/mL vs. 23.7 ng/mL, P<sub>trend</sub> = 0.04); and by frequency of moisturizer use for Methylparaben (123.8 ng/mL vs. 69.4 ng/mL, P<sub>trend</sub> = 0.01).</li> <li>- Girls who used 20 or more products today and yesterday had higher levels of the Propylparaben compared to girls who used fewer than nine products today or yesterday (33.4 ng/mL vs. 6.1 ng/mL, P<sub>trend</sub> = 0.04).</li> </ul>	102

Table 14. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Propylparaben	Human	Human serum samples from 5 males and 11 females (n=16)	Aggregate exposures (undefined sources)	16 commercially available serum samples collected between 1998 and 2003 were purchased from Tennessee Blood Services in Memphis; To determine the concentrations of the free plus conjugated species of the parabens, the enzyme solution, containing $\beta$ -glucuronidase/sulfatase in ammonium acetate buffer, and radio-labeled standards were added into the serum; Six phenols concentrations in the serum sample, including bisphenol A, benzophenone-3, triclosan, 2,5- dichlorophenol, Methylparaben and Propylparaben, were measured by on-line SPE coupled to HPLC - MS/MS	between serum parabens in women who used lipstick containing Methylparaben and Propylparaben (p = 0.0005 and 0.0016, respectively); - A strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202)	<sup>103</sup>
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	<sup>104</sup>
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 <sup>3</sup> 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	<sup>105</sup>
Methylparaben Propylparaben Butylparaben	Human	Human ovarian tumor samples were obtained from Yong Loo Lin School of Medicine, National University of	Aggregate exposures (undefined sources)	15 ovarian malignant tissues and 15 benign tissues were analyzed; technique involves the simultaneous use of MASE and micro-solid SPE, in tandem with HPLC/UV analysis for the determination of parabens concentration; ovarian tissues were not spiked with parabens; the mass fractions of parabens present in human ovarian tissues were then calculated	-The tissue mass fractions of Methylparaben and Propylparaben were higher than Propylparaben and Butylparaben; -The tissue mass fractions of four parabens in all the ovarian cancer tissues are at least twice as much as those present in the benign tissues; -The method detection limits for parabens ranged from	<sup>106</sup>

Table 14. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben heptylparaben 4-Hydroxybenzoic Acid	Human	Singapore, n=30 Human adipose fat samples collected from Wadsworth Center, New York City, n = 20	Aggregate exposures (undefined sources)	Human adipose fat samples were collected from volunteers who underwent liposuction surgery between 2003 and 2004; tissues were spiked with methanol solution containing isotope labeled internal standards and analyzed by HPLC-MS/MS for the presence of parabens as well as several environmental phenols and aromatic compounds	0.005 to 0.0244 ng/g  -Among the six parabens analyzed, Ethylparaben and Propylparaben were more frequently detected than the other parabens, at a detection frequency of 60% and 50%, and a GM concentration of 0.90 and 0.49 ng/g, respectively; -4-Hydroxybenzoic Acid was detected in almost all samples, at concentrations as high as 17,400 ng/g; -The GM concentration of the sum of six parabens and 4- Hydroxybenzoic Acid ( $C_{\Sigma\text{parabens}}$ ) in adipose fat was 3420 ng/g; - Among the 20 samples analyzed, high $C_{\Sigma\text{parabens}}$ ( $>10^5$ ng/g) were found in 5 females and 2 males, indicating high exposure to parabens by some individuals; -No gender-related difference in $C_{\Sigma\text{parabens}}$ was found, and the age related difference between the two age groups (18–33 yr and 34–58 yr) was equivocal; -Paraben concentrations in adipose fat samples of Caucasian volunteers (GM: 7050 ng/g) were higher than those of African Americans (GM: 3440 ng/g); - The authors stated it should be noted that high concentrations of 4-Hydroxybenzoic acid ( $\log K_{ow} = 1.39$ ) found in adipose samples could be an artifact from the reaction of paraben esters with $\text{NaHCO}_3$ solution used in liposuction procedures (i.e., alkaline hydrolysis), thus further studies are warranted	108
Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben heptylparaben 4-Hydroxybenzoic Acid	Human	Human urine samples collected from 40 children (17 males and 23 females, 3–10 yr ) in Albany, 70 Chinese children (38 males and 32 females, 9–10 yr), and 26 Chinese adults (15 males and 11 females, most of 22–30 yr) in Shanghai and Tianjin, China.	Aggregate exposures (undefined sources)	Urine samples were spiked with methanol solution containing isotope labeled internal standards and analyzed by HPLC-MS/MS for the presence of parabens and their metabolite, 4-Hydroxybenzoic Acid	- Parabens were present predominantly (>90%) as conjugated species in urine; - Among the six parabens analyzed, Methylparaben and Propylparaben were the predominant compounds, which accounted for 57–98% and 1.4–12%, respectively, of the total concentrations; - The median concentration of Methylparaben and Propylparaben in US adults was 43.9 and 9.1 ng/mL, respectively; - The median concentrations of the sum of Six parabens in urine from US children were 54.6 ng/mL; - The GM concentrations of 4-Hydroxybenzoic Acid in urine from US children were 752 ng/mL for girls and 628 ng/mL for boys, which were 2 - 3 times lower than the concentrations determined for Chinese children	109
Methylparaben Ethylparaben Propylparaben Isopropylparaben Butylparaben Isobutylparaben Benzylparaben	Human	Human adipose tissue collected from San Cecilio University hospital and Santa Ana Hospital in Sprain (n=144, 88 males and 56 females)	Aggregate exposures (undefined sources)	114 adipose tissue samples were collected from participants of GraMo cohort study; The participants were recruited between July 2003 and June 2004 among patients undergoing non-cancer-related surgery and at two public hospitals in Southern Spain; Study inclusion criteria were age over 16 years, absence of diagnosed hormone-related disease or cancer and residence in one of the two study areas for $\geq 10$ years;	- Detection frequencies and median concentrations were: Methylparaben (100.0%, 0.40 ng/g tissue), Ethylparaben (20.1%, <LOD), - Propylparaben (54.2%, 0.06 ng/g tissue), Isopropylparaben (0, <LOD), Butylparaben (5.6%, <LOD), Isobutylparaben (2.1%, <LOD) and Benzylparaben (0, <LOD); - Isopropylparaben and Benzylparaben were not detected in any of the samples; - Isobutylparaben concentrations above LOD were	110

Table 14. Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				Adipose tissue samples were intraoperatively collected and stored in aliquots at -80 °C until analysis; Main tissue sources were pelvic waist (46.5%), front abdominal wall (44.4%), and limbs (9.0%); Samples were spiked with isotope labeled internal standard stock solution and subjected to HPLC-MS/MS for the presence of parabens as well as several environmental phenols; Spearman correlation tests were performed, followed by stepwise multivariable linear regression analyses to assess determinants of the exposure	recorded in 8 and 3 of the 144 samples; - Older participants showed higher concentrations of Methylparaben; the author stated that the positive association of Methylparaben with age might be a consequence of a lower metabolic activity in older individuals, which may delay the metabolism and clearance of these chemicals; - Methylparaben, Ethylparaben, Propylparaben and bisphenol-A levels were significantly and positively correlated; - A wide variability in exposure levels was found among participants, with some samples showing 10 to 50-fold higher levels than the median level in the population	
Methylparaben Propylparaben Butylparaben	Human	Human urine samples from US NHANES program, male and female participants ≥ 20 years of age (men, n=1399; women, n=1350)	Aggregate exposures (undefined sources)	-A PBPK model for Methylparaben, Propylparaben, and Butylparaben were developed which were parameterized through a combination of quantitative QSAR for tissue solubility and quantitative IVIVE for hydrolysis in portals of entry including intestine, skin, and liver; -The human paraben PBPK model was then used to estimate the plasma free paraben concentration in adults consistent with 95th percentile urine concentration reported in US NHANES program (2009 - 2010 collection period); - The model assume that 4-Hydroxybenzoic Acid and the conjugated metabolites were exclusively excreted in urine; - The EC10 used in this assessment were generated from two assays, ERLUX (reporter gene) and E-Screen (cell proliferation), which were used to assess estrogenicity of the parabens; - In vitro metabolic parameters (nmol/min/ mg microsomal protein) were converted to an intrinsic clearance (Cl <sub>int</sub> ) expressed in terms of L/h-mg protein; The Cl <sub>int</sub> was then scaled to the whole tissue based on the amount of microsomal protein per gram of tissue; - An in vitro based cumulative MOS was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the free plasma paraben concentrations predicted by the model to be associated with the 95th percentile urine concentrations reported in NHANES (2009–2010 collection period)	- For the 2009 - 2010 sampling period, the estimated plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 70 kg male was 0.73, 0.21 and 0.052 µg/L, respectively; -The estimated plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 60 kg female was 1.19, 0.54 and 0.58 µg/L, respectively; - In vitro estrogenicity assay reported parabens concentration resulting in a 10% change from control (EC10): Methylparaben, 1162-1238 µg/L; Propylparaben, 180-234 µg/L; Butylparaben 96.5-111 µg/L -Based on human paraben PBPK model, the calculated cumulative MOS for adult females was 108, whereas the cumulative MOS for males was 444	57
Methylparaben Propylparaben Butylparaben	Human	400 men (18 - 55 year old) at the Massachusetts General Hospital Fertility Center	Aggregate exposures (undefined sources)	- This was a prospective cohort study, enrolled couples seeking fertility treatment; - At each visit, men completed a questionnaire on PCPs use within the past 24h and at what time they last used each PCP prior to the collection of each urine sample; - PCPs included deodorants, shampoo, conditioner/crème rinse, hairspray/hair gel, combined other hair care products (including mousse, hair bleach, relaxer, perm, and straightener), shaving cream, aftershave, cologne/perfume, mouthwash, bar soap, liquid soap/body wash, hand	- The EARTH study examined the association between PCP use and urinary concentrations of parabens in men; - The largest percent increase for parabens was associated with the use of suntan/sunblock lotion (66–156%) and hand/body lotion (79–147%); - A subset of 10 PCPs that were used within 6 h of urine collection contributed to at least 70% of the weighted score and predicted elevated urinary concentrations of Methylparaben,	111,112

**Table 14.** Biomonitoring

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				sanitizer, hand/body lotion, and suntan/sunblock lotion; - Urine samples were collected at each men's visit. The analytical technique for quantification of the urinary biomarkers involved enzymatic deconjugation of the urinary metabolites, followed by solid phase extraction and HPLC-MS/MS analysis	Propylparaben, and Butylparaben (788%, 1333%, and 254% higher, respectively); - GM concentrations of Methylparaben, Propylparaben, and Butylparaben in urine were 28, 2.86, and 0.26 µg/L, respectively; in comparison, the concentrations of Methylparaben and Propylparaben, in urine reported in US NHANES program (2011 - 2012 collection period) were 23.2 and 2.44 µg/L, respectively (Butylparaben < LOD of 0.1 µg/L); - Self-reported PCP use among men was associated with higher urinary concentrations of three parabens (Methylparaben, Propylparaben, and Butylparaben)	

CDC=Centers for Disease Control and Prevention; EC= Effective concentration; GM= geometric mean; HPLC-MS/MS= High-performance liquid chromatography tandem mass spectrometry; IVIVE=in vitro to in vivo extrapolation; LOD= limit of detection; NHANES= National Health and Nutrition Examination Survey; PBPK= Physiologically based pharmacokinetic; QSAR=quantitative structure-activity relationship; SPE=solid phase extraction; MASE=microwave-assisted solvent extraction

**Table 15 Allergic contact dermatitis studies on paraben mixture (Data collected by ESSCA between 2009 and 2012 from 12 European Countries).<sup>120</sup>**

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

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

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
<i>Prospective Studies</i>						
Methylparaben Propylparaben Butylparaben	245 women who completed $\geq 1$ IVF cycle and provided $\geq 1$ 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	<ul style="list-style-type: none"> <li>- 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</li> <li>- Urinary concentrations of total parabens were measured by HPLC-MS/MS</li> <li>- Clinical information was abstracted from the patient electronic medical records</li> <li>- Serum concentrations of FSH and E2 were measured</li> <li>- Each subject was assigned an infertility diagnosis by a physician</li> <li>- Subjects underwent one of three controlled ovarian stimulation IVF treatment protocols, after completing a cycle of oral contraceptives</li> <li>- Embryologists determined the total number of oocytes retrieved per cycle and classified them</li> <li>- Oocytes underwent either conventional IVF or ICSI, and embryologists determined fertilization rate 17-20 h after insemination</li> <li>- Embryo quality was classified based on morphology and number of blastomeres, ranging from 1 (best) to 5 (worst) on day 2 and 3</li> <li>- In women who underwent an embryo transfer, implantation was assessed and pregnancy was confirmed by ultrasound at 6 weeks</li> <li>- Live birth was defined as birth of a neonate on or after 24 weeks gestation</li> <li>- Exposures were categorized into quartiles of urinary concentrations; the lowest quartile used as the reference group</li> <li>- Associations between urinary concentrations and demographics and baseline reproductive characteristics were evaluated using Kruskal-Wallis and Chi-squared tests</li> <li>- Multivariable generalized linear mixed models were used to evaluate associations between concentrations and IVF outcomes</li> <li>- 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)</li> <li>- 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</li> <li>- Final models were adjusted for age, BMI, race (white vs nonwhite), smoking status (never vs ever), and infertility diagnosis (male factor, female factor, unexplained)</li> </ul> <p><u>Limitations</u></p> <ul style="list-style-type: none"> <li>- Study design may not allow extrapolation of the findings to the general population</li> <li>- Misclassification of paraben exposure based on concentrations from spot urine samples is possible</li> </ul>	<p>Urinary paraben concentrations were not associated with IVF outcomes;</p> <p>Geometric means of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben were 133, 24 and 1.5 <math>\mu\text{g/L}</math>, respectively;</p> <p>The urinary concentrations were not associated with total or mature oocyte counts, proportion of high embryo quality, fertilization rates, implantation rates, clinical pregnancy, or live births</p>	<p>None of the ORs calculated for total oocyte yield, metaphase II oocyte yield, <math>&gt;1</math> best embryo quality, and fertilization rate in the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> quartiles of Methylparaben, Propylparaben, and Butylparaben urinary concentrations were statistically-significantly different from those of the 1<sup>st</sup> quartile, adjusted or unadjusted</p>	121

**Table 16.** Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference																		
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	11,311 pregnant women (19-45 year old) in Wuhan city, China	Subjects recruited from 09/2012 to 10/2014	<ul style="list-style-type: none"> <li>- Concentrations of parabens were measured by UPLC-MS/MS in maternal urine collected before delivery;</li> <li>- Gestational age was calculated based on the date of last menstrual period or assessed by ultrasound data;</li> <li>- General linear models were used to analyze the associations of maternal parabens exposure levels with birth weight and birth length</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Urinary paraben concentrations measured at one spot time may not reflect prenatal paraben exposure levels and thus cause exposure misclassification;</li> <li>- The sum of the isomers (n-Propylparaben vs. Isopropylparaben, n-Butylparaben vs. Isobutylparaben) were measured in this study (they couldn't be separated by the detection method)</li> </ul>	<ul style="list-style-type: none"> <li>- Methylparaben, Ethylparaben and Propylparaben were detected in 98.3%, 70.9% and 96.4% of the urine samples, respectively;</li> <li>- Butylparaben and Benzylparaben were detected in 15.0% and 2.3%, respectively, and thus were excluded from further statistical analyses;</li> <li>- The SG-adjusted GM and medians of Methylparaben, Ethylparaben and Propylparaben were 5.41 ng/mL (4.20 ng/mL), 0.11 ng/mL (0.09 ng/mL), and 0.94 ng/mL (0.71 ng/mL);</li> <li>- For overall infants, no significant associations were found between maternal urinary parabens and length of infants at birth;</li> <li>- Sex stratification analysis indicated a significant association between urinary Methylparaben and birth length in boys;</li> <li>- No significant associations were observed between urinary parabens and birth length in girls;</li> <li>- Boys in the medium and highest Methylparaben tertiles had a 0.30 (95% CI: 0.01, 0.58) cm and 0.30 (95% CI: 0.01, 0.58) cm longer birth length compared to boys in the lowest tertile, respectively;</li> </ul>		122																		
Methylparaben Ethylparaben Propylparaben Butylparaben	922 pregnant women older than 18 years ( 18 $\pm$ 2 weeks gestation) in northern Puerto Rico	2011-2017	<ul style="list-style-type: none"> <li>- Each woman participated in three study visits: visit 1 was targeted at 16–20 weeks gestation; visit 2 at 20–24 weeks gestation; and visit 3 at 24–28 weeks gestation;</li> <li>- Concentrations of parabens were measured by HPLC-MS/MS in urine samples collected during the three study visits;</li> <li>- Individual paraben concentrations were adjusted for SG;</li> <li>- The gestational age for complete pregnancies was calculated according to the American Congress of Gynecologists (ACOG) recommendations;</li> <li>- Birthweight values extracted from medical records were converted to gestational age and sex specific z-scores, calculated according to the INTERGROWTH-21st standards;</li> <li>- Infants were considered SGA if they fell below the 10th percentile of birthweight z-scores, while infants were considered large for gestational age (LGA) if they fell above the 90th percentile of birthweight z-scores;</li> <li>- Multiple linear regression models were conducted to regress gestational age and birth weight z-scores against woman's log average urinary concentrations of parabens;</li> <li>- Logistic regression models were conducted to calculate odds of preterm birth, SGA and LGA</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Data collected at three time points may not be sufficient to understand the effects of the measured biomarkers on gestational age;</li> </ul>	<ul style="list-style-type: none"> <li>- Ethylparaben were detected in less than 50% of the samples;</li> <li>- Average Methylparaben and Propylparaben concentrations were strongly correlated (Spearman correlation=0.78, p &lt;0.001);</li> <li>- Propylparaben was moderately correlated with Butylparaben and Ethylparaben (Spearman correlation=0.42, p &lt;0.001);</li> <li>- A protective effect of parabens on SGA was observed</li> </ul> <p><u>Change in Gestational Age Days per IQR Increase in Paraben Concentrations</u></p> <table> <tr> <td></td> <td><math>\beta</math> Coefficient</td> </tr> <tr> <td>Methylparaben</td> <td><b>1.63(0.37, 2.89)</b></td> </tr> <tr> <td>Ethylparaben</td> <td>-0.11 (-0.44, 0.23)</td> </tr> <tr> <td>Propylparaben</td> <td><b>2.06 (0.63, 3.48)</b></td> </tr> <tr> <td>Butylparaben</td> <td>0.60 (-1.23, 2.42)</td> </tr> </table> <p><u>OR per IQR Increase in Paraben Concentrations</u></p> <table> <tr> <td>Methylparaben</td> <td><b>0.66 (0.47, 0.93)</b></td> </tr> <tr> <td>Ethylparaben</td> <td>1.57 (0.86, 2.89)</td> </tr> <tr> <td>Propylparaben</td> <td><b>0.61 (0.41, 0.91)</b></td> </tr> <tr> <td>Butylparaben</td> <td><b>0.50 (0.28, 0.88)</b></td> </tr> </table>		$\beta$ Coefficient	Methylparaben	<b>1.63(0.37, 2.89)</b>	Ethylparaben	-0.11 (-0.44, 0.23)	Propylparaben	<b>2.06 (0.63, 3.48)</b>	Butylparaben	0.60 (-1.23, 2.42)	Methylparaben	<b>0.66 (0.47, 0.93)</b>	Ethylparaben	1.57 (0.86, 2.89)	Propylparaben	<b>0.61 (0.41, 0.91)</b>	Butylparaben	<b>0.50 (0.28, 0.88)</b>		121
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			<ul style="list-style-type: none"> <li>- The variation of concentrations of the exposure biomarkers over time may introduce potential bias, stemming from random measurement error;</li> <li>- Given the multiple comparisons conducted, there is a possibility of chance findings due to Type I error</li> </ul>																																																
Methylparaben Ethylparaben Propylparaben Butylparaben	346 infants born to 346 mothers (average age of 34.8 year old) and 184 (average age of 35.7 year old) fathers at the Massachusetts General Hospital Fertility Center	2005 – 2016	<ul style="list-style-type: none"> <li>- Urine samples were collected before the index pregnancy in both men and women to estimate mean preconception urinary Butylparaben, Propylparaben, Methylparaben, or Ethylparaben concentrations;</li> <li>- Mean maternal prenatal urinary parabens concentrations were estimated by averaging trimester-specific urine samples;</li> <li>- Birth weight and head circumference were abstracted from delivery records;</li> <li>- The association of natural log-paraben concentrations with birth outcomes were estimated using multivariable linear regression models, adjusting for known confounders, such as paternal and maternal age, BMI, smoking, education, and status of in-vitro fertilization based treatment</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Inherent limitations in measuring exposure in spot urine samples</li> </ul>	<ul style="list-style-type: none"> <li>- None of the maternal preconception parabens concentrations were associated with birth weight;</li> <li>- Maternal preconception Methylparaben concentration was associated with a decreased head circumference of 0.27 cm (95% CI: -0.54, 0), while no associations were observed between other parabens and head circumference;</li> <li>- Prenatal Propylparaben concentration showed a sexually-dimorphic pattern: boys had a 67 g (95% CI: -133, -2) decrease in birth weight compared with only a 2 g (95% CI: -62, 58) decrease among girls</li> </ul>		118																																													
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Males partners ( $\geq 18$ years) of 501 couples from 16 counties in Michigan and Texas, who discontinued contraception for purposes of becoming pregnant, no physician-diagnosed infertility and couple off contraception for $\leq 2$ months	2005- 2009	<ul style="list-style-type: none"> <li>- In-person interviews with male partners ascertained lifestyle and reproductive history followed by measuring BMI and a baseline urine sample collection;</li> <li>- After two days of abstinence, male participants provided a baseline semen sample and a second sample 1 month later;</li> <li>- Labeled internal standards were spiked into all samples; concentrations of free parabens were measured in urine samples by UPLC-ESI-MS/MS; limit of quantification ranged from 0.05 to 5.00 ng/mL;</li> <li>- Sperm concentration was assessed using the IVOS system and the IDENT stain, sperm viability was determined by hypo-osmotic swelling (HOS assay), sperm motility was assessed using the HTM-IVOS computer assisted semen analysis system, and Sperm morphometry was conducted using the IVOS METRIX system;</li> <li>- 35 semen parameters were quantified: sperm concentration, semen volume, total sperm count, straw distance, hypoosmotic swollen average path velocity, straight line velocity, curvilinear velocity, amplitude head displacement, beat cross frequency, straightness, linearity, percent motility; length, area, width, perimeter, elongation factor, and acrosome area of head, strict criteria, traditional normal (%), amorphous (%), round (%), pyriform (%), bicephalic (%), taper (%), megalo head (%), micro head(%), neck and midpiece abnormalities (%), coiled tail (%), other tail abnormalities (%), cytoplasmic droplet (%), immature sperm(n); DNA fragmentation index (%), and high DNA stainability (%);</li> <li>- Significance was assessed using the Chi-Square and Wilcoxon non-parametric tests for categorical and continuous covariates, median and accompanying interquartile ranges (IQRs) of urinary</li> </ul>	<p>Median urinary parabens concentrations among 419 males who both provided urine and semen samples (IQR):</p> <table border="0"> <tr> <td>Methylparaben</td> <td>Urinary con (ng/mL)</td> <td>6.51 (2.16, 26.4)</td> </tr> <tr> <td>Ethylparaben</td> <td></td> <td>0.36 (0.17, 1.24)</td> </tr> <tr> <td>Propylparaben</td> <td></td> <td>1.39 (0.49, 5.52)</td> </tr> <tr> <td>Butylparaben</td> <td></td> <td>0.03 (0.01, 0.17)</td> </tr> <tr> <td>Benzylparaben</td> <td></td> <td>0.02 (0.00, 0.04)</td> </tr> </table> <p>Significant associations between urinary parabens concentrations and semen quality parameters:</p> <table border="0"> <tr> <td colspan="2"><u>Sperm concentration (<math>\times 10^6/mL</math>)</u></td> <td><b><math>\beta</math> Coefficient</b></td> </tr> <tr> <td>Methylparaben</td> <td></td> <td>-1.91 (-8.03, 4.21)</td> </tr> <tr> <td>Ethylparaben</td> <td></td> <td><b>-6.96 (-12.8, -1.08)</b></td> </tr> <tr> <td>Propylparaben</td> <td></td> <td>-2.38 (-8.45, 3.69)</td> </tr> <tr> <td>Butylparaben</td> <td></td> <td><b>-6.89 (-12.9, -0.85)</b></td> </tr> <tr> <td colspan="2"><u>Total count (<math>\times 10^6/mL</math> concentration x volume)</u></td> <td></td> </tr> <tr> <td>Methylparaben</td> <td></td> <td>-14.6 (-35.3, 6.05)</td> </tr> <tr> <td>Ethylparaben</td> <td></td> <td>-18.7 (-38.7, 1.30)</td> </tr> <tr> <td>Propylparaben</td> <td></td> <td>-6.67 (-27.3, 13.9)</td> </tr> <tr> <td>Butylparaben</td> <td></td> <td>-11.1 (-31.7, 9.46)</td> </tr> </table> <p><u>Percent motility (%)</u></p>	Methylparaben	Urinary con (ng/mL)	6.51 (2.16, 26.4)	Ethylparaben		0.36 (0.17, 1.24)	Propylparaben		1.39 (0.49, 5.52)	Butylparaben		0.03 (0.01, 0.17)	Benzylparaben		0.02 (0.00, 0.04)	<u>Sperm concentration (<math>\times 10^6/mL</math>)</u>		<b><math>\beta</math> Coefficient</b>	Methylparaben		-1.91 (-8.03, 4.21)	Ethylparaben		<b>-6.96 (-12.8, -1.08)</b>	Propylparaben		-2.38 (-8.45, 3.69)	Butylparaben		<b>-6.89 (-12.9, -0.85)</b>	<u>Total count (<math>\times 10^6/mL</math> concentration x volume)</u>			Methylparaben		-14.6 (-35.3, 6.05)	Ethylparaben		-18.7 (-38.7, 1.30)	Propylparaben		-6.67 (-27.3, 13.9)	Butylparaben		-11.1 (-31.7, 9.46)		124,125
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**Table 16.** Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
			paraben concentrations were calculated; - Urinary concentrations of parabens were modeled individually for each semen parameter and adjusted based age, urinary creatinine, BMI, and race (non-Hispanic White, non-Hispanic Black, Hispanic, other)	Methylparaben Ethylparaben Propylparaben Butylparaben	-1.56 (-2.87, -0.26) -1.5 (-2.76, -0.24) -1.03 (-2.33, 0.27) -0.95 (-2.25, 0.35)	
			<u>Limitations:</u> - An observational study design: reliance on a single spot urine, uncorrected comparisons, and potential for residual confounding; - Only 339 men provided sufficient semen samples for the quantification of parabens in seminal plasma	Significant associations between seminal plasma parabens concentrations and semen motility parameters:	<u>Percent motility (%)</u> Methylparaben 0.05 (-0.23, 0.34) Ethylparaben <b>0.28 (0.00, 0.56)</b> Propylparaben -0.13 (-0.42, 0.15) Butylparaben 0.15 (-0.18, 0.49) Benzylparaben <b>0.32 (0.04, 0.60)</b>	
				- Inverse associations were observed between urinary concentration increase of Ethylparaben and Butylparaben and sperm count; - Inverse associations were observed between urinary concentration increase of Methylparaben and Ethylparaben and percent motile sperm; - Butylparaben was associated with reductions in most sperm motility parameters: including average path velocity, straight-line velocity, curvilinear velocity, beat cross frequency, percent straightness, and percent linearity; -Hydroxylated paraben metabolites (methyl-protocatechuic acid and ethyl-protocatechuic acid) significantly positively associated with sperm morphology (enhanced semen quality); -Seminal plasma concentrations of Ethylparaben and Benzylparaben were associated with an increased percentage of sperm motility, while urinary concentrations were negatively associated with Ethylparaben		
Methylparaben Propylparaben	936 men of couples seeking infertility treatment at the Massachusetts General Hospital	2000-2017	- Self-reported demographic, nutritional and reproductive characteristics were collected using standardized questionnaires; - Urinary concentrations of parabens was quantified by isotope-dilution MS/MS; - Semen samples were analyzed for volume, sperm concentration, count, motility and morphology following WHO guidelines; -Estimate the differences in semen parameters over time by fitting generalized linear mixed models with random intercepts and adjust for abstinence time; - Adjust for demographic, nutritional and environmental factors	-Decreasing trends were observed for sperm concentration, count, total motility and morphologically normal sperm; - Urinary concentrations of parabens remained stable over the study period; - However, the observed trends in sperm concentration and total count were not substantially affected by including parabens in the model		126

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
			<p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>-It is uncertain whether the outcomes from an infertility clinic population can be generalized to men in the general population and in non-Western countries;</li> <li>- Lack of data on all potential predictors, i.e., demographic, nutritional and environmental factors, in all study participants over the study period, which resulted in deficiency of evaluating potential contributors to the trends in semen quality</li> </ul>			
Methylparaben Ethylparaben Propylparaben Butylparaben	482 pregnant women (130 women delivered preterm <37 weeks gestation and 352 women who delivered after 37 weeks gestation) at the Brigham and Women's Hospital in Boston	2006-2008	<p>- Participants attended four study visits during their pregnancy: visit 1 (4.71–19.1 weeks), visit 2 (14.9–32.1 weeks), visit 3 (22.9–36.3 weeks), and visit 4 (33.1–38.3 weeks);</p> <p>- Demographic and health-related information were collected at the first visit;</p> <p>- Physical examinations were conducted during each visit and both urine and plasma samples were collected;</p> <p>- Parabens were quantified by isotope dilution LC-MS/MS;</p> <p>- Inflammatory biomarkers were measured by ELISA, including pro-inflammatory markers CRP, IL -10 as an anti-inflammatory marker IL -10</p> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Unable to assess causality between exposure and inflammatory markers;</li> <li>- The four cytokines measured represented only a fraction of the cytokine repertoire within the maternal immune system;</li> <li>- The study focused on characterizing single pollutant associations for specific toxicants</li> </ul>	<p>- An interquartile range increase in Ethylparaben (10.4 ng/mL) was associated with a 7.7% decrease in IL -10 (95% CI: -14.1, -0.86);</p> <p>- However, the association between Ethylparaben and IL -10 differed across study visits, becoming positive by visit 4;</p> <p>- A greater inverse association between Butylparaben and IL-1<math>\beta</math> among preterm birth cases compared to controls</p>		127
Methylparaben Propylparaben Butylparaben	338 children (159 boys and 179 girls) in the Center for the Health Assessment of Mothers and Children of Salinas	Pregnant women were recruited in 1999–2000	<p>- Mothers were interviewed at two time points during pregnancy (mean: 14.0 and 26.9 weeks' gestation) and when their children were 9 years old;</p> <p>- Information collected during pregnancy included maternal age, marital status, race/ethnicity, country of birth, years in the USA, educational attainment, household income and the number of people in the household.</p> <p>- Timing of puberty was assessed by clinical Tanner staging: Children were examined every 9 months between 9 and 13 years of age (i.e. at age 9 (n = 312), 9<math>\frac{3}{4}</math> (n = 268), 10<math>\frac{1}{2}</math> (n = 300), 11<math>\frac{1}{4}</math> (n = 275), 12 (n = 301) and 12<math>\frac{3}{4}</math> (n = 264);</p> <p>- Spot urine samples were collected from mothers at the time of the two pregnancy interviews (prenatal samples) and from the children at the 9-year-old visit (peripubertal samples);</p> <p>- Urinary concentrations of three parabens were quantified by isotope dilution LC-MS/MS;</p> <p>- LOD was 1.0 ng/mL for Methylparaben, 0.2 ng/mL for Propylparaben and Butylparaben;</p> <p>- For prenatal exposure, average of the creatinine-corrected concentrations were used in the two pregnancy urine samples, while for peripubertal exposure, single creatinine-corrected concentration was quantified in children's urine;</p>	<p>- With peripubertal exposure in girls at age 9, associations of earlier thelarche (mean shift = -1.1 months, 95% CI: -2.1, -0.0), pubarche (mean shift = -1.5 months, 95% CI: -2.5, -0.4), and menarche (mean shift = -0.9, 95% CI: -1.6, -0.1) were observed with each doubling of urinary concentrations of Methylparaben, and earlier pubarche (mean shift = -0.8, 95% CI: -1.6, -0.1) with each doubling of Propyl paraben concentrations;</p> <p>- In boys, no prenatal parabens were associated with pubertal timing; with peripubertal concentrations, an association of earlier gonadarche with each doubling of Propylparaben (mean shift = -1.0 months, 95% CI: -1.8, -0.1) was observed;</p> <p>- Butylparaben was detected in &lt;40% of samples and was not included in the analyses;</p> <p>- In prenatal urine samples collected in pregnancy, the GM concentrations of Methylparaben and Propylparaben were 36.4, and 34.5 ng/g creatinine, respectively;</p> <p>- In peripubertal urine samples collected at 9 years of age, the GM concentrations of Methylparaben and</p>		128

**Table 16.** Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
			<p>- Parametric accelerated failure time (AFT) models were conducted to determine the association of urinary biomarker concentrations with timing of pubertal onset using the Stata intcens program</p> <p><u>Limitations:</u></p> <p>- Given parabens are quickly metabolized, urinary parabens typically reflect exposure in the past 24–48 h and can not accurately reflect usual exposure;</p> <p>- One or two urinary measurements are not sufficient to characterize usual parabens exposure over the prenatal and peripubertal periods;</p> <p>- Because the study participants lives in an agricultural community, potential confounding factors exist, such as pesticides exposure;</p> <p>- The study population was limited to Latino children of low socioeconomic status and may not be widely generalizable;</p> <p>- Unable to assess causality between peripubertal measurements and parabens exposure: children going through puberty early are more likely to use PCPs</p>	<p>Propylparaben were 44.9, 4.9 ng/g creatinine, respectively</p>		
<b>Retrospective Studies</b>						
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	<p>- Random “spot” urine specimens were provided once per participant during last 4 months of pregnancy;</p> <p>- Convenience subset of the subjects were followed to delivery, when umbilical cord blood was collected;</p> <p>- Maternal urinary concentrations were measured;</p> <p>- Random subset of umbilical-blood plasma samples were analyzed for free and total parabens;</p> <p>- Questionnaire was used to gather demographic;</p> <p>- Neonate outcome data were from patient charts;</p> <p>- Urinary biomarker concentrations were corrected for creatinine levels and were log-transformed;</p> <p>- Non-detect values were treated as the MDL divided by the square root of 2;</p> <p>- Covariates were selected if they achieved <math>p &lt; 0.05</math> in Spearman correlations or Chi-square tests in relation to biomarker concentrations or birth outcomes;</p> <p>- Measures of birth outcomes (body length, gestational age at birth, birth weight, and head circumference) were analyzed using linear models;</p> <p>- 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 <math>\geq 5\%</math>;</p> <p>- Relationships between concentrations and dichotomous outcomes were analyzed by logistic regression</p>	<p>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 (<math>p &lt; 0.05</math>). No associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated</p>		129
				<p><u>Low Birth Weight and Maternal Urine Concentrations</u></p>	<p style="text-align: center;"><b>OR</b></p> <p>Methylparaben 0.83 (0.37-1.87)</p> <p>Ethylparaben 1.18 (0.74-1.89)</p> <p>Propylparaben 0.92 (0.44-1.94)</p> <p>Butylparaben 1.45 (0.88-2.39)</p> <p>Benzylparaben NA</p>	
				<p><u>Low Birth Weight and Cord Blood Concentrations</u></p>	<p>Methylparaben NA</p> <p>Ethylparaben 1.89 (0.62-5.81)</p> <p>Propylparaben 1.52 (0.66-3.45)</p> <p>Butylparaben 10.27 (0.68-156.07)</p> <p>Benzylparaben 0.18 (0.01-2.63)</p>	
				<p>Preterm Birth and Maternal Urine Concentrations</p>	<p>Methylparaben 0.78 (0.40-1.54)</p> <p>Ethylparaben 1.15 (0.78-1.69)</p>	
			<p><u>Limitations:</u></p> <p>- Maternal urine was used as a proxy for fetal exposure, except</p>			



Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
			<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.	$\beta$ coefficients calculated for Ethylparaben and Butylparaben, body weights estimated at the 3 <sup>rd</sup> 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		
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 1 month 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 $\beta$ 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	Methylparaben <0.4 ng/g 0.44-1.91 ng/g 1.96-11.69 ng/g Concentration as continuous variable  Ethylparaben <LOD 0.07-0.89 ng/g 0.91-5.49 ng/g Concentration as continuous variable  Propylparaben <LOD 0.06-1.15 ng/g 1.16-5.52 ng/g Concentration as continuous variable  Butylparaben <0.08 ng/g 0.16-0.74 ng/g 0.79-1.60 ng/g Concentration as continuous variable  Methylparaben <0.4 ng/g 0.44-1.91 ng/g 1.96-11.69 ng/g Concentration as continuous variable  Ethylparaben <LOD 0.07-0.89 ng/g 0.91-5.49 ng/g Concentration as continuous variable  Propylparaben <LOD 0.06-1.15 ng/g 1.16-5.52 ng/g Concentration as continuous variable	<b>OR (unadjusted)</b> 1.00 1.00 (0.32-3.09) <b>3.18 (0.88-11.48)</b> 1.17 (0.94-1.46)  1.00 0.29 (0.08=1.06) 1.51 (0.44-5.15) 1.07 (0.74-1.55)  1.00 1.23 (0.30-5.04) <b>4.72 (1.08-20.65)</b> <b>1.90 (1.12-3.22)</b>  <b>OR (adjusted)</b> 1.00 2.29 (0.65-8.05) 2.31 (0.72-7.46) 2.27 (0.8-6.42) OR (adjusted)  1.00 1.04 (0.33-3.26) 3.24 (0.83-12.69) 1.17 (0.93-1.48)  1.00 0.26 (0.07-1.00) 1.25 (0.34-4.60) 1.00 (0.68-1.47)  1.00 1.39 (0.33-5.91) 6.42 (1.16-35.47) 2.16 (1.16-4.01)	131
			<u>Limitations:</u> - Relatively small sample size prevented adjustment for some potential confounders, such as the type of delivery, fetal; presentation, weeks of gestation, child length, head size, presence of other malformations and season of birth; - Exposure assessment made in term placentas may have resulted in exposure misclassification; - Cryptorchidism and hypospadias grouped together for statistical analysis discounts the fact that these conditions are related to inset mechanisms occurring at different critical stages in gestation			

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
				Butylparaben <0.08 ng/g 0.16-0.74 ng/g 0.79-1.60 ng/g Concentration as continuous variable	1.00 2.26 (0.62-8.21) 2.11 (0.62-7.16) 2.07 (0.71-6.06)	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	436 3-year old children recruited from Sheyang Maternal and Child Health Care Centre (China)	Subjects recruited between 7/2012 and 4/2013	<ul style="list-style-type: none"> <li>- Questionnaire survey was administered to each child's caregiver by trained interviewers, covering sociodemographics, living environment and lifestyles;</li> <li>- Pregnancy and maternal health information was obtained from medical records and questionnaires;</li> <li>- Spot urine sample was collected from each child, and urinary paraben concentrations were measured by LVI-GC-MS/MS;</li> <li>- EDI<sub>urine</sub> of parabens was calculated based on urinary concentrations and a steady-state toxicokinetic model;</li> <li>- Anthropometry measurements were compared with sex-specific WHO child growth standards, and age- and sex-standardized z scores were calculated;</li> <li>- Generalized linear models were used to examine associations between SG-adjusted concentrations and body growth outcomes;</li> <li>- Individual paraben concentrations and the P<sub>parabens</sub> were adjusted for SG;</li> <li>- Analyses of quartiles of P<sub>parabens</sub> were conducted separately</li> <li>- Urinary concentrations were log transformed for univariate and multivariate analyses;</li> <li>- Associations between concentrations and sociodemographic characteristics were examined using a Wilcoxon rank-sum or Kruskal-Wallis rank sum test;</li> <li>- Log-transformed concentrations were assessed using Pearson correlation coefficients;</li> <li>- Concentrations below LOD were substituted with LOD divided by the square root of two;</li> <li>- 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;</li> <li>- Potential confounders that were separately include: urinary bisphenol A, triclosan, and benzophenone-3 concentrations</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Spot urine samples may cause exposure misclassification;</li> <li>- Specific diet information was not sufficiently obtained and evaluated</li> </ul>	<p><u>Weight z Score (Boys)</u></p> <p>Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <math>\Sigma</math>Parabens</p> <p><u>Height z Score (Boys)</u></p> <p>Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <math>\Sigma</math>Parabens</p> <p>All <math>\beta</math> coefficients calculated for girls and all other <math>\beta</math> coefficients for boys were not statistically significant</p>	<p><u><math>\beta</math> Coefficient</u></p> <p>0.08 (-0.06-0.23) <b>0.16 (0.03-0.28)</b> 0.00 (-0.16-0.17) 0.12 (-0.09-0.32) -0.04 (-0.18-0.10) 0.17 (-0.04-0.39)</p> <p>0.11 (-0.02-0.26) <b>0.15 (0.03-0.27)</b> 0.05 (-0.11-0.21) 0.14 (-0.06-0.34) 0.08 (-0.06-0.21) <b>0.23 (0.03-0.43)</b></p>	132
Methylparaben Propylparaben Butylparaben	Female participants of a prospective fertility study at the MGH Fertility Center, undergoing	2004-2010	<ul style="list-style-type: none"> <li>- Subjects had at least one hormonal or ultrasonographic marker of ovarian reserve measured and contributed at least one urine sample;</li> <li>- Clinical information was abstracted from medical records;</li> <li>- Intravenous blood sample was drawn on the 3<sup>rd</sup> day of the;</li> </ul>	<p><u>Methylparaben</u></p> <p>Tertile 1 (5.13-132 <math>\mu</math>g/L) Tertile 2 (145-377 <math>\mu</math>g/L) Tertile 3 (381-2,428 <math>\mu</math>g/L)</p>	<p><u>MPC in AFC</u></p> <p>0 (Reference) -6.8 (-23.5-13.7) -10.6 (-28.2-11.2)</p>	133

**Table 16.** Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference					
infertility evaluation, n=109 to 142, depending parameter measured			menstrual cycle, and the serum was analyzed for FSH - AFC and OV were measured for both ovaries using transvaginal ultrasound; - Each patient was given an infertility exam and diagnosis by a physician at the MGH Fertility Center; - Demographic data were collected using a nurse-administered questionnaire at entry into the study; - Convenience spot urine sample was collected at recruitment and at subsequent visits during infertility treatment cycles; - Paraben concentrations were measured by HPLC-MS/MS; - Distribution of exposures was summarized using the median, IQR, and range of urinary paraben concentrations; - Urinary concentrations below LOD were assigned a value equal to the LOD divided by the square root of two; - Concentrations were corrected for SG; - Spearman's rank correlation coefficients ( $r_s$ ) were calculated for markers of ovarian reserve, age, and BMI; - Multivariable linear regression was used to estimate associations between within-person paraben concentrations (divided into tertiles) and day-3 FSH and OV; OV was ln-transformed before all regression analyses; - Poisson regression was used to estimate associations between within-person paraben concentrations (tertiles) and AFC; - Covariates considered included age at time of outcome and BMI determinations at study entry into the study; - MPC in outcome from the lowest tertile of paraben concentrations was calculated for both OV and AFC; - Secondary analysis combined concentrations of parabens using two methods: an EEQ factor approach, and summation of concentrations; - Multivariable linear regression was used to evaluate association between EEQ (parabens) and $\Sigma$ (parabens) with day-3 FSH and OV;	$p_{trend}=0.31$	0 (Reference) -5.0(-23.7-18.4) -16.3 (-30.8-1.3)						
				<u>Propylparaben</u>			Tertile 1 (<LOD-25.2 $\mu\text{g/L}$ ) Tertile 2 (26.3-81.8 $\mu\text{g/L}$ ) Tertile 3 (87.8-727 $\mu\text{g/L}$ )				
				$p_{trend}=0.07$			<u>Butylparaben</u>	Tertile 1 (<LOD-0.73 $\mu\text{g/L}$ ) Tertile 2 (0.75-5.12 $\mu\text{g/L}$ ) Tertile 3 (5.44-177 $\mu\text{g/L}$ )			
				$p_{trend}=0.86$	All MPCs and $p_{trens}$ calculated for AFC and OV were not statistically significant						
				<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 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		2000-2004	- A single spot urine sample was collected on day of each subject's clinic visit; 2 <sup>nd</sup> and 3 <sup>rd</sup> 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;	<u>Comet Tail %</u>	<u><math>\beta</math> Coefficient (adjusted)</u>	134
									Butylparaben	0 6.81 (-1.80-15.4) 8.23 (-0.41-16.9)	
									<0.2 $\mu\text{g/L}$		
									0.2-0.6 $\mu\text{g/L}$		
									>0.6 $\mu\text{g/L}$		

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
	Laboratory, Massachusetts General Hospital (MGH)		<ul style="list-style-type: none"> <li>- Serum testosterone, E2, sex-hormone-binding globulin, inhibin B, FSH, LH, prolactin, free thyroxine (T4), total triiodothyronine (T3), and TSH were measured;</li> <li>- Free androgen index (FAI), testosterone:LH ratio, FSH:inhibin B and E2:testosterone ratios were calculated;</li> <li>- Semen quality parameters and motion characteristics were measured: sperm concentration, motility, and motion parameters;</li> <li>- Total sperm count was calculated and sperm morphology was assessed;</li> <li>- Sperm damage was assessed by comet assay: comet extent, tail distributed moment (TDM), and percent DNA located in the tail (Tail%) were determined;</li> <li>- Multivariable linear regression was used to explore relationships between urinary paraben concentrations and hormone levels, semen quality parameters, and sperm DNA damage measures;</li> <li>- Distribution of sperm count, sperm concentration, FSH, LH, SHBG, prolactin, TSH, all calculated hormone ratios, and paraben concentrations were ln-transformed for statistical analyses;</li> <li>- Paraben concentrations &lt; LOD were assigned values of LOD/2</li> <li>- Inclusion of covariates in the multivariable models was based on statistical and biologic considerations;</li> <li>- Age and BMI were modeled as continuous variables; abstinence period was treated as an ordinal categorical variable;</li> <li>- Race, smoking status, and timing of the clinic visit by season and time of day were considered for inclusion as dichotomous variables;</li> <li>- Covariates with <math>p &lt; 0.2</math> in their relationship with one or more paraben or <math>\geq 1</math> outcome measure in preliminary bivariate analyses were included in a "full" model;</li> <li>- Covariates with <math>p &gt; 0.15</math> 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</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Urine samples were collected weeks or months after, rather than before, serum and semen samples were collected;</li> <li>- Only a single blood or semen sample was available for assessment of hormone levels, semen quality, and sperm DNA damage;</li> <li>- Cross-sectional design restricts the ability to draw conclusions about causal relationships;</li> <li>- Relatively small sample size provided low statistical power</li> </ul>	<p><math>P_{\text{trend}}=0.03</math></p> <hr/> <p>No other comparisons were statistically significant in this study</p>		

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference	
<i>Cross-sectional Studies</i>							
Methylparaben Ethylparaben Propylparaben Butylparaben, Isobutylparaben	315 men who attended the infertility clinic for diagnostic purposes in Lodz, Poland	2008-2011	<ul style="list-style-type: none"> <li>- Semen samples were analyzed for sperm concentration, motility, and motion parameters using a computer-aided semen analysis (Hamilton-Thorne Version 10HTM-IVOS);</li> <li>- Three principal parameters for the vigor and pattern of sperm motion were examined: straight-line velocity, curvilinear velocity, and linearity;</li> <li>- Sperm morphology was quantified using strict Kruger criteria to classify men as having normal or below normal morphology;</li> <li>- Sperm chromatin structure assay was performed using flow cytometry to assess sperm DNA damage;</li> <li>- Levels of follicle-stimulating hormone, testosterone, and estradiol were determined in human plasma using a Chemiluminescent Microparticle Immunoassay</li> </ul> <p>Limitations:</p> <ul style="list-style-type: none"> <li>- A single urine sample was used to assess parabens exposure, to describe the level of reproductive hormones, and to assess semen quality;</li> <li>- Temporal reliability was less for concentrations of urinary metabolites of parabens than for phthalate;</li> <li>- As conducted among men recruited through an infertility clinic, the study is limited to generalize the results to the general population;</li> <li>- As a large number of analyses were performed, some of the observations could be chance findings due to multiple testing</li> </ul>	<ul style="list-style-type: none"> <li>- The statistically significant associations were found between urinary parabens concentrations and an increase the percentage of sperm with abnormal morphology and percentage of sperm with high DNA stainability;</li> <li>- Neither categories of urinary concentrations of parabens nor continuous concentrations of parabens were associated with the level of reproductive hormones;</li> <li>- Urinary concentrations of Methylparaben and Propylparaben were not related to any of the examined semen quality parameters, sperm DNA damage, or the level of reproductive hormones</li> </ul>		135	
				<u>Percentile of Exposure</u>	<u><math>\beta</math> Coefficient (adjusted)</u>	<u>P</u>	
				Ethylparaben			
				Morphology	$\leq 25^{\text{th}}$ $>75^{\text{th}}$	Reference 1.97 (0.05-12.16)	0.048
				Butylparaben			
				Morphology	$\leq 25^{\text{th}}$ $>75^{\text{th}}$	Reference 9.51 (0.80-18.21)	0.03
				Isobutylparaben			
				High DNA stainability	$\leq 25^{\text{th}}$ $>75^{\text{th}}$	Reference 3.52 (1.02-16.03)	0.03
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	27 healthy pregnant women aged $33 \pm 4.1$ years in Czech Republic	Subjects recruited between 10/2016 and 01/2017	<ul style="list-style-type: none"> <li>- 5 parabens and 15 steroids including estrogens, corticoids, androgens and immunomodulatory ones in maternal and cord plasma were measured by liquid chromatography - tandem mass spectrometry methods;</li> <li>- Samples of venous blood from the mothers were taken from the cubital vein during the 37<sup>th</sup> week of pregnancy, and at birth, a sample of mixed cord blood was taken</li> </ul> <p>Limitations:</p> <ul style="list-style-type: none"> <li>- Sample size is small</li> </ul>	<ul style="list-style-type: none"> <li>- Multiple regression models showed that in cord blood, Methylparaben (<math>\beta=-0.027</math>, <math>p=0.027</math>), Propylparaben (<math>\beta=-0.025</math>, <math>p=0.03</math>) and the sum of all measured parabens (<math>\beta=-0.037</math>, <math>p=0.015</math>) were inversely associated with T levels;</li> <li>- No influence of parabens on estrogen levels were observed</li> </ul>		136	

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	215 healthy unselected young university students (18–23 years old) in Southern Spain (Murcia Region).	2010-2011	<ul style="list-style-type: none"> <li>- All men provided a urine, blood and semen sample on a single day;</li> <li>- Urinary paraben concentrations were measured by DLLME and UHPLC-MS/MS;</li> <li>- Semen quality was evaluated by measuring volume, sperm concentration, total sperm count, motility and morphology following WHO guidelines;</li> <li>- Serum samples were analyzed for reproductive hormones, including follicle-stimulating hormone, luteinizing hormone, testosterone, inhibin B and estradiol using immunoassays;</li> <li>- Associations between urinary concentrations of parabens and semen quality parameters and reproductive hormone levels were examined using linear regression, adjusting for potential covariates</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- As with all observational studies, causal inference is limited. Residual confounding should always be considered and low statistical power might have played a role in the null findings;</li> <li>- Both urinary parabens and our outcomes were based on a single blood serum, urine or semen sample;</li> <li>- Exposure measurement error or misclassification cannot be ruled out</li> </ul>	<ul style="list-style-type: none"> <li>- Taking into account important covariates, urinary concentrations of parabens or their molar sum were not significantly associated with any semen parameters or any of the reproductive hormone levels;</li> <li>- 94% of the men had detectable urinary concentrations of parabens</li> </ul>	Relative to men in the lowest quartile of sum of urinary paraben concentrations, the adjusted difference (95% CI) of sperm count for men in the 2nd, 3rd, and 4th quartiles were 4.1% (-37.1-45.3), -1.6% (-41.9-38.8), and -9.8% (-52.5-32.8), respectively (P-trend = 0.55)	137
Methylparaben Ethylparaben Propylparaben Butylparaben	42 men (36.8 ± 5.4 years old) of couples who visited a gynecology clinic in Tokyo for infertility consultation	2010	<ul style="list-style-type: none"> <li>- Urinary parabens analysis was carried out by HPLC MS/MS;</li> <li>- LODs were 0.24, 0.021, 0.065 and 0.0090 ng/mL for Methylparaben, Ethylparaben, Propylparaben and Butylparaben, respectively;</li> <li>- Recoveries of the internal standards were 34–44% for the 4 parabens;</li> <li>- Specific gravity (SG)- and creatinine-adjusted urinary concentrations of parabens were measured;</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Sample size was small (<math>n = 42</math>);</li> <li>- The subjects of this study included people had normal semen quality and those who did not, therefore the association between exposure and effects might be obscured;</li> <li>- The level of parabens exposure was assessed by the parabens concentrations in a single spot urine, not representing long-term exposure level</li> </ul>	<ul style="list-style-type: none"> <li>- The relative contribution of Methylparaben, Ethylparaben, Propylparaben and Butylparaben to estrogen-equivalent total paraben (ETP, sum of the individual concentrations of the 4 parabens) was 12, 12, 38 and 38%, respectively;</li> <li>- Average semen volume, sperm concentration and sperm motility of the present subjects were similar to the levels of fertile Japanese men;</li> <li>- Significantly positive relationship between semen volume and urinary Ethylparaben was observed;</li> <li>- No significant association was found between semen parameters (semen volume, sperm concentration and motility) and urinary paraben concentrations in multiple regression analyses and logistic regression analyses</li> </ul>		138

**Table 16.** Epidemiological studies of parabens

<b>Ingredient(s)</b>	<b>Population/ Geographical Area</b>	<b>Study/ Diagnosis Years</b>	<b>Methods and Limitations</b>	<b>Findings</b>	<b>OR, <math>\beta</math>, or MPC (95% C.I.)*</b>	<b>Reference</b>
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 subsample of US NHANES participants  n=185 adolescent males (ages 12 to 19) males, 171 adolescent females, 785 adult (ages $\geq 20$ ) males, and 708 adult females	2007-2008	<ul style="list-style-type: none"> <li>- Stratified multistage probability sample of civilian US population was surveyed via household interviews, physical exams, and collection of medical histories and biologic specimens;</li> <li>- Urinary parabens concentrations were measured;</li> <li>- Spot urine samples were analyzed by HPLC-MS/MS;</li> <li>- LOD values were estimated as 3 x standard deviation as concentrations approached zero;</li> <li>- Serum thyroid measures included free and total T3 and T4, thyroglobulin, and TSH (or thyrotropin);</li> <li>- Potential confounders considered: age, sex, BMI, urinary creatinine levels, race/ethnicity, poverty income ratio, education, serum cotinine levels and alcohol intake;</li> <li>- 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;</li> <li>- 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;</li> <li>- 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%;</li> <li>- Final regression models included age, sex, BMI, and urinary creatinine;</li> <li>- Concentrations of urinary parabens below the LOD were replaced with values equal to the LOD divided by the square root of two;</li> <li>- Parabens were analyzed on a creatinine-adjusted basis for univariate and bivariate analyses; unadjusted urinary concentrations were used in regression models with urinary creatinine included as a covariate;</li> <li>- 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)</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>- Causality cannot be established because NHANES is an observational, cross-sectional study;</li> <li>- Exposures were evaluated based on spot urine measurements;</li> <li>- 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</li> </ul>	<p><u>Adults, Total T4 (<math>\mu\text{g/dL}</math>)</u></p> <p>Methylparaben Ethylparaben Propylparaben Butylparaben</p> <p><u>Adult Females, ln-Free T3 (pg/mL)</u></p> <p>Methylparaben Ethylparaben Propylparaben Butylparaben</p> <p><u>Adult Females, ln-Free T4 (ng/mL)</u></p> <p>Methylparaben Ethylparaben Propylparaben Butylparaben</p> <p><u>Adult Females, T4 (<math>\mu\text{g/dL}</math>)</u></p> <p>Methylparaben Ethylparaben Propylparaben Butylparaben</p> <p>All other <math>\beta</math> coefficients calculated were not statistically significant</p>	<p><math>\beta</math> Coefficient</p> <p>-0.04 (-0.12-0.03) <b>-0.5 (-0.10 - -0.002)</b> -0.19 (-0.46-0.07) <b>-0.20 (-0.36 - -0.03)</b></p> <p>0.005 (-0.01-0.000) <b>-0.006 (-0.001- -0.0001)</b> <b>-0.02 (-0.04- -0.002)</b> <b>-0.02 (-0.03- -0.002)</b></p> <p><b>-0.01 (-0.03- -0.000)</b> <b>-0.01 (-0.02- -0.003)</b> -0.02 (-0.05-0.01) <b>-0.04 (-0.07- -0.004)</b></p> <p>-0.09 (-0.26-0.08) -0.08 (-0.20-0.05) -0.30 (-0.65-0.06) <b>-0.36 (-0.57- -0.16)</b></p>	139

Table 16. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 sub-sample of the US NHANES participants $\geq 6$ years of age, n=860 (450 males, 410 females)	2005-2006	<ul style="list-style-type: none"> <li>- Sociodemographic data, urinary paraben levels, total and specific IgE levels, respiratory disease and medical condition questionnaire data were included in the dataset;</li> <li>- Urinary parabens levels were collected;</li> <li>- 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?</li> <li>- Atopic asthma was defined as having doctor-diagnosed asthma in addition to at least 1 positive aeroallergen-specific IgE level;</li> <li>- Nonatopic asthma was defined as having doctor-diagnosed asthma with negative specific IgE test results;</li> <li>- 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;</li> <li>- Nonatopic wheeze was defined as having a history of wheezing in the past 12 months with negative specific IgE test results;</li> <li>- Parabens were measured in urine samples by HPLC-MS/MS;</li> <li>- 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, <i>Alternaria</i> species, and <i>Aspergillus</i> species;</li> <li>- Food-specific IgE levels measured were for milk, egg, peanut, and shrimp;</li> <li>- Subjects were considered to have aeroallergen or food sensitization if the specific IgE level was <math>\geq 0.35</math> kU/L;</li> <li>- 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);</li> <li>- Linear regression was used to determine whether mean urinary concentrations varied by race/ethnicity;</li> <li>- 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;</li> <li>- Test for trend was performed by using the variable for tertiles of the paraben concentrations;</li> <li>- Multivariate models were adjusted for age, sex, race/ethnicity, urinary creatinine level, and PIR</li> </ul> <p><u>Limitations:</u></p> <ul style="list-style-type: none"> <li>-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);</li> <li>- Use of allergen sensitization as an outcome was limited by lack of clinical correlation of allergic disease;</li> <li>- Urinary paraben levels were used as biomarkers of exposure, which might not reflect actual exposure</li> </ul>	<p><u>Aeroallergen and Food Sensitization (males and females)</u></p> <p>Methylparaben</p> <p>Tertile 1 Tertile 2 Tertile 3 <math>P_{\text{trend}}=0.4</math></p> <p>Propylparaben</p> <p>Tertile 1 Tertile 2 Tertile 3 <math>P_{\text{trend}}=0.04</math></p> <p>Propylparaben</p> <p>Tertile 1 Tertile 2 Tertile 3 <math>P_{\text{trend}}=0.2</math></p> <p>Butylparaben</p> <p>Tertile 1 Tertile 2 <math>P_{\text{trend}}=0.9</math></p> <p><u>Nonatopic Asthma (males and females)</u></p> <p>Methylparaben</p> <p>Tertile 1 Tertile 2 Tertile 3 <math>P_{\text{trend}}=0.04</math></p> <p><u>Nonatopic Wheeze (males and females)</u></p> <p>Tertile 1 Tertile 2 Tertile 3 <math>P_{\text{trend}}=0.47</math></p> <p>In addition, the OR and <math>p_{\text{trend}}</math> calculated for Propylparaben concentrations and aeroallergen and food sensitization in males were statistically significant</p> <p>The ORs and <math>p_{\text{trend}}</math>s calculated for all other comparisons were not statistically significant</p>	<p><b>OR (unadjusted)</b></p> <p>1.0 (Reference) 1.11 (0.82-1.47) <b>1.74 (1.02-3.11)</b></p> <p>1 (Reference) 1.35 (1.00-1.82) 1.74 (0.98-3.08)</p> <p><b>OR (adjusted)</b></p> <p>1.0 (Reference) <b>1.51 (1.15-1.99)</b> <b>2.04 (1.12-3.74)</b></p> <p>1 (Reference) <b>1.55 (1.02-2.33)</b></p> <p>1.0 (Reference) 0.43 (0.47-3.73) <b>0.25 (0.07-0.90)</b></p> <p>1.0 (Reference) 0.51 (0.18-1.46) <b>0.23 (0.05-0.99)</b></p>	140

**Table 16.** Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, $\beta$ , or MPC (95% C.I.)*	Reference
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\* **Bolded text** was used to highlight statistically significant increases; *Italicized text* was used to highlight statistically significant decreases  
AFC=Antral follicle count; ANOVA=Analysis of variance procedures; BMI=Body mass index; CASA= computer-aided semen analysis; CI=Confidence interval; DLLME=dispersive liquid–liquid micro extraction;  
E2=Estradiol; EDI=Estimated daily intake; EEQ=Estrogen equivalency; FSH=Follicle stimulating hormone; GM: Geometric mean; 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<sub>parabens</sub>=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 17. Global exposure estimate for parabens illustrated using the survey data for Butylparaben<sup>24,144,145</sup>**

Type of Exposure <sup>145</sup>	Product <sup>144</sup>	Daily Use <sup>144</sup> (g/day)	Cumulative Exposure (g/day)	Maximum use concentration of Butylparaben <sup>24</sup>	Maximum exposure estimate of Butylparaben (g/day)	Butylparaben Exposure (mg/kg/day) assuming 60 kg person
<b>Oral</b>	Toothpaste	0.14	2.36	0.2% (Lipstick)	0.0047	0.079
	Mouthwash	2.16				
	Lipstick	0.06				
<b>Eye products</b>	Eye makeup	0.02	0.05	0.5% (Mascara)	0.00025	0.0042
	Mascara	0.025				
	Eyeliners	0.005				
<b>Non rinse-off products</b>	Face cream	1.54	13.93	0.24% (Moisturizing products)	0.0334	0.54
	Hand cream	2.16				
	Liquid Foundation	0.51				
	Body lotion	7.82				
	Deodorant	1.50				
	Hair styling products	0.40				
<b>Rinse-off products</b>	Make-up remover	0.50	1.04	0.33% (Skin cleansing)	0.0034	0.04
	Hand wash soap	0.20				
	Shower gel	0.19				
	Shampoo	0.11				
	Conditioner	0.04				
<b>Total</b>			17.4		0.042	0.6632

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**2019 VCRP data – Parabens**

Benzylparaben - 0

BUTYLPARABEN	01A - Baby Shampoos	2
BUTYLPARABEN	01B - Baby Lotions, Oils, Powders, and Creams	6
BUTYLPARABEN	01C - Other Baby Products	3
BUTYLPARABEN	02A - Bath Oils, Tablets, and Salts	4
BUTYLPARABEN	02B - Bubble Baths	4
BUTYLPARABEN	02D - Other Bath Preparations	15
BUTYLPARABEN	03A - Eyebrow Pencil	50
BUTYLPARABEN	03B - Eyeliner	313
BUTYLPARABEN	03C - Eye Shadow	188
BUTYLPARABEN	03D - Eye Lotion	45
BUTYLPARABEN	03E - Eye Makeup Remover	21
BUTYLPARABEN	03F - Mascara	95
BUTYLPARABEN	03G - Other Eye Makeup Preparations	65
BUTYLPARABEN	04A - Cologne and Toilet waters	1
BUTYLPARABEN	04B - Perfumes	2
BUTYLPARABEN	04C - Powders (dusting and talcum, excluding aftershave talc)	14
BUTYLPARABEN	04E - Other Fragrance Preparation	10
BUTYLPARABEN	05A - Hair Conditioner	48
BUTYLPARABEN	05C - Hair Straighteners	5
BUTYLPARABEN	05E - Rinses (non-coloring)	4
BUTYLPARABEN	05F - Shampoos (non-coloring)	117
BUTYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	47
BUTYLPARABEN	05H - Wave Sets	2
BUTYLPARABEN	05I - Other Hair Preparations	58
BUTYLPARABEN	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	23
BUTYLPARABEN	06B - Hair Tints	2
BUTYLPARABEN	06D - Hair Shampoos (coloring)	5
BUTYLPARABEN	06H - Other Hair Coloring Preparation	3
BUTYLPARABEN	07A - Blushers (all types)	65
BUTYLPARABEN	07B - Face Powders	105
BUTYLPARABEN	07C - Foundations	97
BUTYLPARABEN	07D - Leg and Body Paints	7
BUTYLPARABEN	07E - Lipstick	273
BUTYLPARABEN	07F - Makeup Bases	14
BUTYLPARABEN	07G - Rouges	11
BUTYLPARABEN	07H - Makeup Fixatives	1
BUTYLPARABEN	07I - Other Makeup Preparations	85
BUTYLPARABEN	08A - Basecoats and Undercoats	2
BUTYLPARABEN	08B - Cuticle Softeners	15
BUTYLPARABEN	08C - Nail Creams and Lotions	3
BUTYLPARABEN	08E - Nail Polish and Enamel	8
BUTYLPARABEN	08F - Nail Polish and Enamel Removers	2
BUTYLPARABEN	08G - Other Manicuring Preparations	13
BUTYLPARABEN	10A - Bath Soaps and Detergents	124
BUTYLPARABEN	10B - Deodorants (underarm)	8

BUTYLPARABEN	10C - Douches	1
BUTYLPARABEN	10E - Other Personal Cleanliness Products	96
BUTYLPARABEN	11A - Aftershave Lotion	24
BUTYLPARABEN	11D - Preshave Lotions (all types)	1
BUTYLPARABEN	11E - Shaving Cream	8
BUTYLPARABEN	11F - Shaving Soap	1
BUTYLPARABEN	11G - Other Shaving Preparation Products	14
BUTYLPARABEN	12A - Cleansing	175
BUTYLPARABEN	12B - Depilatories	4
BUTYLPARABEN	12C - Face and Neck (exc shave)	355
BUTYLPARABEN	12D - Body and Hand (exc shave)	314
BUTYLPARABEN	12E - Foot Powders and Sprays	2
BUTYLPARABEN	12F - Moisturizing	497
BUTYLPARABEN	12G - Night	66
BUTYLPARABEN	12H - Paste Masks (mud packs)	76
BUTYLPARABEN	12I - Skin Fresheners	16
BUTYLPARABEN	12J - Other Skin Care Preps	166
BUTYLPARABEN	13A - Suntan Gels, Creams, and Liquids	25
BUTYLPARABEN	13B - Indoor Tanning Preparations	48
BUTYLPARABEN	13C - Other Suntan Preparations	10

## Calcium Paraben - 0

ETHYLPARABEN	01A - Baby Shampoos	1
ETHYLPARABEN	01B - Baby Lotions, Oils, Powders, and Creams	12
ETHYLPARABEN	01C - Other Baby Products	2
ETHYLPARABEN	02A - Bath Oils, Tablets, and Salts	4
ETHYLPARABEN	02B - Bubble Baths	6
ETHYLPARABEN	02D - Other Bath Preparations	21
ETHYLPARABEN	03A - Eyebrow Pencil	13
ETHYLPARABEN	03B - Eyeliner	63
ETHYLPARABEN	03C - Eye Shadow	137
ETHYLPARABEN	03D - Eye Lotion	70
ETHYLPARABEN	03E - Eye Makeup Remover	18
ETHYLPARABEN	03F - Mascara	161
ETHYLPARABEN	03G - Other Eye Makeup Preparations	83
ETHYLPARABEN	04B - Perfumes	1
ETHYLPARABEN	04C - Powders (dusting and talcum, excluding aftershave talc)	10
ETHYLPARABEN	04E - Other Fragrance Preparation	9
ETHYLPARABEN	05A - Hair Conditioner	57
ETHYLPARABEN	05B - Hair Spray (aerosol fixatives)	3
ETHYLPARABEN	05C - Hair Straighteners	5
ETHYLPARABEN	05E - Rinses (non-coloring)	2
ETHYLPARABEN	05F - Shampoos (non-coloring)	159
ETHYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	81
ETHYLPARABEN	05H - Wave Sets	3
ETHYLPARABEN	05I - Other Hair Preparations	136
ETHYLPARABEN	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	90
ETHYLPARABEN	06B - Hair Tints	1
ETHYLPARABEN	06D - Hair Shampoos (coloring)	5
ETHYLPARABEN	06F - Hair Lighteners with Color	1

ETHYLPARABEN	06H - Other Hair Coloring Preparation	5
ETHYLPARABEN	07A - Blushers (all types)	28
ETHYLPARABEN	07B - Face Powders	54
ETHYLPARABEN	07C - Foundations	95
ETHYLPARABEN	07D - Leg and Body Paints	3
ETHYLPARABEN	07E - Lipstick	63
ETHYLPARABEN	07F - Makeup Bases	24
ETHYLPARABEN	07G - Rouges	10
ETHYLPARABEN	07H - Makeup Fixatives	1
ETHYLPARABEN	07I - Other Makeup Preparations	57
ETHYLPARABEN	08A - Basecoats and Undercoats	1
ETHYLPARABEN	08B - Cuticle Softeners	12
ETHYLPARABEN	08C - Nail Creams and Lotions	2
ETHYLPARABEN	08E - Nail Polish and Enamel	10
ETHYLPARABEN	08F - Nail Polish and Enamel Removers	2
ETHYLPARABEN	08G - Other Manicuring Preparations	12
ETHYLPARABEN	09B - Mouthwashes and Breath Fresheners	1
ETHYLPARABEN	10A - Bath Soaps and Detergents	125
ETHYLPARABEN	10B - Deodorants (underarm)	10
ETHYLPARABEN	10C - Douches	1
ETHYLPARABEN	10E - Other Personal Cleanliness Products	82
ETHYLPARABEN	11A - Aftershave Lotion	34
ETHYLPARABEN	11D - Preshave Lotions (all types)	1
ETHYLPARABEN	11E - Shaving Cream	10
ETHYLPARABEN	11F - Shaving Soap	1
ETHYLPARABEN	11G - Other Shaving Preparation Products	18
ETHYLPARABEN	12A - Cleansing	218
ETHYLPARABEN	12B - Depilatories	6
ETHYLPARABEN	12C - Face and Neck (exc shave)	422
ETHYLPARABEN	12D - Body and Hand (exc shave)	366
ETHYLPARABEN	12E - Foot Powders and Sprays	4
ETHYLPARABEN	12F - Moisturizing	478
ETHYLPARABEN	12G - Night	119
ETHYLPARABEN	12H - Paste Masks (mud packs)	81
ETHYLPARABEN	12I - Skin Fresheners	21
ETHYLPARABEN	12J - Other Skin Care Preps	185
ETHYLPARABEN	13A - Suntan Gels, Creams, and Liquids	18
ETHYLPARABEN	13B - Indoor Tanning Preparations	60
ETHYLPARABEN	13C - Other Suntan Preparations	8
ISOBUTYLPARABEN	01A - Baby Shampoos	1
ISOBUTYLPARABEN	01B - Baby Lotions, Oils, Powders, and Creams	2
ISOBUTYLPARABEN	01C - Other Baby Products	2
ISOBUTYLPARABEN	02A - Bath Oils, Tablets, and Salts	3
ISOBUTYLPARABEN	02B - Bubble Baths	3
ISOBUTYLPARABEN	02D - Other Bath Preparations	19
ISOBUTYLPARABEN	03A - Eyebrow Pencil	4
ISOBUTYLPARABEN	03B - Eyeliner	28
ISOBUTYLPARABEN	03C - Eye Shadow	37
ISOBUTYLPARABEN	03D - Eye Lotion	25
ISOBUTYLPARABEN	03E - Eye Makeup Remover	9
ISOBUTYLPARABEN	03F - Mascara	69
ISOBUTYLPARABEN	03G - Other Eye Makeup Preparations	41

ISOBUTYLPARABEN	04A - Cologne and Toilet waters	1
ISOBUTYLPARABEN	04B - Perfumes	2
ISOBUTYLPARABEN	04C - Powders (dusting and talcum, excluding aftershave talc)	4
ISOBUTYLPARABEN	04E - Other Fragrance Preparation	4
ISOBUTYLPARABEN	05A - Hair Conditioner	24
ISOBUTYLPARABEN	05E - Rinses (non-coloring)	1
ISOBUTYLPARABEN	05F - Shampoos (non-coloring)	56
ISOBUTYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	25
ISOBUTYLPARABEN	05H - Wave Sets	1
ISOBUTYLPARABEN	05I - Other Hair Preparations	33
ISOBUTYLPARABEN	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	23
ISOBUTYLPARABEN	06D - Hair Shampoos (coloring)	4
ISOBUTYLPARABEN	06H - Other Hair Coloring Preparation	3
ISOBUTYLPARABEN	07A - Blushers (all types)	15
ISOBUTYLPARABEN	07B - Face Powders	17
ISOBUTYLPARABEN	07C - Foundations	46
ISOBUTYLPARABEN	07D - Leg and Body Paints	2
ISOBUTYLPARABEN	07E - Lipstick	63
ISOBUTYLPARABEN	07F - Makeup Bases	6
ISOBUTYLPARABEN	07G - Rouges	2
ISOBUTYLPARABEN	07H - Makeup Fixatives	1
ISOBUTYLPARABEN	07I - Other Makeup Preparations	43
ISOBUTYLPARABEN	08A - Basecoats and Undercoats	1
ISOBUTYLPARABEN	08B - Cuticle Softeners	12
ISOBUTYLPARABEN	08C - Nail Creams and Lotions	2
ISOBUTYLPARABEN	08E - Nail Polish and Enamel	7
ISOBUTYLPARABEN	08F - Nail Polish and Enamel Removers	2
ISOBUTYLPARABEN	08G - Other Manicuring Preparations	13
ISOBUTYLPARABEN	10A - Bath Soaps and Detergents	91
ISOBUTYLPARABEN	10B - Deodorants (underarm)	5
ISOBUTYLPARABEN	10C - Douches	1
ISOBUTYLPARABEN	10E - Other Personal Cleanliness Products	75
ISOBUTYLPARABEN	11A - Aftershave Lotion	20
ISOBUTYLPARABEN	11D - Preshave Lotions (all types)	1
ISOBUTYLPARABEN	11E - Shaving Cream	2
ISOBUTYLPARABEN	11F - Shaving Soap	1
ISOBUTYLPARABEN	11G - Other Shaving Preparation Products	12
ISOBUTYLPARABEN	12A - Cleansing	95
ISOBUTYLPARABEN	12B - Depilatories	3
ISOBUTYLPARABEN	12C - Face and Neck (exc shave)	232
ISOBUTYLPARABEN	12D - Body and Hand (exc shave)	210
ISOBUTYLPARABEN	12E - Foot Powders and Sprays	2
ISOBUTYLPARABEN	12F - Moisturizing	254
ISOBUTYLPARABEN	12G - Night	49
ISOBUTYLPARABEN	12H - Paste Masks (mud packs)	41
ISOBUTYLPARABEN	12I - Skin Fresheners	10
ISOBUTYLPARABEN	12J - Other Skin Care Preps	113
ISOBUTYLPARABEN	13A - Suntan Gels, Creams, and Liquids	7
ISOBUTYLPARABEN	13B - Indoor Tanning Preparations	34
ISOBUTYLPARABEN	13C - Other Suntan Preparations	4
ISOPROPYLPARABEN	02A - Bath Oils, Tablets, and Salts	1

ISOPROPYLPARABEN	03B - Eyeliner	6
ISOPROPYLPARABEN	03C - Eye Shadow	16
ISOPROPYLPARABEN	03D - Eye Lotion	3
ISOPROPYLPARABEN	03F - Mascara	17
ISOPROPYLPARABEN	03G - Other Eye Makeup Preparations	3
ISOPROPYLPARABEN	04A - Cologne and Toilet waters	1
ISOPROPYLPARABEN	04B - Perfumes	1
ISOPROPYLPARABEN	05A - Hair Conditioner	13
ISOPROPYLPARABEN	05F - Shampoos (non-coloring)	3
ISOPROPYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	7
ISOPROPYLPARABEN	07A - Blushers (all types)	9
ISOPROPYLPARABEN	07B - Face Powders	6
ISOPROPYLPARABEN	07C - Foundations	6
ISOPROPYLPARABEN	07E - Lipstick	31
ISOPROPYLPARABEN	07F - Makeup Bases	1
ISOPROPYLPARABEN	07G - Rouges	2
ISOPROPYLPARABEN	07I - Other Makeup Preparations	13
ISOPROPYLPARABEN	08B - Cuticle Softeners	2
ISOPROPYLPARABEN	08C - Nail Creams and Lotions	1
ISOPROPYLPARABEN	08G - Other Manicuring Preparations	3
ISOPROPYLPARABEN	10A - Bath Soaps and Detergents	3
ISOPROPYLPARABEN	10E - Other Personal Cleanliness Products	17
ISOPROPYLPARABEN	11A - Aftershave Lotion	1
ISOPROPYLPARABEN	12A - Cleansing	4
ISOPROPYLPARABEN	12B - Depilatories	1
ISOPROPYLPARABEN	12C - Face and Neck (exc shave)	4
ISOPROPYLPARABEN	12D - Body and Hand (exc shave)	17
ISOPROPYLPARABEN	12F - Moisturizing	69
ISOPROPYLPARABEN	12G - Night	2
ISOPROPYLPARABEN	12H - Paste Masks (mud packs)	1
ISOPROPYLPARABEN	12J - Other Skin Care Preps	7
ISOPROPYLPARABEN	13B - Indoor Tanning Preparations	3
METHYLPARABEN	01A - Baby Shampoos	3
METHYLPARABEN	01B - Baby Lotions, Oils, Powders, and Creams	20
METHYLPARABEN	01C - Other Baby Products	13
METHYLPARABEN	02A - Bath Oils, Tablets, and Salts	15
METHYLPARABEN	02B - Bubble Baths	18
METHYLPARABEN	02D - Other Bath Preparations	26
METHYLPARABEN	03A - Eyebrow Pencil	60
METHYLPARABEN	03B - Eyeliner	416
METHYLPARABEN	03C - Eye Shadow	645
METHYLPARABEN	03D - Eye Lotion	135
METHYLPARABEN	03E - Eye Makeup Remover	46
METHYLPARABEN	03F - Mascara	318
METHYLPARABEN	03G - Other Eye Makeup Preparations	177
METHYLPARABEN	04A - Cologne and Toilet waters	42
METHYLPARABEN	04B - Perfumes	13
METHYLPARABEN	04C - Powders (dusting and talcum, excluding aftershave talc)	72
METHYLPARABEN	04E - Other Fragrance Preparation	20
METHYLPARABEN	05A - Hair Conditioner	417
METHYLPARABEN	05B - Hair Spray (aerosol fixatives)	8
METHYLPARABEN	05C - Hair Straighteners	19

METHYLPARABEN	05D - Permanent Waves	5
METHYLPARABEN	05E - Rinses (non-coloring)	9
METHYLPARABEN	05F - Shampoos (non-coloring)	316
METHYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	362
METHYLPARABEN	05H - Wave Sets	9
METHYLPARABEN	05I - Other Hair Preparations	352
METHYLPARABEN	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	143
METHYLPARABEN	06B - Hair Tints	11
METHYLPARABEN	06C - Hair Rinses (coloring)	10
METHYLPARABEN	06D - Hair Shampoos (coloring)	11
METHYLPARABEN	06E - Hair Color Sprays (aerosol)	3
METHYLPARABEN	06F - Hair Lighteners with Color	1
METHYLPARABEN	06G - Hair Bleaches	8
METHYLPARABEN	06H - Other Hair Coloring Preparation	50
METHYLPARABEN	07A - Blushers (all types)	170
METHYLPARABEN	07B - Face Powders	274
METHYLPARABEN	07C - Foundations	258
METHYLPARABEN	07D - Leg and Body Paints	26
METHYLPARABEN	07E - Lipstick	286
METHYLPARABEN	07F - Makeup Bases	55
METHYLPARABEN	07G - Rouges	5
METHYLPARABEN	07H - Makeup Fixatives	8
METHYLPARABEN	07I - Other Makeup Preparations	167
METHYLPARABEN	08A - Basecoats and Undercoats	3
METHYLPARABEN	08B - Cuticle Softeners	17
METHYLPARABEN	08C - Nail Creams and Lotions	5
METHYLPARABEN	08E - Nail Polish and Enamel	17
METHYLPARABEN	08F - Nail Polish and Enamel Removers	5
METHYLPARABEN	08G - Other Manicuring Preparations	21
METHYLPARABEN	09A - Dentifrices	12
METHYLPARABEN	09B - Mouthwashes and Breath Fresheners	3
METHYLPARABEN	09C - Other Oral Hygiene Products	4
METHYLPARABEN	10A - Bath Soaps and Detergents	273
METHYLPARABEN	10B - Deodorants (underarm)	20
METHYLPARABEN	10C - Douches	1
METHYLPARABEN	10E - Other Personal Cleanliness Products	195
METHYLPARABEN	11A - Aftershave Lotion	86
METHYLPARABEN	11D - Preshave Lotions (all types)	2
METHYLPARABEN	11E - Shaving Cream	31
METHYLPARABEN	11F - Shaving Soap	1
METHYLPARABEN	11G - Other Shaving Preparation Products	43
METHYLPARABEN	12A - Cleansing	504
METHYLPARABEN	12B - Depilatories	7
METHYLPARABEN	12C - Face and Neck (exc shave)	853
METHYLPARABEN	12D - Body and Hand (exc shave)	994
METHYLPARABEN	12E - Foot Powders and Sprays	4
METHYLPARABEN	12F - Moisturizing	2472
METHYLPARABEN	12G - Night	209
METHYLPARABEN	12H - Paste Masks (mud packs)	194
METHYLPARABEN	12I - Skin Fresheners	88
METHYLPARABEN	12J - Other Skin Care Preps	488
METHYLPARABEN	13A - Suntan Gels, Creams, and Liquids	38
METHYLPARABEN	13B - Indoor Tanning Preparations	110

METHYLPARABEN	13C - Other Suntan Preparations	17
Potassium Butylparaben - 0		
Potassium Ethylparaben - 0		
Potassium Methylparaben - 0		
Potassium Paraben - 0		
Potassium Propylparaben - 0		
PROPYLPARABEN	01A - Baby Shampoos	3
PROPYLPARABEN	01B - Baby Lotions, Oils, Powders, and Creams	21
PROPYLPARABEN	01C - Other Baby Products	11
PROPYLPARABEN	02A - Bath Oils, Tablets, and Salts	12
PROPYLPARABEN	02B - Bubble Baths	14
PROPYLPARABEN	02D - Other Bath Preparations	23
PROPYLPARABEN	03A - Eyebrow Pencil	73
PROPYLPARABEN	03B - Eyeliner	410
PROPYLPARABEN	03C - Eye Shadow	590
PROPYLPARABEN	03D - Eye Lotion	87
PROPYLPARABEN	03E - Eye Makeup Remover	28
PROPYLPARABEN	03F - Mascara	240
PROPYLPARABEN	03G - Other Eye Makeup Preparations	136
PROPYLPARABEN	04A - Cologne and Toilet waters	2
PROPYLPARABEN	04B - Perfumes	5
PROPYLPARABEN	04C - Powders (dusting and talcum, excluding aftershave talc)	48
PROPYLPARABEN	04E - Other Fragrance Preparation	24
PROPYLPARABEN	05A - Hair Conditioner	196
PROPYLPARABEN	05B - Hair Spray (aerosol fixatives)	4
PROPYLPARABEN	05C - Hair Straighteners	12
PROPYLPARABEN	05D - Permanent Waves	2
PROPYLPARABEN	05E - Rinses (non-coloring)	7
PROPYLPARABEN	05F - Shampoos (non-coloring)	171
PROPYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	206
PROPYLPARABEN	05H - Wave Sets	5
PROPYLPARABEN	05I - Other Hair Preparations	143
PROPYLPARABEN	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	119
PROPYLPARABEN	06B - Hair Tints	10
PROPYLPARABEN	06C - Hair Rinses (coloring)	6
PROPYLPARABEN	06D - Hair Shampoos (coloring)	9
PROPYLPARABEN	06F - Hair Lighteners with Color	1
PROPYLPARABEN	06G - Hair Bleaches	1
PROPYLPARABEN	06H - Other Hair Coloring Preparation	22
PROPYLPARABEN	07A - Blushers (all types)	139
PROPYLPARABEN	07B - Face Powders	224
PROPYLPARABEN	07C - Foundations	190
PROPYLPARABEN	07D - Leg and Body Paints	19
PROPYLPARABEN	07E - Lipstick	577
PROPYLPARABEN	07F - Makeup Bases	34
PROPYLPARABEN	07G - Rouges	4
PROPYLPARABEN	07H - Makeup Fixatives	4
PROPYLPARABEN	07I - Other Makeup Preparations	169

PROPYLPARABEN	08A - Basecoats and Undercoats	3
PROPYLPARABEN	08B - Cuticle Softeners	19
PROPYLPARABEN	08C - Nail Creams and Lotions	4
PROPYLPARABEN	08E - Nail Polish and Enamel	13
PROPYLPARABEN	08F - Nail Polish and Enamel Removers	2
PROPYLPARABEN	08G - Other Manicuring Preparations	17
PROPYLPARABEN	09A - Dentifrices	6
PROPYLPARABEN	09B - Mouthwashes and Breath Fresheners	1
PROPYLPARABEN	09C - Other Oral Hygiene Products	2
PROPYLPARABEN	10A - Bath Soaps and Detergents	206
PROPYLPARABEN	10B - Deodorants (underarm)	13
PROPYLPARABEN	10C - Douches	1
PROPYLPARABEN	10E - Other Personal Cleanliness Products	141
PROPYLPARABEN	11A - Aftershave Lotion	45
PROPYLPARABEN	11D - Preshave Lotions (all types)	2
PROPYLPARABEN	11E - Shaving Cream	27
PROPYLPARABEN	11F - Shaving Soap	1
PROPYLPARABEN	11G - Other Shaving Preparation Products	31
PROPYLPARABEN	12A - Cleansing	324
PROPYLPARABEN	12B - Depilatories	14
PROPYLPARABEN	12C - Face and Neck (exc shave)	562
PROPYLPARABEN	12D - Body and Hand (exc shave)	785
PROPYLPARABEN	12E - Foot Powders and Sprays	2
PROPYLPARABEN	12F - Moisturizing	2028
PROPYLPARABEN	12G - Night	132
PROPYLPARABEN	12H - Paste Masks (mud packs)	115
PROPYLPARABEN	12I - Skin Fresheners	44
PROPYLPARABEN	12J - Other Skin Care Preps	372
PROPYLPARABEN	13A - Suntan Gels, Creams, and Liquids	38
PROPYLPARABEN	13B - Indoor Tanning Preparations	70
PROPYLPARABEN	13C - Other Suntan Preparations	13
SODIUM BUTYLPARABEN	12F - Moisturizing	1
SODIUM BUTYLPARABEN	12I - Skin Fresheners	1
SODIUM ETHYLPARABEN	03D - Eye Lotion	1
SODIUM ETHYLPARABEN	03F - Mascara	1
SODIUM ETHYLPARABEN	03G - Other Eye Makeup Preparations	8
SODIUM ETHYLPARABEN	07C - Foundations	1
SODIUM ETHYLPARABEN	07I - Other Makeup Preparations	1
SODIUM ETHYLPARABEN	10C - Douches	2
SODIUM ETHYLPARABEN	12C - Face and Neck (exc shave)	2
SODIUM ETHYLPARABEN	12D - Body and Hand (exc shave)	2
SODIUM ETHYLPARABEN	12F - Moisturizing	4
SODIUM ETHYLPARABEN	12I - Skin Fresheners	1
SODIUM ETHYLPARABEN	12J - Other Skin Care Preps	4
SODIUM ISOBUTYLPARABEN	12F - Moisturizing	1
SODIUM ISOBUTYLPARABEN	12I - Skin Fresheners	1

Sodium Isopropylparaben -

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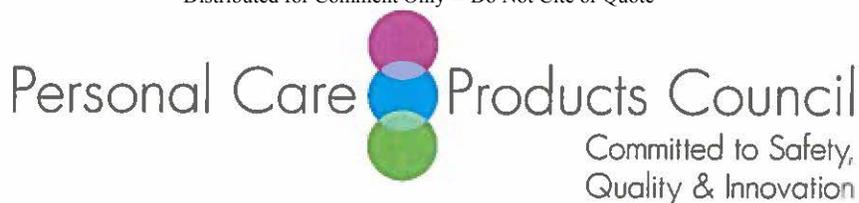
SODIUM METHYLPARABEN	02B - Bubble Baths	8
SODIUM METHYLPARABEN	02D - Other Bath Preparations	1
SODIUM METHYLPARABEN	03B - Eyeliner	12
SODIUM METHYLPARABEN	03C - Eye Shadow	3
SODIUM METHYLPARABEN	03D - Eye Lotion	6
SODIUM METHYLPARABEN	03E - Eye Makeup Remover	4
SODIUM METHYLPARABEN	03F - Mascara	7
SODIUM METHYLPARABEN	03G - Other Eye Makeup Preparations	14
SODIUM METHYLPARABEN	04E - Other Fragrance Preparation	1
SODIUM METHYLPARABEN	05A - Hair Conditioner	7
SODIUM METHYLPARABEN	05B - Hair Spray (aerosol fixatives)	1
SODIUM METHYLPARABEN	05D - Permanent Waves	1
SODIUM METHYLPARABEN	05F - Shampoos (non-coloring)	42
SODIUM METHYLPARABEN	05G - Tonics, Dressings, and Other Hair Grooming Aids	9
SODIUM METHYLPARABEN	05H - Wave Sets	1
SODIUM METHYLPARABEN	05I - Other Hair Preparations	11
SODIUM METHYLPARABEN	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	65
SODIUM METHYLPARABEN	06B - Hair Tints	1
SODIUM METHYLPARABEN	06C - Hair Rinses (coloring)	2
SODIUM METHYLPARABEN	06D - Hair Shampoos (coloring)	4
SODIUM METHYLPARABEN	06F - Hair Lighteners with Color	1
SODIUM METHYLPARABEN	06H - Other Hair Coloring Preparation	2
SODIUM METHYLPARABEN	07C - Foundations	1
SODIUM METHYLPARABEN	07I - Other Makeup Preparations	11
SODIUM METHYLPARABEN	10A - Bath Soaps and Detergents	9
SODIUM METHYLPARABEN	10C - Douches	3
SODIUM METHYLPARABEN	10E - Other Personal Cleanliness Products	2
SODIUM METHYLPARABEN	11E - Shaving Cream	1
SODIUM METHYLPARABEN	12A - Cleansing	32
SODIUM METHYLPARABEN	12B - Depilatories	1
SODIUM METHYLPARABEN	12C - Face and Neck (exc shave)	58
SODIUM METHYLPARABEN	12D - Body and Hand (exc shave)	18
SODIUM METHYLPARABEN	12E - Foot Powders and Sprays	3
SODIUM METHYLPARABEN	12F - Moisturizing	26
SODIUM METHYLPARABEN	12G - Night	5
SODIUM METHYLPARABEN	12H - Paste Masks (mud packs)	11
SODIUM METHYLPARABEN	12I - Skin Fresheners	4
SODIUM METHYLPARABEN	12J - Other Skin Care Preps	24
SODIUM METHYLPARABEN	13B - Indoor Tanning Preparations	2

## Sodium Paraben - 0

SODIUM PROPYLPARABEN	01C - Other Baby Products	1
SODIUM PROPYLPARABEN	02B - Bubble Baths	3
SODIUM PROPYLPARABEN	02D - Other Bath Preparations	1
SODIUM PROPYLPARABEN	03B - Eyeliner	2
SODIUM PROPYLPARABEN	03C - Eye Shadow	3
SODIUM PROPYLPARABEN	03D - Eye Lotion	2

SODIUM PROPYLPARABEN	03F - Mascara	3
SODIUM PROPYLPARABEN	03G - Other Eye Makeup Preparations	8
SODIUM PROPYLPARABEN	05F - Shampoos (non-coloring)	2
SODIUM PROPYLPARABEN	05I - Other Hair Preparations	1
SODIUM PROPYLPARABEN	06C - Hair Rinses (coloring)	1
SODIUM PROPYLPARABEN	07C - Foundations	1
SODIUM PROPYLPARABEN	07I - Other Makeup Preparations	5
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4-Hydroxybenzoic Acid - 0



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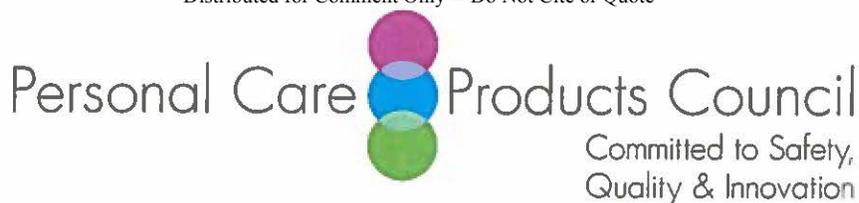
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