Amended Safety Assessment of Parabens as Used in Cosmetics

Status: Draft Tentative Amended Report for Panel Review
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This report was prepared by Priya A. Cherian, Scientific Analyst/Writer and Jinqiu Zhu, Ph.D., Toxicologist.

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MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Priya A. Cherian
Scientific Analyst and Writer

Jinqiu Zhu, PhD, DABT, ERT
Toxicologist

Date: August 29, 2018

Subject: Amended Safety Assessment of Parabens as Used in Cosmetics

Attached is the Draft Tentative Amended Report of 20 parabens and 4-Hydroxybenzoic Acid, as used in cosmetics (*parabe092018rep*). In 2017, the Panel agreed to re-open the parabens report that was published in 2008, and to include the paraben salts and 4-Hydroxybenzoic Acid. At the March 2018 meeting, the Panel reviewed the new data in the category of endocrine activation, developmental and reproductive toxicity (DART), and epidemiology. The Panel discussed the topics and issues related to EU regulations of parabens, bioaccumulation potential, aggregate exposure, and estrogen receptor binding capability of paraben metabolites.

The Panel noted that the European Union (EU) has banned the use of 5 parabens (Isopropylparaben, Isobutylparaben, Phenylparaben, Benzylparaben, and Pentylparaben) as preservatives in cosmetic products, and has set maximum concentration limits of 0.14 % for Butylparaben or Propylparaben (single esters and their salts), 0.4% for Methylparaben or Ethylparaben (single esters and their salts), and 0.8% for the mixture of these four ingredients, wherein the sum of the individual concentration of Butylparaben and Propylparaben cannot exceed 0.14 %. The EU regulations on the parabens were noted in this report for the informative purposes, and the derivation of such maximum authorized concentration of 0.14% for Butylparaben was discussed accordingly.

Also, at the March 2018 meeting, the Panel put into perspective the potential burden of parabens from cosmetics versus multiple other sources of exposure (e.g., food and pharmaceutical uses). In response, a quantitative estimation of the aggregate exposure to parabens used in a variety of cosmetic product types, as well as in food and medical products, was incorporated into this report. Also included were biomonitoring data from the US National Health and Nutrition Examination Survey (NHANES) that measured the concentration of parabens in human urine.

The Panel reviewed additional studies submitted by various stakeholders or discovered by CIR, as well as the data presented in Dr. George Daston's presentation, titled ""Assessing the Developmental and Reproductive Toxicity of Parabens." The Panel requested all relevant new information be included in this report. In addition, the Panel discussed the accumulative properties of parabens in human body and the estrogen receptor binding

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potential of Butylparaben, Isobutylparaben, and Benzylparaben metabolites. One newly discovered study with respect to the estrogenic properties of Butylparaben and Isobutylparaben metabolites was incorporated into the document.

Taking Dr. Daston's presentation into account, the Panel considered whether no-observed-adverse-effect-level (NOAEL) data from new DART studies warranted a dose lower than the 1000 mg/kg/day which was used for margin of safety (MOS) calculation in the previous CIR safety assessment of parabens. After careful consideration of all the new data, the Panel determined an adequate NOAEL of 160 mg/kg/day for Butylparaben. The MOS for Butylparaben was re-calculated accordingly, and can be inferred to other members of the parabens group.

Topics related to paraben aggregation in the tissues, a cumulative MOS calculation, and a refining of aggregate exposure of parabens from various consumer products, were discussed accordingly. The input regarding new studies as well as relevant discussions were highlighted within the text of this report. Please note that the draft Discussion is preliminary and subject to further changes prior to release. In addition, previous Panel discussions were included in the text of this Draft Tentative Amended Report for the purpose of easy review. However, the whole section of *Previous Discussions* will be deleted once the Tentative Amended Report is issued; that is, only the current Discussion will be maintained thereafter.

Also included in this package for review are the CIR report history (*parabe092018hist*), flow chart (*parabe092018flow*), literature search strategy (*parabe092018strat*), ingredient data profile (*parabe092018prof*), 2018 FDA VCRP data (*parabe092018FDA*), previous meeting transcripts (*parabe092018min*), Dr. George Daston's presentation (*parabe092018data*), and comments that were received from the Personal Care Products Council (Council) after the March Panel meeting (*parabe092018pcpc*). The Council's comments have been addressed.

The Panel should review the available data to either affirm or change the conclusion from the 2008 report for the original seven paraben ingredients. The Panel should also determine if this conclusion can be applied to the newly added ingredients, or if a split conclusion is warranted. Whether the conclusion remains the same (and extends to all of the new ingredients) or is to be changed and/or split, the Panel should develop the basis for the Discussion and Conclusion, and issue a Tentative Amended Report.

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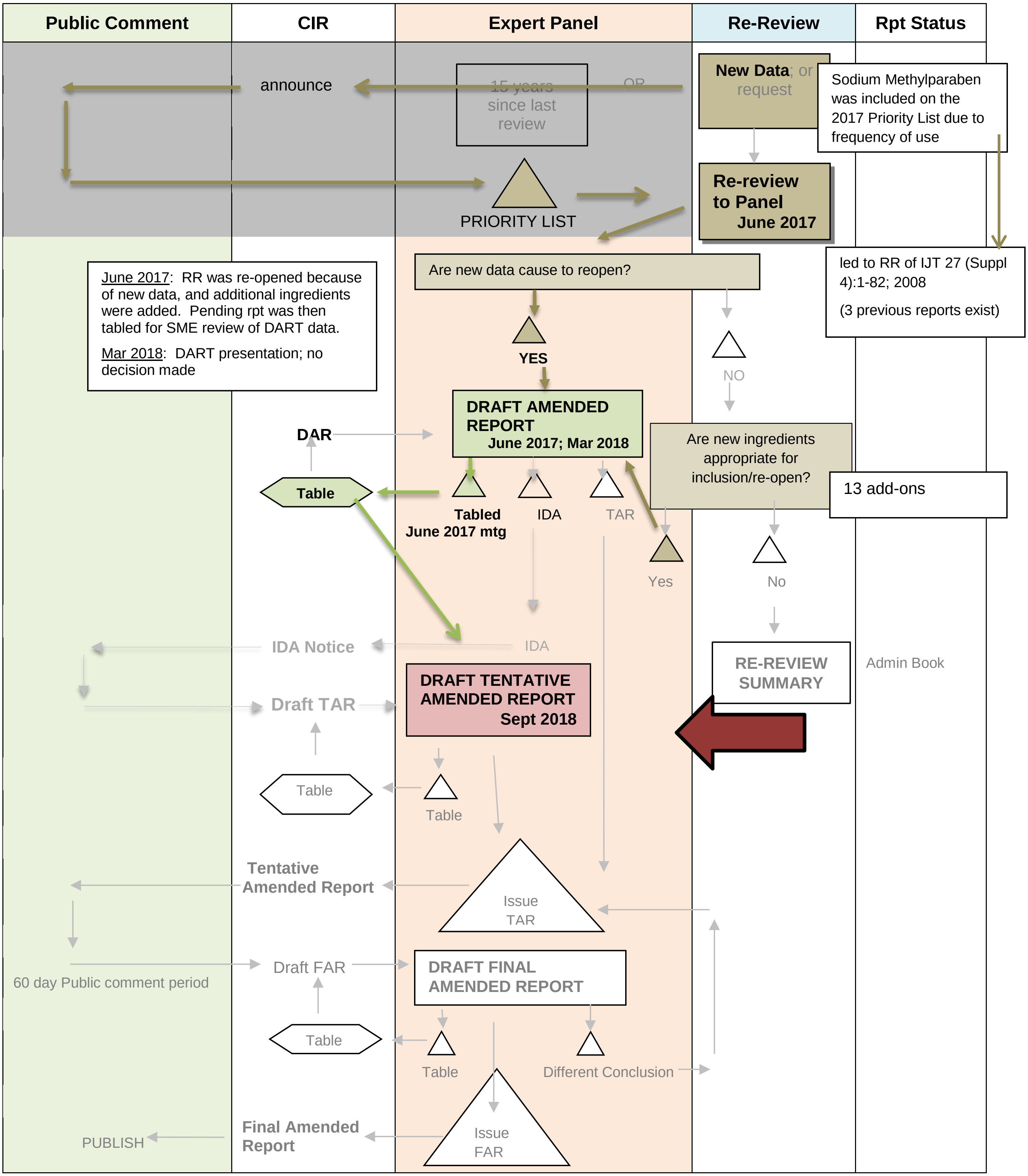
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Parabens

MEETING Sept 2018



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:

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

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.

Parabens Data Profile for ***September 24th - 25th*, 2018**. Writers – Priya Cherian, Jinqiu Zhu

		ADME				Acute toxicity			Repeated dose toxicity			Irritation					Sensitization			_____						
	Dermal Penetration	In Vitro	Dermal	Oral	Aggregate	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Endocrine	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity	Epidemiology	Reported Use
Sodium Methylparaben																										X
Calcium Paraben																										
Potassium Butylparaben																										
Potassium Ethylparaben																										
Potassium Methylparaben																										
Potassium Paraben																										
Potassium Propylparaben																										
Sodium Butylparaben																										X
Sodium Ethylparaben																										X
Sodium Isobutylparaben																										X
Sodium Isopropylparaben																										
Sodium Paraben																										
Sodium Propylparaben																										X
4-Hydroxybenzoic Acid	O	O		O	N																O					
RE-REVIEW:																										
Methylparaben	O	O	O	O N	O N	O	O	O	O	O		O	O	O	O	O	O	O	O	O N	O	O	O I	O	O N	X
Ethylparaben	O	O			O N	O			O			O		O		O	O	O	O	O	O	O	O I		N	X
Propylparaben	O	O	O	O N	O N	O	O		O	O		O		O	O	O	O	O	O	O N	O	O	O I	O	O N	X
Isopropylparaben										O						O			O		O	O				X
Butylparaben	O	O	O	O	O N	O	O		O					O	O	O	O	O	O	O N	O	O	O I	O	O N	X
Isobutylparaben					O				O	O						O			O	O N	O	O	O		N	X
Benzylparaben	O				O N	O			O			O		O		O			O	O					O	

I = In vitro
N = New data
O = Old data
X = Data available

Search Strategy for Parabens

- PubMed – July 1, 2018
  - 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”)

    751 hits, reduced to 290 references of interest based on careful reading of the abstracts

- Scifinder – July 1, 2018
  - 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: 26,569 hits

    Refine by Document types- Biography, Book, Clinical Trial, Commentary, Dissertation, Journal, Letter, Report, and Review: 12,745 hits

    Refine by:

    Acute toxicity; 81 hits
    Repeated dose toxicity; 6 hits
    Subacute toxicity; 3 hits
    Short-term toxicity; 4 hits
    Subchronic toxicity; 11 hits
    Chronic toxicity; 26 hits
    Adverse health effects; 26 hits
    Allergy; 286 hits
    Anaphylaxis; 12 hits
    Asthma; 7 hits
    Hypersensitivity; 57 hits
    Sensitization; 846 hits
    Cancer; 503 hits
    Carcinogenicity; 462 hits
    Cocarcinogenicity; 2 hits

Tumor promotion; 6 hits
Tumor progression; 1 hits
Case report; 297 hits
Case study; 297 hits
Clinical trial; 23 hits
Multicenter study; 12 hits
Clastogenicity, 5 hits
Genotoxicity; 45 hits
Mutagenicity; 177 hits
Comedogenicity; 0 hits
Cytotoxicity; 392 hits
Dermal absorption; 31 hits
Dermal penetration; 14 hits
Dermal irritation; 11 hits
Dermal effects; 181 hits
Dermal pigmentation; 0 hits
Developmental toxicity; 108 hits
Reproductive toxicity; 73 hits
Endocrine toxicity; 56 hits
Endocrine activity; 80 hits
Endocrine disruption; 315 hits
Epidemiology; 55 hits
Hepatotoxicity; 39 hits
Renal toxicity; 6 hits
Inhalation toxicity; 7 hits
Respiratory effects; 83 hits
In vitro toxicity; 59 hits
In vitro test; 1483 hits
Neurotoxicity; 25 hits
Ocular effects; 165hits
Oral exposure; 23 hits
Penetration enhancer; 60 hits
Phototoxicity; 12 hits
Photosensitivity; 1 hit
Risk assessment; 148 hits
Safety assessment; 43 hits
Toxicokinetics; 1193 hits
Pharmacokinetics; 195 hits

Combined: 3,232 hits (after duplicates removed), total; reduced to 450, all years, based on careful reading of the abstracts

- Consolidated and eliminated duplicates in PubMed and SciFinder search results
  - 386 references, all years

- Screened out:
  - Subcutaneous injection studies
  - 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: 53 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/

- 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
- 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/

- 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
- 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

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

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

February 1985

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

The two Teams met separately in closed session to evaluate the additional data submitted by industry during the public comment period. Dr. Bergfeld stated that the eye irritation data lacked details, and that acute oral and dermal tests were submitted although not requested. Dr. Hoffmann recommended deleting the request for teratogenicity studies from the insufficient data report. All Panel members concurred.

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

"DISCUSSION

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

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

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

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

“CONCLUSION

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

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

ISOBUTYLPARABEN AND ISOPROPYLPARABEN

August, 1993

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

November, 1993

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

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

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

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

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

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

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

February/March, 1994

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

METHYLPARABEN, ETHYLPARABEN, PROPYLPARABEN, BUTYLPARABEN, AND BENZYLPARABEN

December 2005

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

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

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

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

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

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

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

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

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

June 2006

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

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

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

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

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

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

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

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

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

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

The Panel voted unanimously in favor of issuing a Final Report with the following conclusion: The CIR Expert Panel concluded that Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben are safe as cosmetic ingredients in the practices of use and use concentrations described in this safety assessment.

MARCH 2012 - NEW DATA/SCCS OPINION

Dr. Belsito's Team

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

DR. LIEBLER: Panel book 73.

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

DR. LIEBLER: Oh, the SCCS comments?

DR. BELSITO: Yes.

DR. LIEBLER: That's 4.6.

DR. BELSITO: Yes, 4.6.

DR. LIEBLER: Panel book 106.

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

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

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

DR. BELSITO: Well, we used that.

DR. LIEBLER: Oh, we used that.

DR. BELSITO: We used 1,000.

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

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

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

DR. EISENMANN: Yes.

DR. BELSITO: Yes.

DR. LIEBLER: Okay, so, I was tracing my way through the literature on this, and it was clear that the CIR document comes up used as 1,000 and in the SCCS document, they cite that as the Holderman, et al., study, but I was confused because of the CIR document, there's no literature citation for anything by Holderman, et al.

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

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

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

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

DR. SNYDER: Okay.

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

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

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

DR. SNYDER: Okay.

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

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

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

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

DR. BRESLAWEC: Yes.

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

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

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

DR. BELSITO: Three months.

DR. ANDERSEN: No, three years.

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

DR. ANDERSEN: Yes. And my reading of that second SCCS document said we can find no basis for the Danish position, but it does seem like there's not a lot of data on exposure to any population under six months of age. So, they at least opened a small door, but they didn't take a step through it. They just made the comment.

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

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

DR. LIEBLER: Well, that changes our outcome.

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

DR. BELSITO: Yes.

DR. ANDERSEN: By our approach.

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

DR. KATZ: What page?

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

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

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

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

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

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

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

Paul, you were saying page 6 of that report?

DR. SNYDER: Page 7.

DR. BELSITO: Page 7.

DR. LIEBLER: Page 7.

DR. SNYDER: The first bullet point.

DR. BELSITO: 3.1 introduction.

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

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

DR. BRESLAWEC: All right.

DR. BELSITO: 3.1.

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

DR. BELSITO: Yes.

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

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

DR. KLAASSEN: I think so, too.

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

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

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

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

DR. SNYDER: We've never seen before.

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

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

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

DR. BELSITO: Well, five papers.

DR. ANDERSEN: Five.

DR. BELSITO: The Boberg, as well.

DR. ANDERSEN: Yes.

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

DR. LIEBLER: Prusakiewicz.

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

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

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

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

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

DR. SNYDER: Shaw.

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

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

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

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

DR. BELSITO: Okay, where's Ivan?

SPEAKER: He's not here.

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

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

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

DR. BELSITO: Yes.

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

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

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

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

DR. KLAASSEN: Dermal penetration and metabolism.

DR. BELSITO: Right.

DR. KLAASSEN: I would suggest --

DR. ANDERSEN: Yes, yes.

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

SPEAKER: Right.

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

DR. KLAASSEN: Yes, yes.

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

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

SPEAKER: That's fine.

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

Dr. Marks' Team

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

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

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

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

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

DR. BERGFELD: It's six months.

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

DR. ANDERSEN: The Danish decision was under three.

DR. MARKS: Under three.

DR. SLAGA: Under three?

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

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

DR. ANDERSEN: Yes.

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

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

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

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

DR. MARKS: Okay.

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

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

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

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

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

DR. SHANK: Carboxy esterase.

DR. MARKS: Esterase, okay.

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

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

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

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

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

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

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

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

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

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

DR. SLAGA: On anything.

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

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

DR. MARKS: Yeah, that would come out.

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

DR. MARKS: Tom?

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

DR. BERGFELD: Infants were separate because --

DR. SLAGA: Yeah, we already say that.

DR. BERGFELD: We already said it in the discussion.

DR. MARKS: Pardon?

DR. SLAGA: Infants were separately considered because they would be a sensitive subpopulation for any agent capable of causing male reproductive effects.

DR. MARKS: Right, and this was actually when we had the outside -- as I recall -- expert discuss endocrine disruptors, so we are very so to speak sensitive about that potential issue relevant to parabens.

So Ron Shank, in light of looking at now that memo that Alan pointed out and looking at our going back to the margin of safety calculations and specifically relevant to infants, do you think we need to reopen?

DR. SHANK: I can't find in the Danish report yet where these -- I thought they actually had experimental evidence that the carboxy esterase activity in infant skin was lower. But I can't find it, so --

DR. MARKS: It's kind of interesting, Alan, if I were to -- the reason the Danish mention the sun exposure is because of the presence of parabens in sunscreens. I'm not sure of their practices in infants, but I'm not sure whether they leave the nappy area open when they're out getting sun exposure or not. It certainly is probably more barrier compromised, but again, looking at the margins of safeties, they're in the thousands calculating for infants.

DR. BERGFELD: I think this is rather a political problem rather than a scientific one. And whether you reopen or not is immaterial to me actually, but the reality is I think with a re-review statement we don't need to reopen. However, if one thinks you have to specifically address the baby skin under six months of age, then I think we have to pull other kinds of scientific documentation on skin absorption in infant skin.

DR. MARKS: So we can certainly address this in the re-review statement, say that it was considered -- that would be published, be public knowledge, that we re-reviewed it and did not re-open and addressed those two issues that were in the memo.

Jay, you were going to --

DR. ANSELL: I would just agree with Wilma that if we want to start working on boilerplate, our experience with the aerosol suggests that it would best be done outside of a specific chemical.

DR. MARKS: Yes.

DR. ANSELL: And addressed much more broadly.

DR. MARKS: Okay, so Tom --

DR. SLAGA: I agree with Wilma, too.

DR. MARKS: So handle it as a re-review statement, not reopen? Ron, what do you feel? Does that sound okay?

DR. SHANK: Yeah, that's all right.

DR. MARKS: Okay.

DR. ANDERSEN: I think, Jim, the question of exactly what would this be, we have some flexibility on. The council used the word "reexamine." So they've asked you to reexamine it. If you want to look at more data, for example the couple of new Danish citations and more detail on what data are exactly available for infant skin, then you could ask CIR to prepare a re-review package. This isn't technically a re-review package. This is kind of pre-re-review. So if you wanted to look at those data, you would ask us to prepare a re-review package. Then you would have the opportunity to look at all of those data and say yes, we want to reopen it or no we don't. The council is very elegantly I think here given us a pre-step so that we have that flexibility of gathering additional information. It would allow any interested party to throw other data on the table for consideration by the panel in a re-review package that would occur later this year. I don't want to promise June, but later this year. So I think we have that flexibility because this is a non-usual request. They didn't say re-review it. They said reexamine it.

DR. MARKS: So I think that's quite reasonable. I mean, we have for today or tomorrow two re-review summaries, but they were pretty straightforward. This is slightly different, so we could just say we're going to see the re- review summary before it actually becomes the final summary so to speak. Does that -- is that what you're envisioning?

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

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

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

DR. ANSELL: I'm with you there.

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

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

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

DR. MARKS: Okay, so if --

DR. ANDERSEN: David, would you identify yourself?

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

DR. MARKS: Thank you.

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

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

DR. HILL: Okay.

DR. ANDERSEN: And that seems to not exist.

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

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

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

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

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

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

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

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

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

DR. ANDERSEN: Sounds good.

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

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

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

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

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

DR. MARKS: Yes.

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

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

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

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

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

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

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

DR. ANDERSEN: That would work.

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

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

Full Panel

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

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

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

DR. BERGFELD: Don?

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. BERGFELD: Alan?

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

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

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

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

DR. BERGFELD: Second?

DR. BELSITO: Second.

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

[Discussion of Parabens is mixed with discussion of Triclosan]

SEPTEMBER 2012

Dr. Belsito's Team

New Data

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

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

DR. SNYDER: Methylparabens.

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

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

DR. ANDERSEN: My strong desire would be to be scientifically correct and then let the political part play out as it will. Now I've got to see if I can remember which meeting we last talked about parabens. I think it was last December when Denmark had raised a series of questions about the use of parabens in baby products, and the Panel -- the Council had asked the Panel to look at those data, not to reopen or not, just look at those data. You did and you said that there was no need to change the Panel's opinion regarding the use of parabens, that the margin of safety adequately dealt with the issue at hand. I see this as the same thing. You don't have to make a decision to open or not reopen. I think you can simply say that the available data -- and again, in the triclosan report you have repeated dose toxicity study after study after study in which there was no identification of any muscle-related endpoint of concern. So while this is an interesting exercise at high exposure levels, in the available data that you did look at, this endpoint was not of concern. I think that's a scientifically-based view of how important is this information and there's no need to further consider this. As did the researchers, you can always throw in the thing at the end that says "more data would be useful." That's always true. I don't know that it gets you anything to say that. I think you need to make that scientific judgment that these data are not significant as regarding the question of triclosan safety.

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

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

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

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

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

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

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

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

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

DR. ANDERSEN: Exactly.

DR. SNYDER: My only comment, Alan, was regarding procedures. And so when an individual article is brought to our attention, do you do any expanded review of the literature, see if there's anything else that has kind of popped up? Or do we just take this as a standalone, ignoring that there may be some other reports affirming or contradicting? So procedure wise, what is our -- what do our procedures say that we do when these are presented? I understand what happens when we reopen or consider for reopening. We do an extensive literature search and try to data mine and see if there's anything else out there. But in this instance, do we do any additional data mining?

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

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

DR. ANDERSEN: Yup. The conclusion stands.

Dr. Marks' Team

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

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

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

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

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

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

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

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

DR. MARKS: Ron Hill?

DR. HILL: I basically agree. This is used in mouthwashes sometimes, is it not? Toothpaste? Yeah, but toothpaste, most of the time we're talking fluoride toothpaste, so we don't consider that, right? That's not a drug because --

DR. SHANK: Toothpaste.

DR. HILL: Toothpaste? Yeah, but toothpaste, most of the time we're talking fluoride toothpaste, so we don't consider that, right? That's not a drug because --

DR. BRESLAWEC: That is a drug.

DR. HILL: But not mouthwash?

DR. BRESLAWEC: The relevant use here is deodorant.

DR. HILL: Is what? Is deodorant?

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

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

DR. BRESLAWEC: Those are considered drugs as they are anti-gingivitis.

DR. HILL: They give a gingivitis indication and therefore fall out of our scope. Okay.

DR. MARKS: Rachel?

MS. WEINTRAUB: Yeah, so, I spent a lot of time looking through this material and I think one of the comments I think that Dr. Shank made was that, well, if you look at cosmetics use and the interaction of people with cosmetics, that's one thing, but if -- but the problem is that no one's looking at total exposure. And each sort of -- there are different entities, not necessarily one entirely parallel to ours, but I think that's a huge problem here.

I mean, I think this study shows, especially what I found concerning, was sex differences and aeroallergen sensitization. So, what is this explanation? Could there be some link to cosmetics? Some link to the use in deodorant?

I found this data to be of concern and thought that this should be reopened to consider this and see -- and for us to review the impact of this specifically on cosmetics as used in deodorant.

DR. MARKS: Halyna.

DR. BRESLAWEC: If I remember correctly, when CIR last considered the Triclosan report, at the end of the report, Dr. Katz, who was representing FDA at that point, asked the panel to consider the dosage that came out of cosmetic use together with other uses and that the panel determination on Triclosan safety was to have reflected that. That's my recollection. I would like, you know, to check the record on that because I do think that that was something that was a very, very thorough review that the panel did last time.

DR. MARKS: Okay, but --

DR. BRESLAWEC: We have, again, please note for the record the comments that we have provided on the individual studies. There are, we believe, some very serious issues with the study in terms of the relevance to human use and particularly cosmetic use, but, again, my main point here is I think the panel looked at that the last time it did its very thorough review of Triclosan, and I would like the record to be checked to see if that recollection is correct.

DR. MARKS: So, what I recall the prototype of do you consider just cosmetic use or do you consider all uses was with the phthalates in nail polish, and so there was concern of phthalate exposure from many different sources and we limited our consideration, again, to cosmetics because I think once we open up to all exposures it becomes a very difficult to handle, but I would like -- perhaps, Alan, obviously, you comment, but also the two Rons and Tom. I would be more in favor, as Dr. Shank indicated, we're looking at this as a cosmetic use, not in the total use of the universe.

But Alan, do you want to comment?

DR. ANDERSON: Yeah, I think Halyna's recollection is exactly correct, that for Triclosan at the end of the discussion, the panel was focusing on the use in cosmetics and the question was posited whether all of the exposures, and there were a great of information in the safety assessment on Triclosan in a wide range of product types, and the panels conclusion was, well, none of them, even if you added them all up, reached a threshold of toxicologic concern. And the way you phrased it was available study data, wide variety of studies, then the end points are listed. "Triclosan may be used safely in a wide variety of products in the present practices of use and concentration even if all product types were to contain Triclosan were used concurrently on a daily basis."

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

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

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

DR. BRESLAWEC: Many of them are IP studies.

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

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

DR. MARKS: So that, again --

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

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

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

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

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

MS. WEINTRAUB: Yeah.

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

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

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

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

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

Now, where CIR would also be obligated to send a response back to Dr. Scranton to Women's Voices for the Earth, that explains what we did as well, because they are on record as encouraging us to look at these new data and see what their impact is, so we owe her a response and we would do that.

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

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

Any other comments? I mean --

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

DR. MARKS: That's a formal --

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

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

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

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

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

DR. MARKS: Right.

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

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

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

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

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

DR. BERGFELD: Found in foods?

DR. MARKS: In foods?

DR. BERGFELD: It might be ingested.

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

DR. SLAGA: Yep.

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

Okay, so we --

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

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

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

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

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

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

DR. BERGFELD: Update.

DR. BRESLAWEC: Press release.

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

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

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

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

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

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

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

DR. ANDERSON: We need to --

DR. MARKS: I guess there though --

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

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

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

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

DR. ANDERSON: Yeah.

DR. MARKS: Which is searchable.

DR. BERGFELD: Yeah.

DR. ANDERSON: Yeah.

DR. MARKS: Good. So --

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

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

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

DR. ANDERSON: And press release.

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

DR. BERGFELD: And the website.

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

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

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

(Recess)

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

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

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

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

DR. BRESLAWEC: I don't know.

DR. HILL: Wave 2.

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

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

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

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

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

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

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

MS. GILL: Yes.

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

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

DR. SHANK: I did.

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

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

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

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

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

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

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

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

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

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

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

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

MS. GILL: Yes.

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

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

Full Panel

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

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

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

DR. BERGFELD: Don?

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

What they didn't look at that I thought was an important issue is atopic dermatitis, because we encourage people who are atopic staph carriers to use antibacterials, so they are likely to use more antibacterial soaps because of that. We don't know that data at all.

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

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

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

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

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

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

DR. BERGFELD: Separately.

DR. MARKS: -- triclosan.

DR. BELSITO: Second.

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

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

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

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

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

DR. BELSITO: Second.

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

Alan?

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

DR. BELSITO: Yes.

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

[Discussion of Parabens]

JUNE 2017

Dr. Belsito's Team

DR. BELSITO: So, now parabens. So seven ingredients that were previously reviewed, there are four total reports, the last was in 2008, and then being asked to add on 13 ingredients which we have not looked at. So sodiumethyl, this came up because sodium methyl paraben was included in the CIR 2017 priority list based on number of uses. And so even though it has been less than 15 years for many of the other parabens, it's like we need to state it or support it, so let's create this regroup the parabens. So we've done that, and we're now being asked for the data sufficient so support this whole new paraben family. Did I summarize that pretty correctly, essentially? So I guess the first question goes to Dan about the carboxcylic salts or parabens. Do they belong here.

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

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

DR . LIEBLER: No.

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

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

DR. BELSITO: What about manufacture? Is there

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

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

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

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

DR. LIEBLER: Not really.

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

DR. LIEBLER: Yeah.

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

DR. LIEBLER: Say that again?

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

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

DR. KLASSSEN: 43.

DR. BELSITO: Page 43.

DR. LIEBLER: Okay. Sorry.

DR. KLAASSEN: Under Toxicokinetics.

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

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

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

DR. BELSITO: What?

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

DR. BELSITO: I know.

DR. LIEBLER: So that's not a primary reference. So that won't really tell you where that data comes from.

DR. ANSELL: Yes. You'd have to be looking at the 2008 report.

DR. LIEBLER: So we would need to look carefully at that report to make sure that there wasn't something misinterpreted, or what type of study supports that assertion from the 2008 report.

DR. BELSITO: Okay. Because here on page 53 in diffusion cells, it was just the opposite.

DR. LIEBLER: You mean 43?

DR. BELSITO: Fifty three. Now, it's saying the penetration decreases with increasing chain length. So in the Franz diffusion cell, methyl paraben was greater than ethyl, greater than probyl, greater than butyl.

DR. LIEBLER: It's 43 in my docket.

DR. BELSITO: No, it's

DR. LIEBLER: Fifty three is EPI in my

DR. BELSITO: Oh, yeah, summary of new data. Sorry. Yeah. The original is probably 43. So, you know, we're contradicting ourselves here within the document. Yeah, so it's right below where we say it's inversely proportional. Now, it

DR. LIEBLER: So we need to resolve that discrepancy. We need to look at the other report.

DR. BOYLE: Okay.

DR. BELSITO: And then

DR. LIEBLER: But as a chemist, I could explain it either way. So (laughter). Just wanted to give you some confidence.

MS. FIUME: Very easy. You can explain it even better. The smaller the numbers, the greater the penetration.

Kind of like being a lawyer. And since we're close to this, on page 44 under the 1984 report, it says that, "Parabens are quickly absorbed from the blood? By definition that makes no sense. You can only you absorb into the blood. You don't absorb from the blood. I don't know what that's talking about.

DR. LIEBLER: I wonder if they're referring to partitioning from blood to tissue.

DR. ANSELL: Could be.

DR. SNYDER: Where's this

DR. KLASSSEN: That's on page 44 of the report under the 1984 the first sentence, "Parabens are quickly absorbed from the blood."

DR. BOYLE: Yeah, these are basically

VOICE: Quotes.

DR. BOYLE: excerpted as the come in those original reports.

DR. BELSITO: Neither of us were on the panel. We can't take the blame.

DR. ANSELL: Well, those people that were on that report at that time, well, explain them.

DR. BELSITO: So what do we make of the breast cancer studies? I think this is what the (inaudible) issue is now.

DR. KLAASSEN: Right.

DR. BELSITO: Endocrine disruption affects some breast cancer. Paul? Jan? Curt, help me out. So that's page 49 of the PDF.

DR. LIEBLER: So these are in vitro studies in cells. Some of the end points are relevant to cancer, but they're not necessarily predictive of carcinogenicity. So, you know, for example being, "Methyl paraben exhibit increased expression of aldehyde hydrate (inaudible) 1, (marker of human mammary stem cells.)" Well, it's true that, you know, something that could do that could be I mean, that's a characteristic of stabilizing stem cells could be a characteristic of a carcinogen, but it doesn't mean that it's carcinogenic. I was scrolling down to the EPI, and it is substantial epi for breast cancer.

DR. BOYER: (inaudible), right? In the epidemiological study section?

DR. SNYDER: But not for cancer anymore. For endocrine activity, right?

DR. BELSITO: Yeah. Lots. So where are you, Dan?

DR. LIEBLER: Well, I looked through the EPI studies (inaudible) breast cancer. So anything specific to breast cancer.

And then under the other relevant studies on PDF 50, Endocrine Activity, everything is cell model stuff. Some of it is with NCF 7 cells because these are breast cancer cell (inaudible). In other words, these are NCF 12A and NCF 10 (inaudible) all breast cancer (inaudible). And they observe paraben driven effects in the micro molar range. On molecular end points like ALB H 1 expression. The effects on mammospheres, which are cellular structures, multi cellular structures that have some organ like properties, but don't necessarily recapitulate (inaudible) an organ.

I don't think any of those would be considered to be predictive of carcinogenic potential unless you were predisposed to think that any effect is a carcinogenic effect. This section actually goes from back and forth between different cell types. I'm trying to remember what BT 474 is. I think those are other I think that's another breast cancer cell (inaudible).

DR. BOYER: I think so.

DR. LIEBLER: I think that's right. And it stimulated proliferation at half micro molar concentration. Again, a pretty nonspecific effect.

DR. BELSITO: Unless you have breast cancer.

DR. LIEBLER: But there are a lot of things that can stimulate proliferation of breast cancer cells in vitro that aren't carcinogenic. I mean, it's, you know, it's just an observation.

DR. BELSITO: Yes, I understand that, but we're not talking okay. So we're not saying that parabens cause breast cancer. Let me just throw this out. But a woman who is applying a nipple cream that is preserved with parabens, and has an introductal carcinoma, does this increase her risk of metastacies? Is this safe under those situations? I guess that's the question I'm asking.

DR. LIEBLER: Those are very clear for phenotype, and the thing is that none of these cell models is a model for addressing the question about the relationship between exposure and that phenotype? If you had, you know, some epidemiologic association, you know, with, for example, a particular subtype of breast cancer, you know, ER positive or triple negative, or something like that, (inaudible) breast cancers, then you'd go to an appropriate model system and ask the specific mechanistic questions. If these are just breast cancer cell lives and, in fact, in the paragraph about the BT 474s, for example, the effect was enhancing

DR. BELSITO: Where are you?

DR. LIEBLER: Oh. On PDF 50, the second paragraph. It's about isobutyl paraben.

DR. BELSITO: Okay.

DR. LIEBLER: So this is actually kind of a mixture of cell models and the narrative kind of goes in out of breast cancer cells lives and other cell lives. So it starts out, "Isobutyl paraben antagonize the estrogen receptor in Chinese hampster ovary cells. The effect was statistically significant at great than 25 micro (inaudible)." In other words, a very high concentration.

"Butyl paraben increased the number of BT 474 cells entering S phase concentration half micro molar. The effect was enhanced in the presence of ligand heregulum which is a stimulator of the EGF receptor, or it's a possible stimulator of the EGF receptor."

And then glucocoticoid like activity was 1.5 milli molar for butyl paraben, and 13 milli molar for propyl paraben. These are very high concentrations.

I mean, this is just kind of one off cell, throw in a chemical, make measurement some end points, and this is the type of thing I rail against all the time on this panel when we get data like this because it really doesn't mean anything.

DR. BELSITO: Okay.

DR. LIEBLER: Just throwing in chemical into particular cell lives, and you're observing something, and you put it in a low impact journal.

DR. BELSITO: Okay. So you'll write the defense in the discussion?

DR. LIEBLER: Sure.

DR. BELSITO: And will craft the defense

DR. LIEBLER: I will, sir.

DR. BELSITO: why we're not concerned about the effects on

DR. LIEBLER: Yeah.

DR. BELSITO: breast cancer. The other thing that I found that was sort of just not logical to me was this in Haines study, and the association with (inaudible) and some food sensitizations where the effect was seen only for ethyl paraben, but not for any of the other parabens.

Can anyone come up with an explanation other than it doesn't make sense?

DR. LIEBLER: It makes little sense.

DR. BELSITO: Yeah. I mean, why would ethyl paraben create a respiratory issue when methyl, and propyl, and butyl don't? So this was looking at data, and looking at urine parabens, right? That's where they got urinary concentrations. I'm looking for an association between

(inaudible) allergen and food sensitization or both.

DR. BOYER: This is another study like many

(inaudible) studies where they're really looking for associations between many different things, and they looked at 35, 40, 50 possible associations, and just by chance you'd expect at least some of them to show up as statistically significant.

So it could very well be that that explains why sometimes (inaudible) to tox out like this. It's just chance.

DR. ANSELL: Yeah. For these really data rich chemicals, you really need to rely more heavily on a weight of evidence approach. You know, if you look at a 95 percent percentile significance, and you measure 20 parameters, one of them is going to show a statistical relationship, and I think in the parabens if I'm not mistaken, we often see that. We'll see a statistical significance on the use with a paraben that isn't even used in those products. You just have to aggregate it, (inaudible) together to try to clarify the picture.

DR. BELSITO: Okay. Explain this (inaudible).

MS. LORETZ: And kind of along the same lines, one suggestion we had was two add for hydroxybenzoic acid to the report. It does have an inky name. It's not used by itself so much, but it is common metabolite, and it kind of gets at that question why would be (indiscernible 4:40:59:). I've used it. It wouldn't just be (inaudible)). There is a common metabolite.

So we think that's kind of is important to it makes more sense of the data then.

DR. LIEBLER: Okay.

DR. BELSITO: So do we want to add

DR. LIEBLER: That's fine with me.

DR. BELSITO: Okay.

DR. LIEBLER: I saw the recommendation. Seems reasonable. Other uses?

MS. LORETZ: No.

DR. BELSITO: No. It's not a cosmetic chemical.

DR. LIEBLER: Oh, it's not a not

MS. LORETZ: No, (inaudible).

DR. LIEBLER: Hasn't anything in it. Okay. But there are no uses. But there are data.

MS. LORETZ: Yes.

DR. LIEBLER: Okay.

DR. BELSITO: So the do we need to address the new data also on the thyroid effects? I guess this goes to Paul or Dan.

DR. LIEBLER: This is on page 50 at the end of the endocrine activity section?

DR. BELSITO: Yeah.

DR. ANSELL: It's in the 26th healthy paragraph?

DR. BELSITO: Uh huh.

DR. ANSELL: Well, it ends up there. It says the differences could not be attributed to the treatment. Can someone elaborate a little bit on that?

DR. BOYER: In the way this study was done, for the first week, the subjects were treated with the ointment, with the lotion without the parabens in them, in it, and the

(inaudible) hormone levels were measured in the blood samples. And during the second week, during that daily treatment, a full body application of the ointment with the parabens, again they generated that sort of data, and statistically that could we tell the difference. And there's such a variation from day to day, and hormone levels, and so on, even from hour to hour that there was no way to attribute any differences specifically to the exposures.

DR. KLAASSEN: Okay. So this is really talking about the minor differences.

DR. BOYER: Right. I think the were we were talking about differences. They weren't particularly statistically significant, and they were just simply pointing out that there were these minor differences, but they couldn't explain them.

BELSITO: Okay.

DR. KLAASSEN: I guess I think maybe that needs to be reworded a little bit. I don't know. It almost you know, while it says, "minor differences," I guess that's the tricky word in the whole paragraph is that minor differences I mean to me when they say the word, "differences," it is statistically different.

DR. BOYER: And in this case, they used the word that's their word, "minor," and it to them means that they weren't statistically significant, but they were pointing out they were indicating that their data showed some differences.

DR. KLAASSEN: I think maybe we need to put something in there, "minor differences, however, not statistically significant." Could be if they used the word, "differences," I'd want to use the word, "differences." You might say there was a trend or something, but, yeah, go ahead. You know, in a parentheses, "not statistically significant That would make that paragraph much

DR. BELSITO: Are we sure that they were not statistically significant?

DR. BOYER: I'm positive, yes.

DR. BELSITO: Okay. Okay. So getting back to the addition of the carboxcylic salts, we have absolutely no data on them. You're comfortable with read across from everything else?

DR. LIEBLER: Yes.

DR. BELSITO: Okay. And you're going to draft the

DR. LIEBLER: Couple of sentences on the in vitro well on the endocrine effects of the parabens. It's mostly cell model down at least what's cited here

DR. BELSITO: Right.

DR. LIEBLER: except for the thyroid, thyroxin stuff we just talked about.

DR. BELSITO: Right.

DR. LIEBLER: But for all the cell model stuff, I can draft a two or three sentence section for the discussion and send it to Lillian.

DR. BELSITO: Okay. Then on page 84, or did I just tab it there? Anyway, in the report where you had this whole margin of exposure calculation, it's on page 105 of this report. I guess I flagged it on page 84. So based upon the new data, do we need to recalculate this margin of exposure table?

DR. KLAASSEN: Well, it was based on the (inaudible) for single, and (inaudible) for multiple, right?

DR. BELSITO: Right.

DR. KLAASSEN: If that still holds, it's still valid.

DR. BOYER: Well, it's also based on a NOAEL of 1,000 milligrams per kilograms per day.

DR. BELSITO: Right.

DR. KLAASSEN: And the Hoberman paper that was considered back in 2008

DR. BELSITO: Right. So does our need data change our NOAEL for any of the endocrine end points, or repro end points, or breast cancer end points, or any end points.

DR. BOYER: And the Women's Voices for the Earth comments in particular, they pointed out specifically a study by Bolberg in 2016, which has been incorporated into the safety assessment report. It's an old study done with rats, and they are reporting that for end points like distances and so on, there is an effect of 100 milligrams per kilogram per day. And they're also they also reported that there are some effects on a male that the parameters down to 10 milligrams per kilogram per day. And they also reported that there are some effects on a male reproductive parameters down to 10 milligrams per kilogram per day.

And, in fact, the SCCS opinion that did a similar calculation before the CAR did their calculation, they more or less dismissed the Hoberman study. They didn't use the 1,000 milligrams per kilogram per day. They used a older study that was published by OEC that indicated again based on some effects, did not necessarily consider the adverse effects on male reproductive organs, that the NOAEL should be something like 2 milligrams per day, grams per day. So that's what they used in their calculation is close to 1,000 milligrams per kilogram per day.

So the question really is if you take into consideration the Bolberg 2016 paper, does that provide enough motivation to shift the NOAEL using these calculations from 1,000 down to 10,000 down to 10 milligrams per kilograms per day, or even down to 2 milligrams per kilograms per day?

DR. BELSITO: That was my question.

DR. BERGFELD: It's a big change.

DR. KLAASSEN: Where what page is that study described on?

DR. BOYER: It's actually (inaudible). I think it's page 54.

DR. BELSITO: Yeah. That's where I put my note, I think. Page 84,

DR. BOYER: Page 84, yes.

DR. BELSITO: It's Table 12.

DR. BOYER: If you look at the last column under that entry, and the second paragraph if you look at the last column on that entry, the second paragraph, that pretty much summarizes it. Identifies the end points that were deemed to be statistically different at the 10 milligram per kilogram per day dosage rate.

DR. BELSITO: But, in fact, there was not a NOAEL at 10. Effects were seen at all doses, so it's a LOAEL.

DR. BOYER: That's true, yes.

DR. BELSITO: So the last time that we reviewed this, we were concerned and we calculated the margins of exposure and came out with levels of 1,000 or greater for adults and children. And so my question to you is based upon this new data, do we need to recalculate that and look at this before we sign off on the parabens?

DR. LIEBLER: Unless there's a flaw in the study, I don't think it's anything we can ignore.

DR. BELSITO: I'm sorry. Unless there's a flaw, there's nothing what, we can ignore?

DR. LIEBLER: Unless there's a flaw in the study, I don't think we can ignore this.

DR. BELSITO: So then we have to do the recalculation?

DR. BOYER: What study specifically are we looking at here?

DR. LIEBLER: Table 12, the first entry. Butyl paraben (inaudible).

MS. BECKER: Reference 59.

DR. KLAASSEN: Table 12.

DR. BELSITO: Here we go. Okay. Search for CYP19A1 is probably the quickest way to get to it.

DR. LIEBLER: He's got it.

DR. SNYDER: And then again, there's lots of data there. The only thing that was altered at 10 was the sperm counts, and sperm counts are not considered to be a very sensitive are considered to not be a very strong parameter for effects, epididymal sperm counts, and so there were effects, but they were all in 100 or greater. Even that's less than 1,000, I guess, so

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

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

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

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

DR. BELSITO: For negative studies.

MS. LORETZ: Yeah.

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

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

DR. KLAASSEN: Right.

DR. BERGFELD: But usually mammalian outweighs AMES.

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

DR. BERGFELD: Oh.

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

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

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

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

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

DR. KLAASSEN: You know anything about that?

DR. LIEBLER: Nothing about that.

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

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

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

DR. BELSITO: Right. Using the broad definition of environmental to include (indiscernible 4:01;34) exposures, but, yeah.

DR. KLAASSEN: So, in essence, he's kind of like taking a oral contraceptive drug?

DR. BELSITO: Well, except the effects seem to be more in male than female.

DR. KLAASSEN: But that's why we're seeing this is kind of decreasing the maleness of a male. All right.

DR. BELSITO: Right. Well, no. But there is epidemiologic data, I believe, that there is there is increasing incidents of hyperspatius among male children being born in the United States. There's a lot of that data, and then there was data on chemo to paraben levels in women of child bearing age too, wasn't

MS. FIUME: (inaudible).

DR. BELSITO: Yeah. I mean, so there's a lot of anecdotal data, you know, just like the phthalate, and adipose tissue increasing and all of that.

So I mean, it's a real hot button issue without clear answers, so I think we need to be as scientifically rigorous as possible. So, I mean, this guy that he's a repro tox person?

DR. KLASSSEN: Oh, yes.

DR. BELSITO: And, I mean, he's certainly closer than Hamburg, Germany and might be

DR. KLAASSEN: Well, two.

DR. LIEBLER: I think we talked to both of them.

DR. KLAASSEN: That's what I was thinking.

DR. LIEBLER: Yeah.

DR. BELSITO: Okay.

DR. LIEBLER: I mean, we know judging, you know, from our experience and working with

DR. BELSITO: Yeah.

DR. LIEBLER: he's excellent, and has really got broad knowledge, and he's got a great sense of what the relevance of different model animal model end points would be to possible exposure effects, and that's really important in interpreting, you know, from these studies in rats, for example, and but I think we get too reads from outside experts and be important.

DR. BELSITO: Okay. So my recommendation would be to table this, and to invite two different experts in reproductive and toxicity, specifically, to review with us the data that's available on parabens, and how we can interpret that in terms of safety as used in cosmetics.

DR. LIEBLER: Right.

DR. KLASSSEN: One of the problems with this is that what can you add (off Mic.). Correct?

DR. SNYDER: But we do have other repro studies. We discussed this before (inaudible) discussion before, there was another study with trimethylpental isobuterate where there were minimal reductions in sperm counts in the testes or epididytimies of treated male rats, but there was no treatment related growths or microscopic lesions, and no effect on reproductive performance. So I think it's the same story.

I think the sperm count thing is not a very good indicator because there's so many things that could affect that outside of toxicity. And so if all other parameters are normal, particularly gross and microscopic examination, and reproductive performance, I think it has to be kind of taken very, very lightly, and as a direct effect of the chemical that's been applied.

So I think that's what this what we need to ask the experts, but I'm pretty certain that's what's going to be the the bottom line on this.

DR. BELSITO: But it would be nice to have the expert explain it.

DR. LIEBLER: Yes, I agree. Well, because it is a very high risk use so we need to go to somebody who is considered a reproductive expert. So I'd like to hear more about this Swarthily Ludwig cell marker in our 5A1. I've looked briefly online, and I saw a series of there was at least ten references to that as a surrogate marker for Swarthily cell differentiation, and it's a apparently, it's a it's a transcriptional regulator, and its expression is related to the downstream that are known to regulate differentiation of Swarthily cells.

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

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

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

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

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

DR. BELSITO: We would provide

DR. BERGFELD: Yes.

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

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

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

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

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

DR. BELSITO: Right.

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

DR. KLAASSEN: Yeah.

DR. BELSITO: Yeah.

DR. KLASSSEN: Especially this one.

DR. BERGFELD: Okay.

DR. KLAASSEN: And especially this one.

DR. LIEBLER: So basically, external consultants.

DR. BERGFELD: Right.

DR. BELSITO: No.

DR. BERGFELD: Okay.

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

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

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

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

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

DR. BOYER: Right.

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

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

DR. LIEBLER: Right. You need a longitudinal study

DR. BELSITO: Right.

DR. LIEBLER: to assess bioaccumulation.

DR. BELSITO: Right.

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

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

DR. BELSITO: Right.

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

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

DR. KLAASSEN: Two tens.

DR. BELSITO: What?

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

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

DR. KLAASSEN: Okay.

DR. LIEBLER: As opposed to uniten?

DR. BELSITO: Oh.

DR. LIEBLER: Kansas humor.

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

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

(Laughter).

DR. LIEBLER: (inaudible).

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

DR. SNYDER: No.

DR. BELSITO: no. Okay.

DR. LIEBLER: I concur.

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

Dr. Marks' Team

DR. MARKS: I'll first start with the May 19th memorandum from Ivan and Lillian with the subject "Re review of Parabens" and they said the Panel already agreed to reopen, so I take their word on it for reopening this. And that's one bad new ingredients and then secondly, that assess any updates on that.

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

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

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

DR. HILL: No. MAN: No.

DR. MARKS: Okay.

MR. STEINBERG: I have a comment.

DR. MARKS: Sure.

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

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

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

MS. EISEMAN: Oh.

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

DR. MARKS: (Inaudible) difference.

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

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

MR. STEINBERG: Yes.

DR. EISENMANN: Potassi

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

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

DR. HILL: No, they're not.

DR. EISENMANN: Yes, they are.

MR. STEINBERG: the salts of the esters are.

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

MAN: Oh, yeah.

MR. STEINBERG: But that by definition

DR. EISENMANN: those three are in.

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

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

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

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

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

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

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

DR. BOYER: That's right.

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

DR. BOYER: It's huge.

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

DR. BOYER: That's perfectly doable.

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

DR.BOYER: Right.

DR. MARKS: Would you repeat

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

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

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

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

(inaudible) didn't mention that study too.

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

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

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

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

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

DR. BOYER: Tortillas?

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

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

DR. EISENMANN: They must occasionally show up in food

DR. BOYER: yeah.

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

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

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

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

DR. SHANK: In the original report?

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

DR. HILL: Mm hmm.

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

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

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

DR. MARKS: Mm hmm.

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

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

DR. BOYER: Well, we certainly let me pull the paper that addresses the measurements of parabens in breast milk. But it's basically, you want to be able to show that we've done a complete review of the literature. We've included considered everything just about everything out there. Everything that certainly that's important. But still, it doesn't help us to tease out just what fraction of the parabens that appear in breast milk or any other tissue that's been mentioned, what fraction can be attributable to cosmetic use. In fact, it probably represents a very small fraction of the overall exposure. So we can soon discuss that and see (inaudible).

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

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

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

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

DR. HILL: Right.

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

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

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

DR. EISENMANN: No, it already was surveyed.

DR. SHANK: It was surveyed.

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

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

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

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

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

MR. STEINBERG: Metabolite.

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

DR. BOYER: That's correct.

DR. EISENMANN: So

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

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

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

(inaudible).

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

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

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

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

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

MAN: (Inaudible)

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

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

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

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

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

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

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

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

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

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

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

MS. BECKER: Spencer VC.

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

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

DR. HILL: Yeah.

DR. BOYER: on that observation?

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

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

DR. SHANK: Yes, it does.

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

DR. HILL: Well, I was going to say that one of the things that jumped out at me and trying to take my focus off estrogens for awhile when estrogenic activity was if you look at places where you do see some affects on either strand breaks or gene repair, in almost all cases you see higher activity under metabolic activation. So that's the other thing that sticks out in my mind is, metabolic activation would have nothing to do with estro raises and clinging to Parahydroxybensoic Acid, that would be metabolizing one end of the molecules or the other presumably for seeing differences between metabolic activation and not. Some compounds and not others, so some are clean, some are not going back to ames and then there are a few other agents. So, anyway.

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

DR. SLAGA: No.

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

DR. SLAGA: Right.

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

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

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

MR. STEINBERG: That was the original Darby.

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

DR. HILL: I know there is.

DR. BOYER: And the paper also

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

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

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

DR. HILL: No.

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

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

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

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

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

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

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

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

Okay. Any other comments?

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

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

DR. HILL: I know, right.

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

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

Full Panel

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

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

DR. MARKS: Second.

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

All those in favor of tabling? Unanimous.

(The motion passed unanimously.)

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

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

DR. BERGFELD: Okay. Bart?

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

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

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

All right.

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

[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 used 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 to 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.

Amended Safety Assessment of Parabens as Used in Cosmetics

Status: Draft Tentative Amended Report for Panel Review
Release Date: August 29, 2018
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ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of parabens as preservatives in cosmetic products. The Panel reviewed relevant data relating to the safety of these ingredients under the intended conditions of use in cosmetic formulations, and the issuance of a conclusion is expected.

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. According to the web-based *Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the ingredients in this paraben group are primarily reported to function in cosmetics as preservatives, and five are reported to also function as fragrance ingredients (Table 1).[1]

In 2017, the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) agreed to re-open the parabens report that was published in 2008,[2] and to include the paraben salts and 4-Hydroxybenzoic Acid. The previous CIR safety assessments of parabens were 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.

This re-review was initiated because 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. 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/day for Butylparaben, and margin of safety was re-calculated accordingly.

An exhaustive search was conducted for new data on the safety of parabens as well as 4-Hydroxybenzoic Acid in preparation of this previous iteration of the 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. Published data are identified by conducting an exhaustive search of the world's literature. 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 (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Pertinent data were discovered in the European Chemicals Agency (ECHA) database.[3-9] Data were also discovered in reports by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Union's (EU) Scientific Committee on Consumer Products (SCCP).[10-18]

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

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

CHEMISTRY

Definition and Structure

The ingredients in this safety assessment are paraben phenolic acids and their salts and carboxylic acids. The basic paraben structure is provided in Figure 1, and an example of a specific paraben (Butylparaben) is provided in Figure 2.

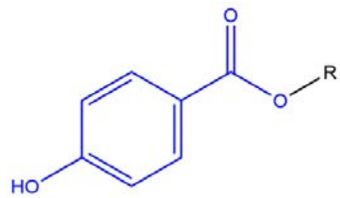


Figure 1. Paraben phenolic acids: a generic structure wherein R is an alkyl group from 1 to 4 carbons long, or is benzyl (or, in the case of 4-Hydroxybenzoic Acid, is hydrogen).

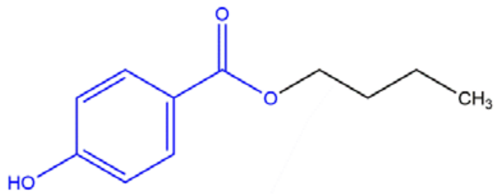


Figure 2. Paraben phenolic acids: an example, Butylparaben (wherein R from the generic structure in Figure 1, is an alkyl group 4 carbons long).

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

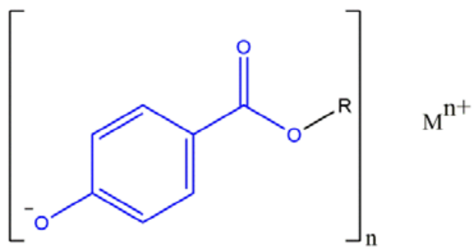


Figure 3. Paraben phenolic salts: generic structure wherein R is an alkyl group from 1 to 4 carbons long and M is sodium or potassium.

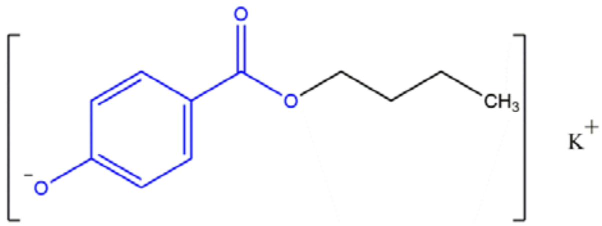


Figure 4. Paraben phenolic salts: an example, Potassium Butylparaben (wherein R, from the generic structure in Figure 3, is an alkyl group 4 carbons long and M is potassium).

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

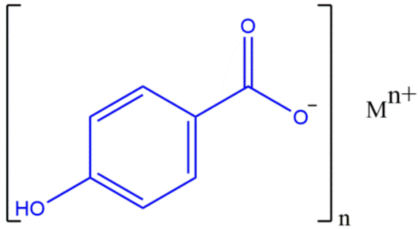


Figure 5. Paraben carboxylic salts: a generic structure wherein M is sodium, potassium, or calcium.

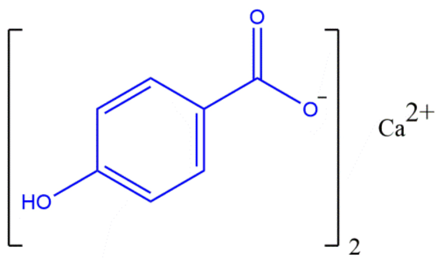


Figure 6. Paraben carboxylic salts: an example, Calcium Paraben (wherein M, from the generic structure in Figure 5, is calcium and n is 2).

Physical and Chemical Properties

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

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

The median particle diameter (D_{50}) of Sodium Methylparaben was reported to be 117.1 ± 17.5 µm; Ethylparaben was 307.5 ± 21.9 µm; Sodium Ethylparaben was 49.5 ± 6.4 µm; and Sodium Propylparaben was 37.8 ± 4.9 µm (Table 4).[3,4,6,7]

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

Method of Manufacture

Paraben phenolic acids (and salts) are prepared by esterifying 4-Hydroxybenzoic Acid with the corresponding alcohol in the presence of an acid catalyst, such as sulfuric acid, and an excess of the specific alcohol.[2] The acid is then neutralized with caustic soda, and the product is crystallized by cooling, centrifuged, washed, dried under vacuum, milled, and blended. Benzylparaben can also be prepared by reacting benzyl chloride with sodium 4-Hydroxybenzoic Acid. Paraben carboxylic 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).[20]

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 2018, Methylparaben was reported to be used in 11,626 formulations (9188 in leave-on formulations, 2380 in rinse-off formulation, and 58 diluted for (bath) use); this is an increase from the 8786 formulations reported in 2006 (Table 5 and Table 6).[2,21,22] Propylparaben had the next highest number of reported uses at 8885 (7331 of which are leave-on formulations); this was an increase from 7118 formulations reported in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to none.

The results of the concentration of use survey conducted by the Council in 2016 indicate Methylparaben had the highest reported maximum concentration of use; it is used at up to 0.9% in shampoos.[2,22] 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.

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.[21,22] 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) and could possibly be inhaled.[21,22] These ingredients are reportedly used at concentrations up to 0.41% in spray products (e.g., Methylparaben in the category of other fragrance products). In practice, 95% - 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays.[23-25] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[23,25] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[23] 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.[26-28]

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.[29] 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 available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak oestrogenic activity, but there are no established adverse outcome pathways for this effect. Should further information on adverse outcome pathways in mammals associated with weak oestrogenic activity become available, further assessment of these chemicals at Tier III could be required." [29]

The Scientific Committee on Consumer Safety (SCCS) of the EU has published several opinions on parabens over the last few years (Table 8).[10-16] 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…."[14,16]

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,[30] and has established maximum concentration limits of 0.4% for Methylparaben or Ethylparaben (single esters and their salts), 0.14% 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 0.14 %.[30,31] In addition, "…Butylparaben and Propylparaben should be prohibited in leave-on cosmetic products designed for application on the nappy area of children…."

Non-Cosmetic

<u>2008</u>

The European Food Safety Authority opinion cited reduction in daily sperm production in juvenile male rats fed Propylparaben at 10 mg/kg/day as the lowest-observable-adverse-effect-dose and contrasted these findings with the absence of effect for Methylparaben and Ethylparaben at doses up to 1000 mg/kg/day-.[2] The opinion restated the acceptable daily intake (ADI) of 0 to 10 mg/kg/day for the sum of Methylparaben and Ethylparaben. The opinion stated that Propylparaben should not be included in the ADI, and failed to recommend an alternative ADI because of the lack of a clear no-observed-adverse-effect-level (NOAEL).

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

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.[32]

An evaluation by the JECFA determined that the acceptable daily intake (ADI) of the sum of the Ethylparaben and Methylparaben to 0-10 mg/kg.[17] In view of the adverse effects in male rats, Propylparaben was excluded from the group ADI for the parabens used in food.[18]

<u>TOXICOKINETIC STUDIES</u>

Dermal Penetration

<u>2008</u>

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

In Vitro

In vitro dermal penetration studies are presented in Table 9.

In Franz-type diffusion cells, 2.3% - 3.3% of the applied concentration (0.1%) of Methylparaben penetrated porcine skin (fresh or after stored frozen) in 4 h.[33] In 24 h, 2.0% - 5.8% and 2.9% - 7.6% penetrated previously frozen intact and tape-stripped skin, respectively. In full-thickness porcine skin stored frozen, permeability coefficients ranged from 31.3 ± 1.6 to 214.8 ± 40 cm/h x 10^{-4}, decreasing (Methylparaben > Ethylparaben > Propylparaben > Butylparaben) with increasing lipophilicity.[34] Increasing the ethanol concentration or the exposure duration increased the retention of the parabens in the dermis, compared to the epidermis. Binary combinations of the parabens reduced their permeation rates, which was attributed by the authors to high retention in the epidermis and dermis.

In a different study, the penetration of parabens in 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.[35] Retention varied widely in the epidermis and dermis depending on the formulation.

Permeability coefficients estimated for Methylparaben, Propylparaben and Butylparaben in human cadaver skin (0.37 to 0.91 cm/h x 10^{-4}) and mouse skin (1.17 to 1.76 cm/h x 10^{-4}) were similar regardless of concentration tested (0.1% - 2%).[36] Residual quantities of parabens remaining in 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.[37] Previously frozen skin samples were thawed and mounted on Franz diffusion cells. A dose of 100 µL of lotion containing the test substance was applied to the skin once at t=0 or multiple times at t=0, t=12 and t=24. Thirty-six hours after a single application, penetration ranged from 0.007% ± 0.003 (Butylparaben) to 0.057% ± 0.03 (Methylparaben). Penetration 12 hours after the t=24 dosing ranged from 0.04% ± 0.01% (Butylparaben) to 0.6% ± 0.1 (Methylparaben).

Human

Butylparaben

Dermal penetration was studied in 26 healthy Caucasian male volunteers after application of 2% (w/w) Butylparaben in basic cream formulation, which also contained 2% diethyl phthalate and 2% dibutyl phthalate.[38] Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week (control week) were followed by daily application of the cream with the test substances for 1 week. Butylparaben serum concentrations in the blood were undetectable in most samples during the control week, with maximum concentrations not exceeding 1.0 µg/L. Butylparaben concentrations increased rapidly (mean peak concentration = 135 ± 11 µg/L in 3 h) after the first application of cream containing the 3 test compounds. Twenty-four hours after the first application, but before the following application, the mean serum concentration was 18 ± 3 µg/L. Butylparaben could be detected in most serum samples collected throughout the second week of this study.

Penetration Enhancement

In Vitro

Methylparaben

Skin samples were collected within 24 h postmortem from the back of a 77-year-old woman and leg of a 73-year-old man and stored frozen.[39] Split thickness (~350 µm) samples were thawed and mounted in vertical flow-Neoflon™ diffusion cells, and exposed to a saturated aqueous solution of Methylparaben, with (saturated) and without 4-cyanophenol (CP). Receptor fluid (phosphate buffered saline [PBS]) and skin samples (diffusion area 0.64 cm^2) were maintained at 32°C. Solutions containing one or both compounds were added to the donor chamber at t = 0, and the receptor fluid was sampled hourly for 18 h for analysis by high-performance liquid chromatography (HPLC). Compared with the single-solute solutions, the steady-state flux was more than 5-fold larger for Methylparaben and 2.6-fold larger for 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. This suggests that CP enhances skin permeation of Methylparaben primarily by increasing the solubility of Methylparaben in the SC (especially in the nonlipid regions), and Methylparaben increases skin permeation of CP by enhancing both the solubility and diffusivity of CP in the SC.

Absorption, Distribution, Metabolism, and Excretion (ADME)

<u>1984</u>

Parabens are quickly absorbed from the blood and gastrointestinal tract, hydrolyzed to 4-Hydoxybenzoic Acid, conjugated, and the conjugate excreted in the urine.[40] *Data obtained from chronic administration studies indicate that parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Varying amounts of parabens are passed in the feces depending upon which paraben is administered and the size of the dose. Little or no unchanged paraben is excreted in the urine. Most of an administered dose can be recovered within 5 to 72 hours as 4-Hydoxybenzoic Acid or its conjugates. Parabens appear to be rapidly absorbed through intact skin.*

<u>1986</u>

Metabolism of Benzylparaben is by sulfate conjugation of the parent compound.[41] *Excretion is in the urine. Small amounts of the ester are excreted unmetabolized or hydrolyzed to the benzyl alcohol and 4-Hydoxybenzoic Acid.*

<u>1995</u>

When male rabbits were administered either 800 mg/kg or 400 mg/kg of Isobutylparaben via a stomach tube, 77-85% of the ingredient was recovered as a form of 4-Hydoxybenzoic Acid; 20% was not recovered.[42]

<u>2008</u>

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

The ADME studies summarized below are presented in Table 10.

In Vitro

Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for α-fetoprotein (AFP).[43] On the other hand, the 50% inhibitory concentration (IC_{50}) of Benzylparaben was 0.012 µM. Butylparaben was biotransformed to 4-Hydoxybenzoic Acid in S9 fraction of skin obtained from 5-week old male rats, with maximum rate at saturating concentration (V_{max}) of 8.8 nmol/min/mg protein.[44]

Methylparaben and Ethylparaben were stable in human plasma, but Propylparaben, Butylparaben and Benzylparaben concentrations decreased by 50% within 24 h.[45] All parabens tested were rapidly hydrolyzed when incubated with human liver microsomes (HLM), depending on the alkyl chain length. Parabens, but not 4-Hydroxybenzoic Acid, were actively glucuronidated by liver microsomes and human recombinant uridine-5'-diphospho (UDP)-glucuronosyltransferases (UGTs).

Methylparaben, Ethylparaben, Propylparaben and Butylparaben were hydrolyzed by rat liver microsomes (RLM) and HLM in in vitro tests.[46] In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. Human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes.

Metabolism rates of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben by HLM were inversely proportional to chain length (the longer the alcohol moiety, the slower the hydrolysis).[47] This trend was also observed for human skin microsomes (HSM), but at much lower rates. Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the paraben. In contrast to human tissue fractions, all rat tissue fractions tested hydrolyzed the parabens at rates that increased as the ester chain length increased. Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin.

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

Animal

<u>Dermal</u>

Nine rats were given a single dermal dose of 100 mg/kg bw 4-hydroxy [ring-U-^{14}C]-labeled Methylparaben, Propylparaben, or Butylparaben in 60% aqueous ethanol vehicle. C_{max} (≥ 693 and ≥ 614 ng eq/g in males and females, respectively) occurred within 8 h post-application, and blood concentrations decreased until the last quantifiable concentration within 24 h.[49] Most of the dosage (≥ 46.4%) was not absorbed, and less than 25.8% was found in the urine. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of ^{14}C- labeled Butylparaben was absorbed 72 h following application to the skin in rats.[48] Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in liver.

Oral

In rats exposed to a single oral dosage of 100 mg/kg bw 4-hydroxy [ring-U-14C]-labeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥ 11,432 and ≥ 21,040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h.[49] Radioactivity was eliminated rapidly, with averages ≥ 69.6% recovered in the urine during the first 24 h. Radioactivity was excreted predominantly in urine in rats orally exposed to a single 10, 100, or 100 mg/kg bw/day dosage of ^{14}C- labeled Butylparaben.[48] The rate of urinary excretion was similar across all dosages, with ≥ 66% recovered in the first 24 h in males. Female rats excreted more Butylparaben in urine in the first 4 h after exposure, but there was no sex difference in the total dose excreted within 24 h.

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.[50] Mean total Butylparaben excreted in urine during exposure was 2.6 ± 0.1 mg/24 h. The concentrations peaked in the urine 8-12 h after application.

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.[51] Minor metabolites discovered had hydroxy groups on the alkyl side chain or oxidative modifications on the aromatic ring.

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.[40]

1986

Benzylparaben was not considered an acute toxic agent to mice or rats… Intravenous injections of Benzylparaben to dogs and cats caused no variation in blood sugar, circulation, and respiration.[41]

1995

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

Short-Term Toxicity Studies

1995

No significant histological changes were observed in mice dosed with 0.6% Isobutylparaben in the feed for 6 weeks. Mice dosed with 1.25% had atrophy of the spleen, thymus, and lymph nodes as well as multifocal degeneration and necrosis of the hepatic parenchyma. Mice dosed with 5% and 10% Isobutylparaben died within the first 2 weeks of the study.[42]

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.[52] Macroscopic and microscopic examinations revealed mild-to-moderate skin damage in female rats. The NOAELs for Isobutylparaben and Isopropylparaben 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, rats exhibited statistically-significant increases in relative liver weights, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, serum urea concentrations, lipid peroxidation and nitric oxide (NO) generation, and 17β-estradiol (E2) concentrations.[53] Statistically-significant decreases in total serum protein and albumin, glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities, serum testosterone concentrations, and T/E2 ratios, were also reported. Livers of affected rats exhibited dilated congested central and portal veins, highly proliferated bile ducts with fibrotic reactions, and multifocal areas of necrotic hepatocytes, and testes exhibited evidence of severe spermatogenic arrest, among other effects. Serum markers of lipid-peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically significantly elevated in rats exposed to 250 mg/kg bw/day Methylparaben.[54] Malondiadehyde levels were elevated in the liver in a statistically significant, dose-dependent manner, among other effects, in mice orally exposed to 1.33 - 40 mg/kg bw/day Butylparaben for 30 days.[55]

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.[40]

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

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

A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses up to 2 g/kg/day.

1995

Mice were orally dosed with 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks.[42] *Upon necropsy, the only effect noted was amyloidosis in 58% of dosed males and 33% of dosed females surviving past 78 weeks, as compared with 25% of control males and 10% of control females.*

2008

Ethylparaben, Propylparaben, and Butylparaben in the diet produced cell proliferation in the forestomach of rats, with the activity directly related to chain length of the alkyl chain.[2] *Isobutylparaben and Butylparaben were noncarcinogenic when given to mice in a chronic feeding study.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

1984

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

2008

Methylparaben was nonteratogenic in rabbits, rats, mice, and hamsters, and Ethylparaben was nonteratogenic in rats.[2] *Parabens, even at levels that produce maternal toxicity, do not produce terata in animal studies. One study examined the* *developmental toxicity of Butylparaben in rats and reported no effect on development up to an oral dose of 1000 mg/kg day-1, even with some maternal toxicity at that dose. The maternal toxicity NOAEL dose was 100 mg/kg/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 reported no spermatotoxic effects. Propylparaben did affect sperm counts at all levels from 0.01% to 1.0%. Epididymis and seminal vesicle weight decreases were reported in rats given a 1% oral Butylparaben dose decreased sperm number and motile activity in F1 offspring of rats maternally exposed to 100 mg/kg/day were reported. Decreased sperm numbers and activity were reported in F1 offspring of female rats exposed to Butylparaben at 100 or 200 mg/kg/day, but there were no abnormalities in the reproductive organs.

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^{-1}) 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. No adverse reproductive effects were found.

<u>Dermal</u>

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

<u>Oral</u>

The oral DART studies summarized below are described in Table 12.

Statistically-significant, dose-dependent reductions in anogenital distance and ovary weights were observed in offspring of female rats exposed orally to 100 or 500 mg/kg bw/day Butylparaben from gestation day (GD) 7- GD 21.[56] Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring were statistically-significantly reduced at 10 mg/kg bw/day or more. Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day. CYP19 and estrogen receptor (ER)α expression was statistically-significantly increased, and the expression of steroidogenic acute regulatory protein (StAR), cytochrome cholesterol side-chain cleavage enzyme (P450scc), estrogen sulfotransferase (SULT1E1), and androgen receptor (AR) in the testes and methylation rate of the ERα promoter were statistically-significantly reduced, in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD7 to GD21.[57] Weights of the testes, epididymal cauda sperm counts, and daily sperm production in male offspring were statistically significantly-reduced in the 400 and 1000 mg/kg bw/day groups of rats orally exposed to Butylparaben on GD7 to post-natal day (PND) 21.[58] 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.[59] Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

Prepubertal female rats exposed orally to 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Isopropylparaben on PND 21 to PND 40 exhibited statistically-significant delays in vaginal opening.[60] In the 1000 mg/kg bw/day groups, there were statistically-significant decreases in the weights of the ovaries (Methylparaben or Isopropylparaben) and kidneys (Ethylparaben or Isopropylparaben), and increases in the weights of the adrenal glands (Methylparaben, Ethylparaben, or Propylparaben) and thyroid glands (Methylparaben). Liver weights increased at all dosage rates of Butylparaben. Morphological studies of the uterus revealed myometrial hypertrophy after exposure to 1000 mg/kg bw/day Propylparaben or Isopropylparaben and in animals of all dose groups of Butylparaben and Isobutylparaben. Among the effects on serum hormone concentrations, estradiol concentrations were statistically-significantly reduced (Ethylparaben or Isopropylparaben) and prolactin concentrations were increased (Methylparaben) in the 1000 mg/kg bw/day groups. Reduced plasma leptin concentrations were observed in male and female offspring of young adult female rats exposed orally to 100 mg/kg bw/day Butylparaben.[61]

F2 pups exhibited statistically-significantly greater mortality at PND 7 and thereafter, compared with controls, in a DART study in which F0 females and their F1 offspring were exposed to 0.105 mg/kg bw/day Methylparaben by gavage.[62] 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.[63]

Methylparaben was associated with a statistically-significantly higher incidence of abnormal sperm in rats exposed to 1000-ppm or 10,000-ppm in the diet for 8 weeks, mostly sperm with no head in 4% to 5% of sperm, compared with 2.3% in 100-ppm and control groups.[44] Measurements of hormone concentrations were generally not altered, except that testosterone (T) and follicle-stimulating hormone (FSH) concentrations were higher in the 10,000-ppm Butylparaben-treated group, compared with the control group. The authors concluded that the NOAEC was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of about 1140 and 1100 mg/kg/day for Methylparaben and Butylparaben, respectively.

GENOTOXICITY STUDIES

1984

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

1995

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

At a concentration of 1 mg/plate, Isobutylparaben and Isopropylparaben had negative Ames tests in 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 parabens are generally non-mutageni.[2] *Ethylparaben, Propylparaben, and Butylparaben induced 1% to 3% increases in polyploid cell production in an in vitro assay using Chinese hamster ovary (CHO) cells; Ethylparaben and Methylparaben were judged to induce significant chromosomal aberrations (11.0% and 15.0% increases, respectively) in the same study.*

In Vitro

Propylparaben

Vero cells (derived from African green monkey kidney) were grown and incubated for 24 h with 0, 50, 200, 300, 400, or 500 µM Propylparaben at 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 5% fetal calf serum (FCS), 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine.[64] A statistically-significant, dose-dependent decrease in percentage of mitotic cells was observed across the concentrations tested (4-fold decrease at 500 µM, compared with control). Flow-cytometric analysis of DNA content revealed that the decline was attributable mainly to cell-cycle arrest at the G0/G1 phase. Immuno-detection techniques revealed statistically-significant induction of DNA DSBs (2-fold compared to control) verified by 8-OhdG staining at all concentrations tested (maximum intensity at 500 µM).

CHO cells were grown, and incubated for 1 or 3 h with 0, 0.5, 1, 1.5, 2, or 2.5 µM Propylparaben.[65] Sister chromatid exchange (SCE), chromosome aberration (CA), and DNA strand break (comet) assays were performed. Statistically-significantly elevated SCEs/cell and CAs/cell were observed in cells incubated with Propylparaben (≥ 1.5 µM) and Propylparaben (≥ 1.0 µM) for 3 h, respectively.

Butylparaben

Chinese hamster ovary cells were grown, and incubated for 1 or 3 h with 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mM or 0, 0.1, 0.25, 0.5, or 0.75 µM Butylparaben.[65] Sister chromatid exchange (SCE), chromosome aberration (CA), and DNA strand break (comet) assays were performed. Statistically-significantly elevated indices of DNA fragmentation were observed in cells incubated for 1 h with ≥ 0.4 µM Butylparaben. Comparatively high incidences of fragmentation was observed. Statistically-significantly elevated SCEs/cell and CAs/cell were observed in cells incubated with 0.75 µM Butylparaben for 3 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 injected subcutaneously in mice or rats when administered intravaginally in rats and was not co-carcinogenic when injected subcutaneously in mice.[40] *Propylparaben was noncarcinogenic in a study of transplacental carcinogenesis.*

1995

No changes in either neoplasm incidence or time to neoplasm development were observed in mice dosed with 0.15, 0.3, or 0.6% Isobutylparaben in the feed for 102 weeks as compared with controls.[42]

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. The estrogenic effect of parabens has been estimated by their competitive binding to the human estrogen receptors α and β. With diethylstilbestrol binding affinity set at 100, the relative binding affinity of the parabens increased as a function of chain length from not detectable for Methylparaben to 0.267 ± 0.027 for human estrogen receptor a and 0.340 ± 0.031 for human estrogen receptor β for Isobutylparaben. In a study of androgen receptor binding, Propylparaben exhibited weak competitive binding, but Methylparaben had no binding effect at all.

Parabens and 4-Hydroxybenzoic Acid have been studied in several uterotrophic assays. 4-Hydroxybenzoic Acid at 5 mg/kg day- 1 (s.c.) was reported to produce an estrogenic response in one uterotrophic assay using mice, but there was no response in another study using rats (s.c. up to 5 mg/kg day-1) and mice (s.c. up to 100 mg/kg day-1) and in a study using rats (s.c. up to 100 mg/kg day-1).

Methylparaben failed to produce any effect in uterotrophic assays in two laboratories, but did produce an effect in other studies from another laboratory. The potency of Methylparaben was 1000 to 20000 less when compared to natural estradiol. The same pattern was reported for Ethylparaben, Propylparaben, and Butylparaben when potency was compared to natural estradiol; in positive studies the potency of Ethylparaben was 346 to 25000 less; the potency of Propylparaben was 1612 to 20000 less; and the potency of Butylparaben was 436 to 16,666 less. In two studies, Isobutylparaben did produce an estrogenic response in the uterotrophic assay, but the potency was 240,000 to 4,000,000 less than estradiol. In one study, Benzylparaben produced an estrogenic response in the uterotrophic assay, but the potency was 330,000 to 3,300,000 less than estradiol.

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

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

The endocrine activity studies summarized below are described in Table 13.

In Vitro

Weak activation of murine peroxisome proliferator-activated receptor (mPPAR)α was seen in murine NIH-3T3-L1 cells at the highest concentrations of Butylparaben tested (100 µM).[66] Butylparaben activated mPPARγ with a lowest observed effect concentration (LOEC) of 30 µM and a maximal (4-fold) induction at 100 µM. The human data for Butylparaben (hPPARα and hPPARγ) were comparable to those obtained with mPPARα and mPPARγ, indicating a similar responsiveness.

Isobutylparaben antagonized the androgen receptor (AR) in CHO cells. The effect was statistically significant at ≥ 25 µM.[67] Butylparaben increased the number of BT-474 cells entering S-phase (concentration for half maximal stimulation of proliferation [EC_{50}] = 0.551 µM); the effect was enhanced in the presence of ligand heregulin (HRG; EC_{50} = 0.024 µM).[68] The EC_{50} for glucocorticoid-like activity in MDA-kb2 cells was 1.75 mM for Butylparaben and 13.01 mM for

Propylparaben.[69] Butylparaben at 25 µM statistically-significantly enhanced the hydrocortisone-induced glucocorticoid receptor (GR) signal by 85%; Methylparaben, Ethylparaben, and Propylparaben did not have this effect.[70]

Butylparaben exhibited estrogen agonism at all concentrations tested in T47D-KBluc cells.[71] The maximum effect was observed at 10 µM.

The EC_{50}s for stimulating proliferation of MCF-7 cells ranged from 0.4-40 µM, LOECs from 0.1-20 µM, and no observed effects levels (NOECs) from 0.05 - 8 µM for the parabens tested.[72] The parabens tested, in descending order of these values, were Isobutylparaben > Butylparaben > Propylparaben > Ethylparaben > Methylparaben. In comparison, corresponding values for E2 were $EC_{50} = 2 \times 10^{-6}$ µM, LOEC = 10^{-6} µM, and 1×10^{-7} µM. Propylparaben at 10 µM resulted in deformed acini and filling of the acinar lumen in non-transformed MCF-12A and MCF-10A cells.[73] MCF-7 and HCI-7-Luc2 mammospheres treated with Methylparaben exhibited increased expression of ALDH1 (marker of human mammary stem cells) and were larger than control and E2-treated mammospheres.[74] 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.[75] 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 compounds.[76] 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.[77] 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). 3OH and 2OH metabolites promoted significant ER-dependent transcriptional activity of an estrogen-response element (ERE)-luciferase reporter construct at 10 and 20 µM for 2OH and 10 µM for 3OH. 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.

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 concentration of 100 mg/kg/day orally for 5 weeks.[78] 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.[79] 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.[80] After 3, 7, and 21 days of treatment, 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, di-(2-ethylhexyl) phthalate (DEHP) and Butylparaben attenuated estradiol output but only when present together.[81] 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. At concentrations relevant to human exposure, DEHP (50 nM) and Butylparaben (100 nM) adversely affected steroidogenesis from the preantral stage onward and the effects of these chemicals were both stage-dependent and modified by co-exposure.

Relative uterine weights were elevated in immature Sprague-Dawley rats after treatment with ≥ 0.16 mg/kg bw/day Benzylparaben on PND 21-PND 23.[82] 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.[83] 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.[84]

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.[85] Transferase uridyl nick end labeling (TUNEL) assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h.

Human

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

Effects on Human Breast Cells

Methylparaben, Propylparaben, Butylparaben

MCF-10A non-transformed, immortalized human breast epithelial cells were exposed to 500 µM Methylparaben, 10 µM Propylparaben or Butylparaben in semi-solid 2% methylcellulose suspension culture, or 1 µM Methylparaben or 0.1 µM Propylparaben or Butylparaben in monolayer culture.[86] Ethanol served as the vehicle. The cells were grown in suspension culture (non-adherent conditions) to assess colony growth after a 17-day incubation period. Cells were grown in monolayer culture (adherent conditions) to assess cellular proliferation after a 7-day incubation period. In suspension culture, MCF-10A cells produced very few colonies and only of a small size. The presence of 500 µM Methylparaben or 10 µM Propylparaben or Butylparaben resulted in greater numbers of colonies per dish ($p < 0.05$) and greater average colony sizes ($p < 0.001$) compared with controls. Average colony sizes of cells grown with a paraben were comparable to those of cells grown with 17β-estradiol (70 nM). Concentration-response experiments showed that maximal numbers of colonies were formed at 100 µM Methylparaben or 1 µM Propylparaben or Butylparaben. Control experiments showed that the parabens did not influence the growth of MCF-10A cells under adherent conditions (i.e., monolayer cultures).

Human high-risk donor breast epithelial cells (HRBECs) were collected from the unaffected contralateral breasts of women undergoing breast surgery with a personal or family history of breast cancer, atypical neoplastic histopathology, and/or high mammographic density.[87] The cells were incubated for 7 days with 10 nM to 1 µM (vehicle not specified) Methylparaben in phenol red-free medium supplemented with 0.2% charcoal-stripped fetal bovine serums.[87] Some cells were exposed to 10 µM 4-hydroxy tamoxifen (OHT) or 1, 10, or 100 nM rapamycin for 24 h before functional analysis. Methylparaben substantially reduced the fraction of OHT-induced apoptotic cells in a concentration-dependent manner ($p = 0.001$) at all three concentrations: 57.82% ± 6.77% at 1 µM, 55.93% ± 10.54% at 100 nM, and 28.14% ± 11.3% at 10 nM. Methylparaben induced a detectable decline in endogenously accumulated reactive oxygen species (ROS) in all cell cultures. In early passage HRBECs, average reduction in ROS by Methylparaben treatment was 38% ($p < 0.02$), without an evident concentration-response relationship. Prior exposure to Methylparaben resulted in a concentration-dependent, complete-to-partial evasion from the G1-phase arrest induced by OHT, and concurrent increase in the S-phase fraction. In contrast, the growth inhibitory effects of OHT were not reversed by a combination of luteal-phase serum concentrations of E2 and progesterone. The maintenance of S-phase in OHT-treated cells, like apoptosis evasion, was correlated with increasing concentrations of Methylparaben ($p < 0.001$).

Aggregate Exposure

The aggregate exposure studies summarized below are described in Table 14.

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.[88] Median concentrations were highest for Propylparaben (16.8 ng/g tissue) and Methylparaben (16.6 ng/g tissue). Propylparaben concentrations were statistically significantly higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts.

Ethylparaben, Butylparaben, and Benzylparaben were detected in all placenta samples collected from healthy mothers.[89] The highest measured concentration was 11.77 ng Methylparaben/g tissue. The amount of Butylparaben, Ethylparaben, Methylparaben and Propylparaben was studied in human ovarian tumor samples.[90] 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.

One or more of 6 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, and Heptylparaben) as well as 4-Hydroxybenzoic Acid were detected in 20 human adipose fat samples collected from volunteers who underwent liposuction surgery.[91] 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 geometric mean (GM) concentration of 0.90 and 0.49 ng/g, respectively. Paraben concentrations in adipose fat samples of Caucasian volunteers (GM: 7050 ng/g) were higher than those of African Americans (GM: 3440 ng/g).

One study reported the free and total paraben concentrations in 16 human serum samples in the US.[92] The mean paraben concentrations in serum are 42.6 µg/L and 7.4 µg/L for Methylparaben and Propylparaben, respectively; whereas the free concentration of Methylparaben and Propylparaben in the serum is 2.2 µg/L and 0.5 µg/L, respectively, indicating that parabens that are not hydrolyzed to 4-Hydroxybenzoic Acid are rapidly conjugated.

National Health and Nutrition Examination Survey (NHANES, Fourth National Report) provides a large dataset for human spot urine levels of parabens collected from 2005 to 2014 with 2013 - 2014 the most recent collection period.[93] Total 2686 urine specimens from a representative sample of persons ≥ 6 years of age in the US general population were analyzed for the exposure level Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. 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 (limit of detection: 1 µg/L).

Epidemiology

The epidemiological study summarized below is described in Table 15.

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).[94]

DERMAL IRRITATION AND SENSITIZATION STUDIES

No new published animal or human irritation and sensitization studies were discovered in the published literature, and no unpublished data were submitted, since the 2008 CIR report.

1984

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

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

Parabens are practically nonsensitizing in the [human] population with normal skin. Paraben sensitization has occurred, especially when paraben-containing medicaments have been applied to damaged or broken skin. Even when applied to patients with chronic dermatitis, parabens generally induce sensitization in less than 3 percent of such individuals. Of 27,230 patients with chronic skin problems, 2.2 percent were sensitized by preparations of parabens at concentrations of 1 to 30 percent. Many patients sensitized to paraben-containing medications can wear cosmetics containing these ingredients with no adverse effects. Skin sensitization tests on product formulations containing from 0.1 to 0.8 percent of one or two of the parabens showed no evidence of significant irritation or sensitization potential for these ingredients.

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

1986

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

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

2008

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

Parabens are practically 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).[95] The keratinocytes were isolated from skin received as residual material from plastic surgery; PBMCs were enriched from buffy coats by density centrifugation. The cells were co-cultured in serum-free KGM-2 on 12-well cell culture plates. The co-culture was incubated for 48 h with or without a paraben. The concentrations tested were not specified, but likely ranged around 1 - 1000 µM, in dimethyl sulfoxide (DMSO; vehicle). Fluorescence-activated cells sorting (FACS) was used to identify and characterize dendritic cell-related cells (DC-rcs). Categorization of compounds as potential irritants and sensitizers was based on EC_{50}s calculated from concentration-response data for cell death (irritancy) and CD86-expression (sensitization), compared with vehicle controls. Substances with EC_{50} for cell death ≤ 50 µM were considered to be irritating, EC_{50} ranging from 50 - 1000 µM weakly irritating, and substances that did not reach the 50% threshold for cytotoxicity, or for which EC_{50} > 1000 µM, were considered non-irritating. Substances with EC_{50} for CD86-expression ≤ 12.5 µM were categorized as extreme sensitizers, > 12.5 µM < 50 µM as strong sensitizers, > 50 µM < 100 µM as moderate sensitizers, and > 100 EC_{50} as non-sensitizers. Methylparaben and Ethylparaben showed no potential for irritation in this test. Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben appeared to be weak irritants. The sensitization potential of the parabens tested was correlated with side-chain length: Methylparaben, Ethylparaben, Propylparaben, and Isopropylparaben were classified as weak sensitizers; and Butylparaben, Isobutylparaben, and Benzylparaben were strong sensitizers in this study.

Photosensitization/Phototoxicity

1984

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

In Vitro

Methylparaben

Normal human keratinocytes (HaCaT cells) were exposed to 0, 0.003%, 0.03%, and 0.3% (0, 0.197, 1.97, and 19.7 mM, respectively) Methylparaben in ethanol vehicle.[96] The cells were grown and incubated, with or without Methylparaben, for 6 or 24 h in DMEM supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine, and 100 U/mL penicillin/streptomycin at 37°C. Methylparaben-treated and -untreated cells were exposed to medium-wavelength ultraviolet light (UVB; 15 or 30 mJ/cm^2) after replacing the 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 statistically-significantly reduced cell viability within 6 h at 0.3% and within 24 h at 0.03%. Fluorescent microscopy using a fluorescent micro-plate reader revealed little evidence of reactive oxygen species (ROS) or nitric oxide (NO) production after Methylparaben exposure. UVB irradiation at 30 mJ/cm^2 (but not at 15 mJ/cm^2) induced small amounts of late apoptosis and necrosis. Methylparaben statistically-significantly elevated ($p < 0.5$) UVB-induced cell death, as evaluated by immunocytochemistry and flow cytometry; the propidium iodide (PI) index increased 3- and 7-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 15 mJ/cm^2, and 2- and 3-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 30 mJ/cm^2. Methylparaben at both concentrations elevated ($p < 0.05$) measurements of ROS and NO production and lipid peroxidation, and activated NFκB and AP-1 in UVB-irradiated cells.

OCULAR IRRITATION STUDIES

No new published ocular irritation studies were discovered in the published literature, and no unpublished data were submitted, since the 2008 CIR report.

1984

Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits.[40] *A primary eye irritation study in humans showed Methylparaben to be nonirritating at concentrations up to 0.3%.*

1986

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

2008

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

In Vitro

Methylparaben

Wong-Kilbourne-derived human conjunctival epithelial cells (WCCs) and immortalized human corneal epithelial cells (HCEs) were exposed to 0, 0.001%, 0.0025%, 0.005%, 0.0075%, 0.01%, 0.025%, 0.05%, 0.075%, and 0.1% Methylparaben.[97] The cells were cultured under standard conditions in Hank's balanced salt solution supplemented with 10% FCS, 1% l-glutamine, and 1% penicillin-streptomycin. HCEs were cultured under standard conditions in keratinocyte serum-free medium supplemented with 0.05 mg/mL bovine pituitary extract, 5 ng/mL epidermal growth factor, 0.005 mg/mL human insulin, and 500 ng/mL hydrocortisone. When the cells reached 75% - 80% of confluency, the medium was replaced with testing solutions and incubation continued for 1 h; after which the solutions were replaced with an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazonium bromide) solution, incubation continued for 4 h, and the MTT solution was replaced with MTT-solubilization solution (10% Triton X-10) that was spectrophotometrically analyzed. Metabolic activity/number of viable cells, measured via the MTT assay, was reduced in both cell lines in a concentration-dependent manner after exposure to Methylparaben; 0.001% Methylparaben (the lowest concentration tested) reduced activity/viability by 36.41% ± 33.95% in HCEs and by 24.48% ± 23.24% in WCCs. The highest concentration tested (0.1%) reduced activity/viability by 77.3% ± 33.8% in HCEs and by 73.92% ± 26.25% in WCCs.

CLINICAL STUDIES

Adverse Event Reports

1984

Industry complaint experience data showed low to moderate numbers of safety-related complaints with the incidence depending on the product.[40]

EPIDEMIOLOGICAL STUDIES

The epidemiological studies summarized below are described in Table 15.

Prospective Studies

In vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility.[98] Another study examined the association between the use of 14 personal care products (PCPs) and the urinary concentrations of parabens in 400 men (18 - 55 year of age).[99] 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 a 254 - 1333% increase in monomethyl phthalate and the three parabens urinary concentrations (Methylparaben, 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.[100] These parabens were also measured in breast milk samples collected at approximately 3 months postpartum (n = 56 women). 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. 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.

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).[101] 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. 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.[102]

The incidence of cryptorchidism and/or hypospadias, combined, was associated with placental concentrations of Methylparaben ≥ 1.96 ng/g (OR = 3.18; CI = 0.88 - 11.48) and Propylparaben concentrations ≥ 1.16 ng/g (OR = 4.72; CI = 1.08 - 20.65).[103] Linear regression analyses indicated an association between urinary Ethylparaben concentrations in 3-year old children and their body weights and heights.[104] The latter parameter was also associated with calculated estimates of aggregate exposures to parabens, including Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben. All regression coefficients calculated for girls and all other coefficients for boys were not statistically significant.

Linear regression analyses of data from the US National Health and Nutrition Examination Survey (NHANES) program indicated an association between reduced serum thyroxine (T4) concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben.[105]

Mean percent change (MPC) and the results of statistical tests for trends were not statistically significant in a study of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben in women undergoing infertility evaluation and ovarian volume (OV) or anthral follicle count (AFC).[106]

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).[107] The results also indicated an associations between urinary concentrations of Methylparaben and nonatopic asthma (OR = 0.025; CI = 0.07 - 0.90) and nonatopic wheeze (OR = 0.23; CI = 0.05 - 0.99).

No statistically-significant associations were found between the urinary concentrations of Methylparaben, Propylparaben, or Butylparaben and serum hormone concentrations, semen quality parameters and motion characteristics or all but one indicator of sperm damage in a comet assay.[108] The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

Cross-Sectional Studies

Analysis of data from the US 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).[109] 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.

Urinary level of Ethylparaben and Butylparaben increases the percentage of sperm with abnormal morphology.[110] In addition, the level of Isobutylparaben in urine increases 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.[111] Urinary concentrations of parabens were not significantly associated with any semen parameters or any of the reproductive hormone levels.

A community-based intervention study indicated that using PCPs that are labeled to be free of parabens for 3 days lowered urinary concentrations of some parabens, but increased concentrations of other parabens, in 100 adolescent 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.[112] However, concentrations of Ethylparaben and Butylparaben increased.

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 disruption and from DART studies.[56,58,62,63,113,114] 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.[58] 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. More 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. Such dose-response relationship was not demonstrated between Butylparaben exposure and reduction of epidermal sperm concentrations observed in another oral rat study following the same exposure scenario, which was therefore not considered as the principal study for the NOAEL derivation.[56]

The Panel considered exposures to cosmetic products containing a single paraben preservative (use level of 0.4%) separately from products containing multiple parabens (use level of 0.8%). Considering the worst-case scenario in which the consumer would use a set of cosmetic products containing the same preservative, adult (60 kg body weight) use of cosmetic products was estimated to be 17.76 g per day and infant (4.5 kg) use of cosmetic products was estimated to be 378 mg per day. In addition, a conservative estimate of 50% dermal penetration rate of parabens was selected in the calculation of the MOS.

For adults, the relevant calculations are (the calculations for infants are summarized in Table 16):

Systemic exposure dose (SED, Butylparaben) = 17.76 g/day of product x 0.4 % use concentration ÷ 60 kg person x 50 % absorption x 1000 mg/g conversion factor = 0.59 mg/kg/day

MOS (adult, Butylparaben) = NOAEL/SED = 160 mg/kg/day / 0.59 mg/kg/day= 270

Systemic exposure dose (SED, multiple parabens) = 17.76 g/day of product x 0.8 % use concentration ÷ 60 kg person x 50 % absorption x 1000 mg/g conversion factor = 1.18 mg/kg/day

MOS (adult, multiple paraben) = NOAEL/SED = 160 mg/kg/day / 1.18 mg/kg/day= 135

Margin of safety was also determined based on systemic exposure doses on infant and different dermal absorption rate, as shown in Table 16. Such conservative MOS for the most lipophilic compound Butylparaben could then be inferred to other less potency members of the parabens group.

Aggregate

In one study, a physiologically based pharmacokinetic (PBPK) model was developed and 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).[115] For the 2009 - 2010 sampling period, the predicted plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 70 kg male was 0.73, 0.21, and 0.052 µg/L, respectively; the predicted plasma free concentration of Methylparaben, Propylparaben, and Butylparaben in a 60 kg female was 1.19, 0.54, and 0.58 µg/L, respectively. An in vitro based cumulative margin of safety (MOS) was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the predicted free plasma paraben concentrations. The calculated cumulative MOS for adult females was 108, whereas the cumulative MOS for males was 444.

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/kg/day: with cosmetics and personal care products accounting for 50 mg/day; drugs, 25 mg/day; and foods, 1 mg/day.[116]

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.[117] 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%.

Methylparaben and Propylparaben are the most widely used preservative system in multiple cosmetic product types in North America.[118] They were found in 42% and 35% of the formulations over the years 1981- 2005, while Butylparaben and Ethylparaben are used much less frequently, with an average use over the same period of time of approximately 10% and 7%, respectively.

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 overestimated, 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.

A new approach has recently been developed to refine the aggregate exposure estimates using four of the more commonly used parabens (i.e., Methylparaben, Ethylparaben, Propylparaben, and Butylparaben).[118] 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 such nine cosmetic and skin care products was 1.61, 0.80, 1.70, and 0.016 mg/kg/day for Methylparaben, Propylparaben, Ethylparaben, and Butylparaben, respectively. When the refining factors were applied, and a conservative dermal penetration rate of 80% was chosen, the aggregate exposure compared to the simple addition approach was reduced by 51%, 58%, 90%, and 92% for Methylparaben, Propylparaben, Butylparaben, and Ethylparaben, respectively. In comparison, estimated internal exposure based on the 95th percentile values of parabens concentration in human urine was 19.9, 8.2, 1.39, and 0.86 µg/kg/day for Methylparaben, Propylparaben, Ethylparaben, and Butylparaben, respectively. This means that in all cases the aggregate exposure estimates are significantly greater than the exposures derived from the biomonitoring data.[118] If exposure via food was included, the aggregate exposure for Methylparaben and Propylparaben, which are used most extensively in foods, would only increase by 1% and 4%, respectively. That is, estimates for exposure to Methylparaben and Propylparaben via food are at last 25-fold lower than the estimates for aggregate exposure resulting from dermal exposure to cosmetic products.[116, 118]

Another study takes population viability of individual characteristics and behavior within the female US population into account.[119] Daily parabens intake was estimated based on skin permeation coefficient models, product use characteristics, and multi-pathway exposure model, i.e., aqueous dermal uptake, gaseous dermal uptake, inhalation intake, and environmentally mediated intake due to disposal after parabens use. The mean (2.5th –97.5th percentiles) modeled population intakes were 0.2 (0.003 - 0.8), 0.03 (0 - 0.2), 0.06 (0 - 0.3), 0.02 (0 -0.1) mg/kg/day for Methylparaben, Ethylparaben, Propylparaben, and Butylparaben, respectively. This intake estimate represents a user 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.[119]

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 2018, Methylparaben was reported to be used in 11,626 formulations; this is an increase from 8786 formulations in 2006. Propylparaben had the next highest number of reported uses at 8885; this was an increase from 7118 formulations in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to 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 Essex cream in 26 healthy Caucasian men, Butylparaben was detected in the serum, with maximum concentrations not exceeding 1.0 µg/L. Butylparaben concentrations increased rapidly within 3 h after the first application of cream containing the three test compounds, and could be detected in most serum samples collected throughout the second week of this study.

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

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

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference. Butylparaben was extensively hydrolyzed to 4-Hydroxybenzoic Acid as the major metabolite for both sexes and species. Methylparaben, Ethylparaben, Propylparaben and Butylparaben were hydrolyzed by RLM and HLM in in vitro tests. In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. 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 4-hydroxy [ring-U-^{14}C]-labeled Methylparaben, Propylparaben, or Butylparaben. C_{max} (≥ 693 and ≥ 614 ng eq/g in males and females, respectively) occurred within 8 h post-application, and blood concentrations decreased until the last quantifiable concentration within 24 h. Most of the dosage (≥ 46.4%) was not absorbed, and less than 25.8% was found in the urine. Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in liver.

In rats exposed to a single oral dosage of 100 mg/kg bw 4-hydroxy [ring-U-14C]-labeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥ 11,432 and ≥ 21,040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h. Radioactivity was eliminated rapidly, with averages ≥ 69.6% recovered in the urine during the first 24 h. The rate of urinary excretion was similar across all dosages, with ≥ 66% recovered in the first 24 h in males.

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

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

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

Serum markers of lipid-peroxidase (i.e., malondialdehyde) and hydroxyl radical production were statistically-significantly elevated in rats exposed to 250 mg/kg bw/day Methylparaben. Malondiadehyde levels were elevated in the liver in a statistically-significant, dose-dependent manner, among other effects, in mice orally exposed to 1.33-40 mg/kg bw/day Butylparaben for 30 days.

Weak activation of PPARα and PPARγ were observed in 3T3-L1 cells exposed to Butylparaben. Isobutylparaben antagonized the androgen receptor (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 or 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 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. The expression of estrogen-inducible gene (GREB1) was induced by 3OH and 2OH metabolites, and blocked by co-administration of an ER. The estrogenic activity of the 3OH and 2OH metabolites is mediated by classical ER mediated signaling. 3OH and 2OH metabolites showed the potential for favorable ligand-binding domain interactions with human ERα.

Longer diestrus phases and shortened the interval 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 and a higher frequency of androgen receptor binding activity.

In isolated mouse preantral follicle and hGC cultures, 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. At concentrations relevant to human exposure, DEHP (50 nM) and Butylparaben (100 nM) adversely affect steroidogenesis from the preantral stage onward and the effects of these chemicals are both stage-dependent and modified by co-exposure.

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

Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring were statistically-significantly reduced at 10 mg/kg bw/day or more. Adult prostate weights were significantly reduced at 500 mg/kg bw/day. CYP19 and ERα expression was significantly increased, and the expression of StAR, P450scc, SULT1E1, and AR in the testes and methylation rate of the ERα promoter were significantly reduced, in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD7 to GD21.

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

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

F2 pups exhibited statistically-significantly greater mortality at PND 7 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.

Dose-dependent decrease in percentage of mitotic cells was observed in Vero cells exposed to Propylparaben. Induction of DNA DSBs was also observed. Elevated indices of DNA fragmentation were observed in CHO cells incubated with Butylparaben. Elevated SCEs/cell and CAs/cell were observed in CHO cells incubated with Propylparaben.

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

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

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 statistically significantly higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts.

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

The amount of Butylparaben, Ethylparaben, Methylparaben and Propylparaben was studied in human ovarian tumor samples. 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.

One or more of 6 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, Heptylparaben) 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 geometric mean (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.

The US NHANES program provides a large dataset for human spot urine levels of parabens collected from 2005 to 2014. 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). The median concentration of Butylparaben in urine was below the limit of detection (0.1 µg/L). In females, the median concentration of Ethtylparaben 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).

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 paraben concentrations in the serum samples of total 16 humans are 42.6 µg/L and 7.4 µg/L for Methylparaben and Propylparaben, respectively, whereas the free concentration of Methylparaben and Propylparaben in the serum is 2.2 µg/L and 0.5 µg/L, respectively.

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

In prospective studies, in vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility. Another study examined the association between 14 PCPs use and urinary concentrations of parabens in 400 men (18 - 55 year of age). The largest percent increase

Women who used lotions in the past 24 h had significantly higher 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 Benzyl paraben (41%). Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben, respectively.

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

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). 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. 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.

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 or all but one indicator of sperm damage in a comet assay. The exception was a trend for increased tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

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.

Urinary level of Ethylparaben and Butylparaben increases the percentage of sperm with abnormal morphology. In addition, the level of Isobutylparaben in urine increases 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.

A community-based intervention study indicated that using personal care products that are labeled to be free of parabens for 3 days lowered some parabens urinary concentrations in 100 adolescent girls: Methylparaben and Propyl paraben concentrations decreased by 43.9% and 45.4%, respectively. However, concentrations of Ethylparaben and Butylparaben increased.

MOS for Butylparaben was determined based on an NOAEL of 160 mg/kg/day. MOS for adults are 270 and 135 for single and multiple parabens, respectively; MOS for infants are 952 and 476 for single and multiple parabens, respectively. A human paraben PBPK model developed to predict the plasma free paraben concentration based on 95th percentile parabens concentration in urine reported in US NHANES program (2009 - 2010 collection period). The model was then used to derive a cumulative MOS of 444 and 108 in adult men and women, respectively.

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 simple summed exposures from all multiple cosmetic product types.

PREVIOUS DISCUSSIONS

1984

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben[40]

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

A number of acute, subchronic, and chronic toxicity tests have been performed on the parabens using a wide variety of routes of administration. From these data, it is readily apparent that these ingredients exhibit a very low order of toxicity and must certainly be considered safe in this respect for cosmetic use in the usual quantities employed as a preservative.

When tested on human skin, each of the parabens began producing evidence of irritation only when concentrations exceeded 5 to 12 percent. Considering the order of magnitude of these concentrations, it may be concluded that the parabens are relatively nonirritating at the concentrations used in cosmetic products.

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

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

1986

Benzylparaben[41]

Section 1 paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the CIR Procedures, the Expert

Panel informed the public of its decision that the data on Benzylparaben are insufficient to determine that this ingredient, under the relevant condition of use, is either safe or not safe. The Panel released a "Notice of Insufficient Data Announcement" on October 10, 1984, outlining the data needed to assess the safety of Benzylparaben. The types of data required included:

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

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

1995

Isobutylparaben and Isopropylparaben[42]

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

2008

Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isopropylparaben, Isobutylparaben, and Benzylparaben[2]

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

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

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

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

The CIR Expert Panel compared exposures to parabens resulting from use of cosmetic products to a no observed adverse effect level (NOAEL). If that exposure is lower than the level shown to have no effect, then safety may be inferred.

The CIR Expert Panel selected a NOAEL of 1000 mg/kg day-1 based on the most statistically powerful and well conducted study of the effects of Butylparabens on the male reproductive system. The Panel did note the several studies in which spermatotoxic effects were noted at lower doses. In the Expert Panel's experience, studies of sperm counts are particularly unreliable and evaluation of reproductive organs is a much more reliable and reproducible indicator. The benchmark study noted above included a careful staging analysis of reproductive organ damage, which was likely to detect even subtle forms of damage.

The Expert Panel acknowledged that one study has reported estrogenic activity in the uterotrophic assay system of the paraben metabolite, 4-HYDROXYBENZOIC ACID . Three other studies did not detect any estrogenic activity. In considering the benchmark end point of male reproductive effects, the Expert Panel noted that the available animal studies of Methylparaben and Ethylparaben (parabens with the shortest ester side chains) have demonstrated an absence of an effect, so it is considered unlikely that 4-Hydroxybenzoic Acid has any significant estrogenic activity.

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

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

For adults, the relevant calculations are:

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

Systemic dose (multiple parabens) = 17.76 g/day of product x 0.8% use concentration ÷ 60 - kg person x 50% absorption x 1000 mg/g = 1.18 mg/kg/day

For infants, the relevant calculations are:

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

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

Based on these systemic doses and the NOAEL for Butylparaben of 1000 mg/kg/day, a MOS may be determined by dividing the NOAEL by the systemic dose to yield the MOS values shown in Table 17. The Expert Panel considers that these MOS determinations are conservative and likely represent an overestimate of the possibility of an adverse effect (e.g., use concentrations may be lower, penetration may be less). As presented, the MOS over the level demonstrated to produce no adverse male reproductive toxicity is around 3 orders of magnitude or greater. The CIR Expert Panel considers this MOS adequate to assure the safety of cosmetic products in which these preservatives are used.

DISCUSSION

The draft Discussion addresses the concerns and topics presented at the Panel Meeting, related to NOAEL determination, bioaccumulation potential, cumulative MOS, EU regulations of parabens, etc. This draft is preliminary and subject to further changes prior to release.

The Panel was concerned that new data from DART studies which indicated lower NOAEL values than the one used in the previous CIR safety assessment of the parabens. The Panel agreed that a subject matter expert should be consulted to review the reproductive toxicity data available for the parabens and identify additional relevant data that the Panel should consider, if any. This expert should provide professional opinions on the relevance of the animal-model toxicity endpoints reported in the DART studies available for assessing the safety of the parabens as used in cosmetics, and, should evaluate the quality of, and facilitate the interpretation of data on which NOAELs and MOS values may be derived to assess the safety of these cosmetic ingredients.

In response, Dr. George Daston, a Victor Mills Society Research Fellow at Proctor & Gamble, presented to the Panel on the topic of parabens and DART. He provided expertise, among other things, on the relevance of routes of exposure, paraben metabolism, and study design, in determining the validity of a multitude of DART studies for inclusion in this assessment. After careful consideration of all the new data in the category of endocrine disruption and from DART studies, the Panel determined the use of no observed adverse effect level (NOAEL) of 160 mg/kg/day to calculate a conservative MOS for Butylparaben, which could then be inferred to other members of the parabens group.

The Panel discussed the conflicting data from 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 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.

The Panel noted that some DART studies involving subcutaneous administration clearly showed adverse effects of the parabens on the endocrine or reproductive functions of rodents. However, route of subcutaneous exposure results in circumventing the physiological barriers and thus bypassing the portal of entry metabolism (e.g., first pass effects in the liver). These studies are not considered suitable for risk assessment and should be avoided when more adequate data are available. The Panel noted that concern was raised on the relevance of the oral animal studies to human risk assessment in that the rapid and effective metabolism of parabens in rodents does not place in humans. As properly conducted dermal absorption and/or toxicokinetic studies in humans are scarce, dermal absorption of parabens is reported by animal studies, ranging from 1% to 55%. Species differences in the esterase affinities and activities must be carefully taken into consideration for deriving a safe level of human exposure.

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 inactivated internally by conjugating with glucuronide, sulfate, or glycine prior to excretion. When applying to human skin, it has been claimed that parabens are extensively and nearly completely hydrolyzed into 4-Hydroxybenzoic Acid, the systemic absorption of un-metabolized parabens is low, and thus, detectable concentrations of parabens or their metabolites in the blood, urine or feces is considered as a result of exposures that are regular and frequent.

The Panel adopted that the parabens are relatively lipid soluble compounds, they would tend to bioaccumulate in the lipid fraction of the biological tissues. Recent studies have showed the presence of parabens in breast, adipose, and placenta tissues. However, the metabolism, the excretion and the pharmacokinetics of the parabens made accumulation in the body not an issue. It remains unknown as to whether the measured paraben results from long-term accumulation, from multiple potential sources, or current exposure. Some studies indicated that no correlations were found between parabens concentration in tissues and age groups of subjects, thereby suggests no bioaccumulation. The high levels of Methylparaben and Propylparaben observed in tissues could be due to the fact that they are the most common compound used as preservative not only in cosmetics and hygiene products, but also in food, beverages, pharmaceuticals household pesticides, cleaning products, paints, pet supplies, and paper products. Nevertheless, no epidemiological evidence suggested a direct causative effect on diseases and conditions be attributed to parabens exposure.

The Panel noted that the EU Cosmetic Regulation has banned the use of Isopropylparaben, Isobutylparaben, Phenylparaben, Benzylparaben, and Pentylparaben as preservatives in cosmetic products due to the lack of human risk evaluation, and has established maximum concentration limits of 0.4% for Methylparaben or Butylparaben (single esters and their salts), 0.14% for Propylparaben or Butylparaben (single esters and their salts), and 0.8% for the mixture of the these four ingredients, wherein the sum of the individual concentration of Butylparaben and Propylparaben can not exceed 0.14 %. The Panel recognized that SCCS opinion on the recommended maximum concentration of 0.14 % for Butylparaben was derived based on the following parameters, be aimed at achieving an adequate MOS ≥ 100: 1) a principal rat study which involved subcutaneous instead of oral administration, in which an NOEL of 2 mg/kg bw/day instead of an NOAEL was chosen for MOS calculation; 2) assuming that parabens were used as preservatives in all cosmetic products (17.4 g/day); and 3) 3.7% dermal absorption rate which was derived from the mean dermal absorption of 37% measured in human split-thickness skin, using a correction factor of 10 to account for skin metabolism as seen in the full thickness skin experiments. The principal study considered for the calculation of MOS as well as the derivation of the maximum concentration limit of 0.14% for Butylparaben herein suffered from additional critical limitations, e.g., not a guideline study, lack of effect on epididymis, and only one dose tested (Butylparaben at 2mg/kg/day).

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 can provide 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 discussed the issue of incidental inhalation exposure to paraben. The Panel noted that some of the parabens were reported to be used in cosmetic power and sprays at very low concentrations in products 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, 95% - 99% of droplets/particles would not be respirable to any appreciable amount. 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 http://www.cir-safety.org/cir-findings.

The Panel discussed the issue of skin sensitization exposure to parabens. The Panel noted that skin sensitization tests on product formulations containing from 0.1 to 0.8 percent of one or two of the parabens showed no evidence of significant irritation or sensitization potential for these ingredients. All animal sensitization tests indicate that the parabens are nonsensitizing. The Panel agreed that the results of these studies indicate that these ingredients do not have skin sensitization potential at cosmetic use concentrations.

CONCLUSION

To be determined.

TABLES

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

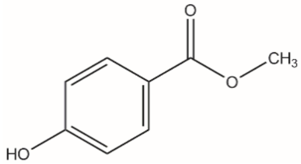
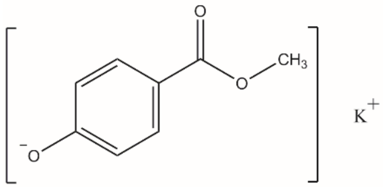
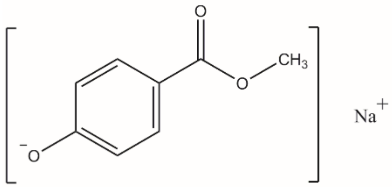
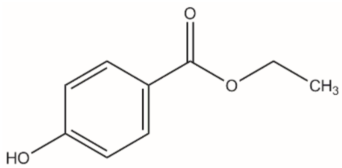
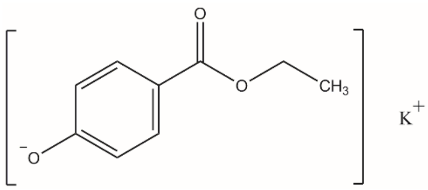
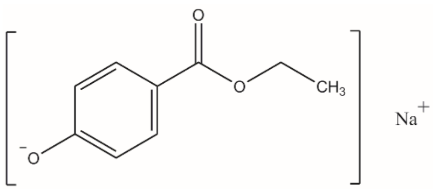
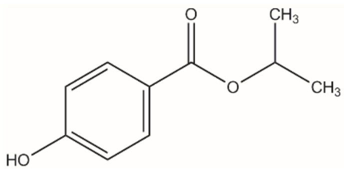
Ingredient CAS No.	Definition & Structure	Function
Parabens and Paraben Salts		
Methylparaben 99-76-3	Methylparaben is the ester of methyl alcohol and 4-Hydoxybenzoic Acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Methylparaben 26112-07-2	Potassium Methylparaben is the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

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

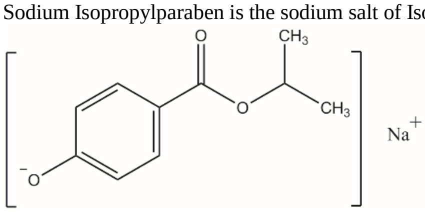
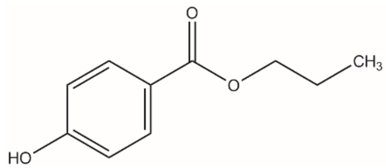
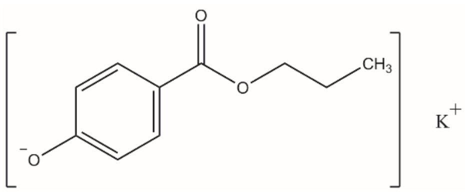
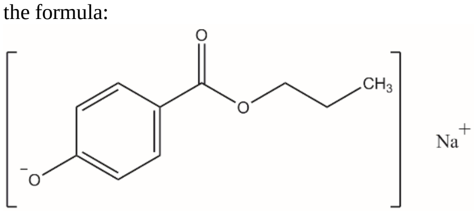
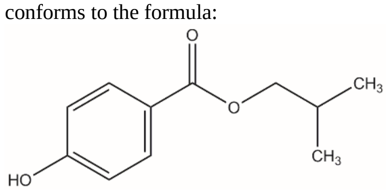
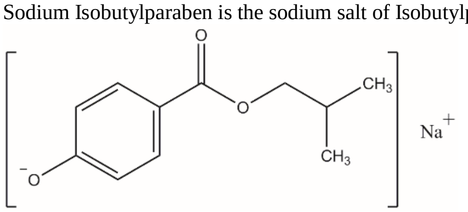
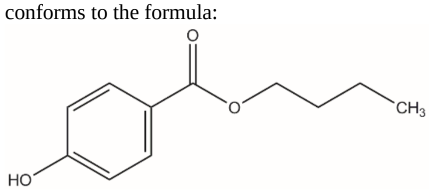
Ingredient CAS No.	Definition & Structure	Function
Sodium Isopropylparaben	Sodium Isopropylparaben is the sodium salt of Isopropylparaben: 	Preservative
Propylparaben 94-13-3	Propylparaben is the ester of n-propyl alcohol and *4-Hydoxybenzoic Acid*. It conforms to the formula: 	Fragrance ingredient,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

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

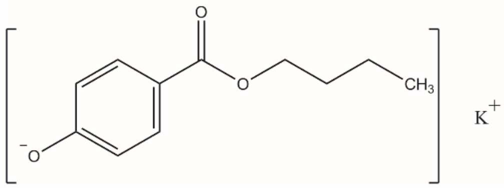
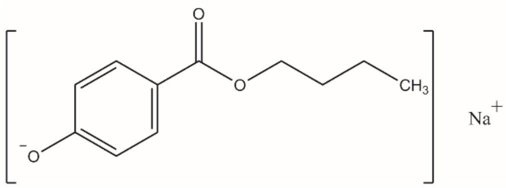
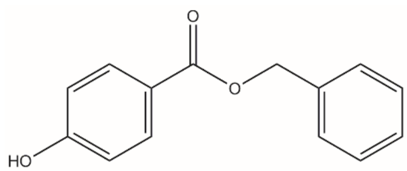
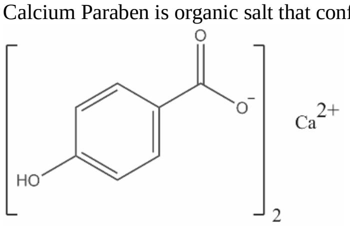
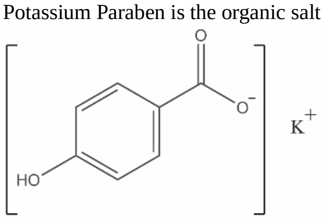
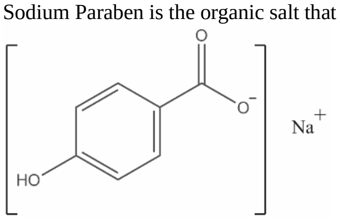
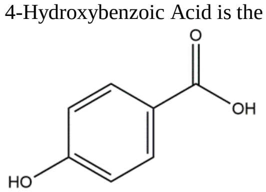
Ingredient CAS No.	Definition & Structure	Function
Potassium Butylparaben 38566-94-8	Potassium Butylparaben is the potassium salt of Butylparaben that conforms to the formula: 	Preservative
Sodium Butylparaben 36457-20-2	Sodium Butylparaben is the sodium salt of Butylparaben that conforms to the formula: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

Table 2. Previous CIR safety assessments of parabens.

Parabens	Conclusion	Reference
Methylparaben, Ethylparaben, Propylparaben, and Butylparaben	Safe as cosmetic ingredients in the present practices of use	1984[40]
Benzylparaben	Available data are insufficient to support the safety	1986[41]
Isobutylparaben and Isopropylparaben	Safe as cosmetic ingredients in the present practices of use	1995[42]
Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, Isopropylparaben, and Isobutylparaben	Safe in the present practices and concentrations	2008[2]

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Sodium Methylparaben		
Physical Form	Crystalline solid	3
Color	White	3
Molecular Weight g/mol	174.131	120
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_{ow}	-0.63	3
Disassociation constants		
pKa @ 23°C	8.4	3
Calcium Paraben		
Molecular Weight g/mol	314.306	121
Potassium Butylparaben		
Molecular Weight g/mol	232.32	122
Potassium Ethylparaben		
Molecular Weight g/mol	204.266	123
Potassium Methylparaben		
Molecular Weight g/mol	190.239	124
Potassium Paraben		
Molecular Weight g/mol	176.212	125
Potassium Propylparaben		
Molecular Weight g/mol	218.293	126
Sodium Butylparaben		
Molecular Weight g/mol	216.212	127
Sodium Ethylparaben		
Physical Form	Solid, powder	6
Color	White	6
Molecular Weight g/mol	188.157	29
Density g/cm^3 @ 20°C	1.34	6
Melting Point °C	268	6
Water Solubility g/L @ 23°C & pH 10.4	> 1000	6
log K_{ow}	-0.14	6
Sodium Isobutylparaben		
Molecular Weight g/mol	216.212	128
Sodium Paraben		
Molecular Weight g/mol	160.104	129
Sodium Propylparaben		
Physical Form	Solid, powder	7
Color	White	7
Molecular Weight g/mol	202.185	130
Density @ 20°C	1.24	7
@ 25°C	1.24	7

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Vapor pressure mmHg @ 20°C	< 0.001	7
Melting Point °C	302	7
Boiling Point °C	310 (decomp)	7
Water Solubility g/L @ 23°C	> 100	7
log P_{ow}	0.27	7
Methylparaben		
Physical Form	Powder	19
	Liquid	19
Color	White or colorless	19
Odor	Characteristic	19
Molecular Weight g/mol	152.16	2
Density g/cm^3 @ 137.2°C	1.1208	131
@ 20°C	1.209±0.06 est.	132
Vapor pressure mmHg @ 25°C	2.37×10^{-4}	19
Melting Point °C	131	2
	125-128	2
Boiling Point °C	270-280	2
	265	133
	140-141	134
Water Solubility g/L @ 25°C	2.50×10^{3}	19
	Slightly soluble	2
Other Solubility		
Alcohol	Very soluble	2
Benzene	Slightly soluble	2
Ether	Very soluble	2
Glycerin	Slightly soluble	2
log K_{ow}	1.93	35
Disassociation constants (pKa, pKb)		
pK_a	8.17	2
Ethylparaben		
Physical Form	Crystals or powder	135
Color	Colorless or white	135
Molecular Weight g/mol	166.18	2
Density @ 20°C	1.291	4
Vapor pressure mmHg @ 25°C	9.29×10^{-5}	135
Melting Point °C	116-118	2
	115-118	2
Boiling Point °C	297-298	2
Water Solubility g/L @ 25°C	0.885	135
Other Solubility		
Alcohol	Very soluble	2
Ether	Very soluble	2
Glycerin	Slightly soluble	2
log K_{ow}	2.47	4,135
	2.27	35
Disassociation constants (pKa, pKb)		
pK_a	8.22	2
	8.34	135
Propylparaben		
Physical Form	Crystal or powder	136
Color	Colorless or white	136
Odor	Odorless or faint	136
Molecular Weight g/mol	180.21	2
Density	1.0630	2
	1.28	136
Vapor pressure mmHg @ 25°C	5.55×10^{-4} est.	136
Melting Point °C	96.2-98	2
	95-98	2
Boiling Point °C	294	133
	271	136
Water Solubility g/L	0.0500	136
	Insoluble	2
Other Solubility		
Alcohol	Soluble	2
Ether	Soluble	2
log K_{ow}	2.34	5
	2.81	35
Disassociation constants (pKa, pKb)		
pK_a	8.35	2

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
	Isopropylparaben	
Molecular Weight g/mol	180.22	2
Melting Point °C	96-97	137
Boiling Point °C	294	133
	Butylparaben	
Physical Form	Crystals or powder	138
Color	White	138
Odor	Odorless	138
Molecular Weight g/mol	194.23	138
Vapor pressure mmHg @ 25°C	1.86×10^{-4}	138
Melting Point °C	68-69	2
	68-72	2
Boiling Point °C	309.2±15.0	132
Water Solubility g/L @ 20°C	0.0027×10^{2}	138
	Insoluble	2
Other Solubility g/L		
Alcohol	Soluble	2
Ether	Soluble	2
Glycerin	Slightly soluble	2
Disassociation constants (pKa, pKb)		
pK_a	8.37	2
	8.47	138
	Isobutylparaben	
Physical Form	Solid, powder	8
Color	White	8
Molecular Weight g/mol	194.25	2
Density g/cm^3 @ 20°C	1.105±0.06	132
Vapor pressure mmHg @ 25°C	0.000381	8
Melting Point °C	72.95 est.	8
Boiling Point °C	302.3±15.0	132
Water Solubility g/L @ 25°C	2.24	8
log P_{ow}	3.04	8
	Benzylparaben	
Physical Form	Solid, crystalline	9
Color	White	9
Odor	Odorless	9
Molecular Weight g/mol	228.25	2
Molecular Volume m^3/kmol		
Density g/cm^3 @ 20°C	1.224±0.06 est.	132
Vapor Density mmHg	0 est.	9
Melting Point °C	110-112	2
Boiling Point °C	389.8±17.0 est.	132
Water Solubility g/L @ 25°C	1.08	9
	10	2
Other Solubility g/L		
Propylene glycol	130	2
log P_{ow}	3.97	9
Disassociation constants (pKa, pKb)		
pK_a	8.18±0.15 est.	132
	4-Hydroxybenzoic Acid	
Molecular Weight g/mol	138.12	139
Melting Point °C	214.5	140
Boiling Point °C	336.2 est.	139
log K_{ow}	1.39 est.	141
Disassociation constants (pKa, pKb)		142
pK_a	4.57±0.10 est	

Decomp=decomposes on melting

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

Ingredient	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	Reference
Sodium Methylparaben	7.9±3	117.1±17.5	693.5±96.8	3
Ethylparaben	50±4.3	307.5±21.9	770.6	4
Sodium Ethylparaben	6.5±0.3	49.5±6.4	147.1±28.3	6
Sodium Propylparaben	6.7±0.3	37.8±4.9	164.5±36.7	7

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

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	Benzylparaben				**Butylparaben**			
	2018[21]	**2006[2]**	**2016[22]**	**2003[2]**	**2018[21]**	**2006[2]**	**2016[22]**	**2003[2]**
Totals*	**NR**	**1**	**NR**	**NR**	**3915**	**3001**	**0.00000006-0.5**	**0.00002-0.54**
Duration of Use								
Leave-On	*NR*	*1*	*NR*	*NR*	*3141*	*2409*	*0.0000000-0.5*	*0.00002-0.4*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*751*	*551*	*0.0000004-0.33*	*0.00004-0.54*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*23*	*41*	*0.00002-0.1*	*0.00004-0.07*
Exposure Type								
Eye Area	NR	NR	NR	NR	822	812	0.000002-0.5	0.00002-0.3
Incidental Ingestion	NR	NR	NR	NR	281	219	0.0000026-0.2	0.0008-0.1
Incidental Inhalation-Spray	NR	NR	NR	NR	13; 629[a]; 656[c]	27; 453[a]; 320[c]	0.00000011-0.1; 0.00059-0.22[a]	0.0004-0.2; 0.03-0.4[a]; 0.0004-0.4[c]
Incidental Inhalation-Powder	NR	NR	NR	NR	132; 6[b]; 656[c]	88; 21[b]; 320[c]	0.0057-0.3; 0.0001-0.24[b]	0.07-0.14; 0.05[b]; 0.0004-0.4[c]
Dermal Contact	NR	1	NR	NR	3160	2406	0.0000004-0.4	0.00004-0.54
Deodorant (underarm)	NR	1[a]	NR	NR	8[a]	10[a]	0.000025[d]	0.002[a]
Hair - Non-Coloring	NR	NR	NR	NR	283	246	0.00000011-0.22	0.0004-0.25
Hair-Coloring	NR	NR	NR	NR	45	28	0.0000005-0.05	0.03
Nail	NR	NR	NR	NR	42	21	0.00000006-0.07	0.003-0.2
Mucous Membrane	NR	NR	NR	NR	531	312	0.0000026-0.2	0.00004-0.11
Baby Products	NR	NR	NR	NR	11	28	NR	0.05
	Ethylparaben				**Isobutylparaben**			
	2018[21]	**2005**[2]**	**2016[22]**	**2003[2]**	**2018[21]**	**2006[2]**	**2016[22]**	**2003[2]**
Totals*	**3860**	**2679**	**0.00000032-0.65**	**0.00002-0.98**	**1984**	**642**	**0.00000006-0.3**	**0.000007-0.5**
Duration of Use								
Leave-On	*2926*	*2066*	*0.00000032-0.65*	*0.00002-0.6*	*1494*	*435*	*0.00000006-0.3*	*0.000007-0.5*
Rinse-Off	*903*	*562*	*0.0000008-0.5*	*0.0001-0.98*	*465*	*178*	*0.0000004-0.23*	*0.0001-0.4*
Diluted for (Bath) Use	*31*	*51*	*0.005-0.1*	*0.00004-0.15*	*25*	*29*	*0.000012-0.005*	*0.00002-0.2*
Exposure Type								
Eye Area	578	543	0.000002-0.65	0.00002-0.49	233	59	0.00000006-0.14	0.000007-0.5
Incidental Ingestion	70	72	0.000008-0.3	0.0002-0.2	69	11	0.000004-0.09	0.0001-0.4
Incidental Inhalation-Spray	13; 763[a]; 778[c]	23; 431[a]; 330[c]	0.000031-0.22; 0.00059-0.2[a]; 0.06-0.15[c]	0.02-0.2; 0.0001-0.6[a]; 0.0004-0.4[c]	7; 381[a]; 452[c]	7; 109[a]; 129[c]	0.00004-0.023; 0.00002-0.18[a]	0.01-0.2; 0.0002-0.3[a]; 0.02-0.4[c]
Incidental Inhalation-Powder	73; 12[b]; 778[c]	122; 12[b]; 330[c]	0.0057-0.5; 0.0002-0.48[b]; 0.06-0.15[c]	0.04-0.5; 0.0004-0.4[c]	22; 2[b]; 452[c]	8; 5[b]; 129[c]	0.0029-0.0086; 0.0000007-0.24[b]	0.00001-0.04; 0.02-0.4[c]
Dermal Contact	3032	2147	0.000002-0.65	0.00004-0.98	1621	525	0.0000006-0.3	0.00001-0.5
Deodorant (underarm)	10[a]	10[a]	0.00005[d]; 0.5[e]	0.002-0.1[a]	5[a]	3[a]	NR	0.002[a]
Hair - Non-Coloring	434	229	0.0000008-0.3	0.001-0.6	138	83	0.0000004-0.17	0.01-0.3
Hair-Coloring	115	92	0.000004-0.2	0.2	42	1	0.000036-0.00008	NR
Nail	40	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**			
	2018[21]	**2006[2]**	**2016[22]**	**2003[2]**	**2018[21]**	**2006[2]**	**2016[22]**	**2003[2]**
Totals*	**283**	**48**	**0.000005-0.32**	**0.00001-0.3**	**11626**	**8786**	**0.000001-0.9**	**0.0003-1**
Duration of Use								
Leave-On	*236*	*39*	*0.00004-0.32*	*0.00001-0.3*	*9188*	*6468*	*0.0000043-0.8*	*0.0008-1*
Rinse-Off	*46*	*8*	*0.000005-0.22*	*0.03-0.2*	*2380*	*2105*	*0.000001-0.9*	*0.001-0.46*
Diluted for (Bath) Use	*1*	*1*	*NR*	*0.005*	*58*	*213*	*0.21-0.5*	*0.0003-0.5*
Exposure Type								
Eye Area	51	10	0.19	0.06-0.2	1837	1610	0.000002-0.8	0.07-0.6
Incidental Ingestion	31	1	0.12	0.2	315	301	0.000032-0.35	0.07-1
Incidental Inhalation-Spray	2; 78[a]; 20[c]	2; 6[a]; 6[c]	0.00004; 0.00004[a]	0.0005-0.3[a]; 0.1-0.2[c]	85; 3000[a]; 1865[c]	111; 1382[a]; 968[c]	0.0000043-0.41; 0.0024-0.5[a]; 0.25-0.6[c]	0.1-0.35; 0.07-0.5[a]; 0.15-0.44[c]
Incidental Inhalation-Powder	5; 20[c]	5; 6[c]	NR	0.00001-0.00002; 0.1-0.2[c]	365; 20[b]; 1865[c]	376; 33[b]; 968[c]	0.004-0.4; 0.001-0.6[b]; 0.25-0.6[c]	0.1-0.5; 0.2-0.4[b]; 0.15-0.44[c]
Dermal Contact	206	39	0.031-0.32	0.00001-0.3	9169	6898	0.000001-0.6	0.0003-0.7
Deodorant (underarm)	NR	NR	NR	NR	21[a]	35[a]	0.000075-0.00012[d]; 0.15-0.4[e]	0.0008-0.3[a]
Hair - Non-Coloring	21	6	0.000005-0.22	0.001	1475	1137	0.0002-0.9	0.1-0.4
Hair-Coloring	NR	NR	NR	NR	270	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	56	2	0.12	0.005-0.2	838	751	0.000001-0.5	0.0003-1
Baby Products	NR	NR	NR	NR	37	60	0.13-0.4	0.2-0.4

	Propylparaben			
	2018[21]	**2006[2]**	**2016[22]**	**2003[2]**
Totals*	**8885**	**7118**	**0.00000014-0.7**	**0.00002-0.7**
Duration of Use				
Leave-On	*7331*	*5585*	*0.00000014-0.7*	*0.00002-0.7*
Rinse-Off	*1497*	*1422*	*0.00000026-0.3*	*0.01-0.5*
Diluted for (Bath) Use	*57*	*140*	*0.0001-0.3*	*0.04-0.3*
Exposure Type				
Eye Area	1600	1477	0.00000014-0.7	0.02-0.5
Incidental Ingestion	601	527	0.000004-0.3	0.03-0.62
Incidental Inhalation-Spray	34; 2248[a]; 1323[c]	62; 996[a]; 706[c]	0.00000014-0.31; 0.0003-0.25[a]; 0.02-0.25[c]	0.1-0.3; 0.001-0.5[a]; 0.03-0.4[c]
Incidental Inhalation-Powder	285; 21[b]; 1323[c]	308; 31[b]; 706[c]	0.0018-0.3; 0.0001-0.3[b]; 0.02-0.25[c]	0.1-0.7; 0.2[b]; 0.03-0.4[c]
Dermal Contact	7064	5598	0.00000014-0.4	0.00002-0.7
Deodorant (underarm)	14[a]	29	0.000025-0.000058[d]; 0.025-0.15[e]	0.002-0.2[a]
Hair - Non-Coloring	737	623	0.0000055-0.4	0.03-0.5
Hair-Coloring	174	150	0.00000026-0.25	0.04-0.5
Nail	59	27	0.0000003-0.2	0.002-0.4
Mucous Membrane	1005	832	0.000004-0.3	0.02-0.62
Baby Products	36	56	0.15	0.05-0.2

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

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

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

NR – no reported use

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

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

[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[d] Spray products

[e] Not spray products

Table 6. Frequency and concentration of use according to duration and exposure of parabens.

	# of Uses[21]	Max Conc of Use (%)[22]	# of Uses[21]	Max Conc of Use (%)[22]	# of Uses[21]	Max Conc of Use (%)[22]
	Sodium Butylparaben		**Sodium Ethylparaben**		**Sodium Isobutylparaben**	
Totals*	**1**	**NR**	**29**	**0.000012-0.062**	**1**	**NR**
Duration of Use						
Leave-On	*1*	*NR*	*27*	*0.000012-0.062*	*1*	*NR*
Rinse-Off	*NR*	*NR*	*2*	*0.0036*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	11	0.0036	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	1[a]	NR	4[a]; 6[c]	NR	1[a]	NR
Incidental Inhalation-Powder	NR	NR	6[c]	0.0036[c]	NR	NR
Dermal Contact	1	NR	25	0.0036-0.062	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	0.0036	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.000012	NR	NR
Mucous Membrane	NR	NR	2	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
	Sodium Methylparaben		**Sodium Paraben**		**Sodium Propylparaben**	
Totals*	**400**	**0.000005-0.4**	**NR**	**0.008**	**136**	**0.000015-0.28**
Duration of Use						
Leave-On	*203*	*0.00001-0.4*	*NR*	*0.008*	102	*0.000017-0.28*
Rinse Off	*188*	*0.000005-0.4*	*NR*	*NR*	30	*0.000015-0.1*
Diluted for (Bath) Use	*9*	*NR*	*NR*	*NR*	4	*NR*
Exposure Type						
Eye Area	45	0.000012-0.4	NR	NR	20	0.004-0.28
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	1; 41[a]; 76[c]	0.00002; 0.00022-0.3[b]	NR	NR	13[a]; 43[c]	NR
Incidental Inhalation-Powder	76[c]	0.00013; 0.00016-0.3[c]	NR	NR	43[c]	0.0051[c]
Dermal Contact	242	0.000005-0.4	NR	0.008	124	0.0004-0.28
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	71	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	1	0.000017
Mucous Membrane	23	0.25	NR	NR	10	0.1
Baby Products	NR	NR	NR	NR	1	NR

Totals=Rinse-off + Leave-on + Diluted for Bath Product Uses.
*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
NR=Not Reported
[a] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[c] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 7. Parabens with no current reported use according to 2018 VCRP data and the Council survey (2016).[2,21,22]

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

Table 8. SCCP opinions on parabens.

Year	Conclusion	Reference
2005	It is the opinion of the SCCP that, viewing the current knowledge, there is no evidence of demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics.	10
2005	Methyl and ethyl paraben can be safely used up to the maximum authorized concentration as actually established (0.4%). The available data do not enable a decisive response to the question of whether propyl, butyl and isobutyl paraben can be safely used in cosmetic products at individual concentrations up to 0.4%. More information is needed in order to formulate a final statement on the maximum concentration of propyl, isopropyl, butyl and isobutyl paraben allowed in cosmetic products.	11
2006	The conclusion of opinion SCCP/0873/05 remains unchanged.	12
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.	12,13
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.	14
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.	15
2013	The concerns of the SCCP/SCCS expressed previously and reiterated in recent Opinions remain unchanged and reinforced after the evaluation of both the reproductive toxicity and the toxicokinetic studies on Propylparaben recently submitted to the SCCS. The same data were extrapolated for the evaluation of the risk by Butylparaben exposure. The additional submitted data does not remove the concern expressed in the previous opinions on the relevance of the rat model for the risk assessment of parabens. Although much toxicological data on parabens in rodents exists, adequate evidence has not been provided for the safe use of propyl- or Butylparaben in cosmetics. For these reasons, the 22 SCCS reiterates its previous conclusions and requests regarding an improvement of the data, in particular a) on the exposure of humans including children to Propyl- and Butylparaben in cosmetic products and b) the toxicokinetics of Propyl- and Butylparaben in humans.	15,16

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 (3% bovine serum albumin in isotonic saline solution) and skin samples (~3.3 cm^2 discs, intact or tape-stripped 20 times; diffusion area 2 cm^2) maintained at 32°C; 20 µL aqueous solution was added to the donor chamber or ~20 mg of hydrogel or emulsion was applied to the skin sample at t=0; 50 µL samples removed from the receptor chamber at intervals for up to 4 h or 24 h (depending on the experiment) for analysis by HPLC and replaced by fresh receptor medium	For freshly excised intact skin and previously frozen intact skin, concentrations of unmetabolized Methylparaben in receptor fluid <LOD-2.3% and 2.3%-3.3% of applied dose, respectively, after 4-h exposure; for previously frozen intact and tape-stripped skin, concentrations of unmetabolized Methylparaben in receptor fluid were 2.0%-5.8% and 2.9%-7.6% respectively, after 24-h exposure; absorption rate was higher from emulsions vs. hydrogels, enhancer-containing formulations vs. enhancer-free formulations, and when skin was tape stripped	33
Methylparaben Ethylparaben Propylparaben Butylparaben	Pig	Ears (~1 mm thick) collected from young animals	0.1% in 20%(v/v) or 50% (v/v) ethanol/PBS	Full-thickness porcine skin, stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (20% or 50% ethanol/PBS) and skin samples (diffusion area 1.77 cm^2); system maintained at 37°C; 2 mL solution added to the donor chamber at t=0; 400 µL samples removed from the receptor chamber at intervals for up to 6 h or 7.5 h (depending on the experiment) for analysis by capillary electrophoresis (CE) and replaced by fresh receptor medium	Permeability coefficients (cm/h x 10^{-4}), in descending order: Methylparaben, 214.8 ± 40, Ethylparaben, 197.5 ± 10; Propylparaben, 101.9 ± 15; Butylparaben 31.3 ± 1.6; skin penetration was inversely proportional to lipophilicity; Increasing ethanol concentration and exposure duration increased parabens retention in dermis compared epidermis; Binary combinations of the parabens reduced their permeation rates, attributed by the authors to high retention in the epidermis and dermis	34
Methylparaben Ethylparaben Propylparaben	Rabbit (mixed breed)	Skin excised from ears of 6-month-old animals	3 commercial facial moisturizing creams containing 0.23%-0.32% (w/w) Methylparaben, 0%-0.1% Ethylparaben, and 0.04%-0.19% Propylparaben.	Full-thickness skin, stored froze, thawed and mounted on Franz-type diffusion cells	Receptor fluid (saline) and skin samples (diffusion area 0.6 cm^2); Donor chamber filled with 2 mg/cm^2 cream at t=0; 300 µL samples removed from the receptor chamber at intervals for up to 86 h for analysis by HPLC and replaced by fresh receptor medium	Percentage of applied dose in receptor fluid after 8 h exposure, in descending order: Methylparaben, 60%; Ethylparaben, 40%; Propylparaben, 20% of PP – penetration decreased with decreasing water solubility, regardless of the formulation tested; Retention varied widely in the epidermis (14.0-253.0 µg/g) and dermis (0-19.3 µg/g), depending on the formulation	35
Methylparaben Propylparaben Butylparaben	Human Mouse (hairless)	Human cadaver epidermis (commercially available) Skin from 8-week-old male mice	0.1%, 0.4%, and 2% in a general oil-in-water cream formulation	Human epidermis (~0.03 mm thick) and mouse skin (~0.25 mm thick), stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (1:1 ethanol/water, v/v) and skin samples (diffusion area 0.785 cm^2) maintained at 32°C; 10 mg cream applied to the skin surface at t=0; 1 mL samples removed from the receptor chamber at intervals for up to 24 h for analysis by LC-MS/MS and replaced by fresh receptor medium	Permeability coefficients (K_ps; cm/h x 10^{-4}) were similar regardless of concentration tested; K_ps were directly related to paraben concentration K_ps for human skin ranged from 0.74 ± 0.19 to 0.91 ± 0.44 for Methylparaben, 0.54 ± 0.14 to 0.91 ± 0.22 for Propylparaben, and 0.37 ± 0.15 to 0.56 ± 0.32 for Butylparaben K_ps for mouse skin ranged from 1.41 ± 0.12 to 1.66 ± 0.21 for Methylparaben, 1.52 ± 0.13 to 1.76 ± 0.39 for Propylparaben, and 1.17 ± 0.15 to 1.27 ± 0.20 for Butylparaben Residual quantities of parabens remaining in	36

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
						skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin; Residual quantities in human epidermis (µg/ml x 10^{-4}): Methylparaben, 235 ± 132 to 7198 ± 4662; Propylparaben, 375 ± 212 to 4120 ± 2344; Butyl paraben, 436 ± 226 to 5480 ± 2593; Residual quantities in mouse skin: Methylparaben, 14 ± 5 to 286 ± 104; Propylparaben, 21 ± 9 to 410 ± 112; Butyl paraben, 15 ± 2 to 358 ± 118 Authors state results show that parabens may be classified as moderate penetrants	
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	Abdominal skin samples collected during surgery from 8 women	Commercial body lotion containing 0.1% (w/w) Methylparaben, 0.08% Ethylparaben, 0.2% Propylparaben, and 0.15% Butylparaben.	Human skin samples, stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (3% bovine serum albumin in isotonic saline solution) and skin samples (diffusion area 3.14 cm^2) maintained at 32°C; single 100 µL (45 mg) lotion applied to skin surface at t=0, which was repeated for some skin samples at t=12 h and t=24 h; fluid was removed from the receptor chamber at intervals for up to 36 h for analysis by HPLC and replaced by fresh receptor medium	Penetration was inversely proportional to lipophilicity of parabens tested, and increased with repeated applications; penetration 36 h after single application (percentage of applied dose): Methylparaben, 0.057% ± 0.03; Ethylparaben, 0.045% ± 0.01; Propylparaben, 0.028% ± 0.01; Butylparaben, 0.007% ± 0.003; Penetration 12 h after last of 3 repeated applications: Methylparaben, 0.6 ± ± 0.1%; Ethylparaben, 0.3% ± 0.1; Propylparaben, 0.2% ± 0.05; Butylparaben, 0.04% ± 0.01	37

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

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
			In Vitro			
Methylparaben Ethylparaben Propylparaben Benzylparaben	Rat (strain not specified)	AFP in rat amniotic fluid	Five to 6 concentrations between 10^{-9} M and 10^{-4} M	Competitive binding to AFP in rat amniotic fluid assayed against 2,4,5,7-[^{3}H]-estrone, with assay tubes containing no "cold" radio-inert test competitor provided the 100% binding level, and 1.5 x 10^{-6} M "cold" competitor maximally competed with 10^{-6} M 2,4,5,7-[^{3}H]-estrone; radioactivity remaining above this standard was considered nonspecific and was subtracted from assay measurements to estimate specific binding	The concentration of Benzylparaben inhibiting the binding of 2,4,5,7-[^{3}H]-estrone to AFP by 50% (IC_{50}) was 0.012 µM; AFP did not exhibit binding affinity for Methylparaben, Ethylparaben, and Propylparaben	43
Butylparaben	Rat (Wistar)	S9 fraction of 5-week old males (n not specified)	Twelve concentrations between about 5 µM and 90 µM	Reactions performed in PBS, pH 7.4, at 37°C in shaking water bath and stopped by adding ice-cold methanol; supernatant was separated by HPLC and formation of *4-Hydoxybenzoic Acid* metabolite was monitored using UV detector at 254 nm; Michaelis-Menten parameters were estimated by Lineweaver-Burk plot (no further details provided)	Butylparaben was biotransformed to *4-Hydoxybenzoic Acid* in the reaction mix with the maximum rate achieved by the system, at saturating substrate concentration (V_{max})=8.8 nmol/min/mg protein and the substrate concentration at which the reaction rate is half of V_{max} (Km)=28.6 mM	44
Butylparaben	Human Rat (Harlan Sprague-Dawley)	Hepatocytes from human subjects (59-year-old woman an 45-year-old man, both non-smokers) and 8 to 12 week old male and female rats	1 µM radiolabeled Butylparaben (phenyl ring-^{14}C(U) – 53.1 mCi/mmol); 10 µM radiolabeled Butylparaben in metabolism studies	The plates were then pre-incubated for 5 min at 37°C and Butylparaben added in acetonitrile (<0.5% final concentration) at t=0; 50 µL aliquots were collected at t=300 min for metabolism studies and at intervals up to t= 300 min for clearance studies for LC-MS/MS analysis	Butylparaben was rapidly cleared in hepatocytes from rats, with little or no sex difference ($t_{1/2}$=3.8 ± 0.3 min and 3.3 ± 0.1 min for hepatocytes from males and females, respectively, corresponding to Cl_{int}=811 ± 53 and 903 ± 28 mL/min/kg); Butylparaben was cleared more slowly in hepatocytes from humans but, again, there was no sex difference ($t_{1/2}$=23.9 ± 1.3 min and 29.6 ± 5.2 min, respectively, corresponding to Cl_{int}=92 ± 5 and 111 ± 22 mL/min/kg); Butylparaben was extensively hydrolyzed to *4-Hydoxybenzoic Acid* as the major metabolite for both sexes and species (92% to 100% in rat, 78% to 84% in human) after 5 h of incubation. The other metabolite observed in human hepatocytes was 4-hydroxyhippuric acid (16% to 22%)	48
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (Sprague-Dawley) Monkey (African green)	Pooled human liver and small intestine microsomes available commercially Rat liver, skin, kidney, pancreas, and small intestine microsomes and blood plasma S9 from COS cells (Monkey-kidney derived, fibroblast	100 nmol paraben and tissue microsomes or plasma in final volume of 1 mL 0.1 M K, Na-phosphate buffer (pH 7.4)	Incubation was for 7 min at 37°C, then 10 mg 2,4-dihydroxybenzophenone (internal standard) and 1 mL acetonitrile added; aliquot of the supernatant was collected for analysis of paraben hydrolase activity by HPLC Carboxylesterase activity was determined by measuring deacetylase activities toward 4-nitrophenol acetate and 4-methylumbelliferyl acetate: 4-nitrophenol acetate deacetylase activity measured by spectrophotometry at 405 nm; 4-methylumbelliferyl acetate deacetylase activity measured by fluorophotometry at 329 nm (excitation) and 448 nm (emission)	Rat liver microsomes showed the highest hydrolytic activity towards Butylparaben, with activity decreasing with decreasing side-chain length – carboxylase 1 exhibited a similar activity pattern; Rat small-intestinal microsomes exhibited higher activity toward longer-side-chain parabens – carboxylase 2 showed a similar activity pattern; In contrast, human liver microsomes showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length; human small-intestinal microsomes showed a specificity pattern similar to that of rat small-	46

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
		like)			intestinal microsomes	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Human	Human liver microsomes (pooled from 21 men and women) Blood plasma (pooled from nine 25 to 35 year old men)	164 µM paraben (dissolved in DMSO)	Biotransformation of parabens to yield 4-hydroxybenzoic acid metabolite studied at 37°C in 67 mM PBS (pH 7.4), human plasma, 580 mM albumin solution in phosphate buffer (pH 7.4), and human liver microsomes (100 mg) in 100 mM Tris-HCl buffer (pH 7.4) Glucuronidation of parabens and 4-hydroxybenzoic acid by human liver microsomes and recombinant UDP-glucuronosyltransfeases (UGT) was performed by a modified of the method of Bansal and Gessner (1980)	Methylparaben and Ethylparaben were stable in human plasma, with 95% of the initial concentration remaining after 24-h incubation; Propylparaben, Butylparaben and Benzylparaben concentrations decreased by 50% within 24 h; All parabens tested were rapidly hydrolyzed when incubated with human liver microsomes, depending on the alkyl chain length ($t_{1/2}$=22 min for Methylparaben and 87 min for Butylparaben; Parabens (but not 4-hydroxybenzoic acid) were actively glucuronidated by liver microsomes and mainly by human recombinant UGT1A1, UGT1A8, UGT1A9, UGT2B7, UGT2B15 and UGT2B17	45
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (strain not specified)	HLM, HSM, HLC, and HSC RLM, RSM, RLC, and RSC	100 µM in 50 mM potassium phosphate, pH 7.4	Reactions were initiated with the addition of 100 µM paraben; mixture incubated for 30 min at 37°C; 4-Hydoxybenzoic Acid formation measured by HPLC-analysis of supernatants	Hydrolysis of parabens by HLM was about 10-fold more rapid than by HLC; Metabolism rates were inversely proportional to chain length (the longer the alcohol moiety, the slower the hydrolysis); this trend was also observed for HSM and HSC, but at much lower rates of hydrolysis; Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the ester compared; Paraben hydrolysis rates in rat liver and skin were greater than in human liver and skin; RLM and RSM metabolized parabens 7-fold and 5-fold faster than RLC and RSC, respectively; In contrast to human tissue fractions, hydrolysis rates of the parabens increased as the ester chain length increased in rat tissue. Methylparaben and Propylparaben was the preferred substrate for human tissue fractions and rat tissue fractions, respectively; Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin	47
ANIMAL						
Dermal						
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	n=9/sex/group for the toxicokinetics study and n=3/sex/group for the mass balance	Single 100 mg/kg bw dosage of radiolabeled (ring-U-^{14}C) paraben, in 60% aqueous ethanol vehicle, applied to the skin	Isotopic mixtures were applied to the interscapular/back region (on an area equivalent to approximately 10% of the total body surface) over a 6-h period; hair at the administration site was clipped before application; animals wore an Elizabethan collar during the 6-h	For all 3 parabens, C_{max} (≥693 and ≥614 ng eq/g in males and female, respectively) occurred within 8 h post-gavage, and blood concentrations decreased until the last quantifiable concentration within 24 h;	49

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
		study		exposure period Blood samples were taken from the retro-orbital sinus of the toxicokinetic animals pre-dose and then at 0.5, 1, 2, 4, 8, 12, 22, and 24 h after oral dosing; 3 rats/sex/group were sampled each time; Animals were killed after the last sampling; Blood, excreta were collected from all mass balance animals pre-dose and then after the periods 0–6, 6–24, 24–48, 48, 72–96, 96–120, 120–144 and 144–168 h after oral dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection Organs were collected, weighed, and analyzed for radioactivity. .	Most of the dosage (≥46.4%) as unabsorbed and recovered in the swabs used for cleaning of the application site at the end of the exposure period; ≤25.8% of the applied radioactivity was found in the urine; urinary excretion was the main route of elimination; radioactivity was eliminated rapidly in the urine with averages ≥11.9% recovered in the first 48 h; ≤0.16 % of the radioactive dose of Methylparaben was found in the skin strips and biopsies from the treated sites after necropsy; for all of the parabens tested, a large part of the radioactivity (≥20.7%) was retained in the carcasses; Metabolic profiling of pooled plasma collected 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of *4-Hydoxybenzoic Acid*	
Butylparaben	Rat (Harlan Sprague-Dawley)	8 to 10 week old males, n=4	Single 10 or 100 mg/kg dosage of radiolabeled Butylparaben (phenyl ring-[14]C(U) – 53.1 mCi/mmol; 50 µCi dose/animal) in 95% ethanol, applied to the skin	Single dermal dosages (0.5 mL/kg bw) were applied onto a 4 cm^2 (2 cm × 2 cm) area of shaved skin on the backs of the rats; a protective foam appliance was glued onto the skin using medical adhesive, the doses were administered evenly to the dose area, and a non-occlusive cloth cover was attached over the appliance Urine and feces of rats were collected separately for up to 72 h post-exposure; the animals were then killed, blood was collected and the tissues were excised and weighed.The protective appliance was removed, dose-site skin was excised and washed with a series of water-wetted gauzes and appliance.	Absorption of 10 mg/kg and 100 mg/kg Butylparaben 72 h following application was about 52% and 8%, respectively; total absorbed dosage was comparable (5.2 mg and 8 mg for 10 and 100 mg/kg, respectively); authors stated that nonlinearity with increasing dosage indicates saturation of the capacity for dermal absorption; About 21% of the 10 mg/kg dosage remained unabsorbed; about 16% was recovered in the dose-site skin; About 3% and 8% of the 100 mg/kg dosage was absorbed at 24 h and 72 h, respectively; the amount recovered in the dose-site skin increased from 19% at 24 h to 43% at 72 h; Urine was the primary route of elimination, with about 46% of 10 mg/kg recovered in urine and in cage rinse at 72 h; fecal elimination of radioactivity accounted for 1.7%; Tissues contained about 4.3% of the 10 mg/kg dosage; highest concentrations of radiolabel were in bladder, liver and kidney, which contained about twice the concentration of residues found in liver	48
				Oral		
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	n=9/sex/group for the toxicokinetics study and n=3/sex/group for	Single 100 mg/kg bw dosage of radiolabeled (ring-U-[14]C) paraben, in 60% aqueous ethanol vehicle, administered by gavage	Blood samples were taken from the retro-orbital sinus of the toxicokinetic animals pre-dose and then at 0.5, 1, 2, 4, 8, 12, 22, and 24 h after oral dosing; 3 rats/sex/group were sampled each time; Rats were	For all 3 parabens, C_{max} (≥11432 and ≥21040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last	49

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
		the mass balance study		killed after the last sampling; Blood, excreta were collected from all mass balance rats pre-dose and then after the periods 0–6, 6–24, 24–48, 48, 72–96, 96–120, 120–144, and 144–168 h after oral dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection. Organs were collected, weighed, and analyzed for radioactivity.	quantifiable concentration at 12 h; Mean total cumulative excretion (urine, feces and cage wash) of the administered radioactive dose over a 168-h collection period was complete and amounted to ≥89%; most of the administered dose (≥71%) was eliminated in urine, while ≤3.3% was eliminated in the feces; radioactivity was eliminated rapidly with averages ≥69.6% recovered in the urine during the first 24 h; A small amount of radioactivity (<0.1%) was observed in the collected tissues, and the levels of radioactivity were below the LOQ in the carcasses of most animals; Metabolic profiling of pooled plasma collected at 0.5, 1, 2, 4, and 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of *4-Hydoxybenzoic Acid*	
Butylparaben	Rat (Harlan Sprague-Dawley)	8 to 10 week old males, n=4	Single 10, 100, or 1000 mg/kg dosage of Butylparaben with radiolabeled Butylparaben (phenyl ring-^{14}C(U) – 53.1 mCi/mmol; 50 µCi dose/animal) in Cremophor EL, administered by gavage	Urine and feces of rats were collected separately for up to 72 h post-exposure; the animals were then euthanized, blood was collected via cardiac, and the following tissues were excised and weighed: liver, kidney, brain, muscle (hind leg), abdominal skin, adipose (perirenal), spleen, heart, lung, ovaries, uterus, and testes samples were analyzed by liquid scintillation spectroscopy for radioactivity and by HPLC for parabens and potential metabolites (4-hydroxybenzoic acid, HHA, n-butyl-3,4-dihydroxybenzoate, 3,4-dihydroxybenzoic acid, and 3,4-dihydroxybenzoic acid)	Radioactivity was predominantly excreted in urine; rate of urinary excretion was similar across all dosages, with ≥66% recovered in the first 24 h in males, for example; in 72 h, around 80% was recovered in urine and 3% to 6% in feces; Total radioactivity in tissues was low (0.02% - 1.25%) in males at all dosages, decreasing with increasing dosage; Female rats excreted more Butylparaben in urine in the first 4 h after exposure, but there was no sex difference in the total dosage excreted within 24 h. In general, tissue levels at 24 h were considerably higher in female rats; Highest levels in non-gastrointestinal tract tissues were found in kidney and liver, followed by ovaries and uterus; Comparing the disposition Butylparaben in males rats at 24 h with that at 72 h revealed that blood and plasma concentrations dropped about 50% or more levels in tissues such as adipose, muscle and kidney remained unchanged, and levels in liver and skin increased by 44% and 36%, respectively during that interval; Metabolites detected in urine included Butylparaben-glucuronide, Butylparaben-sulfate, hydroxybenzoic acid, hydroxyhippuric acid, and newly discovered metabolites arising from ring hydroxylation followed by glucuronidation and sulfation	48

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
			HUMAN			
			Dermal			
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean=26 years old), n=26	2% (w/w) Butylparaben in Essex cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate	Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week, followed by daily application of cream with test substances for 1 week; 24-h urine samples were collected and analyzed for total and unconjugated Butylparaben by LC-MS/MS	All 26 subjects showed increased excretion of Butylparaben following topical application; Mean total Butylparaben excreted in urine during treatment was 2.6 ± 0.1 mg/24 h; on average, 0.32% of the applied dose was recovered in urine as Butylparaben; the concentration peaked in urine 8-12 h after application; on average, 1.5% and 2.1% Butylparaben was excreted as free Butylparaben in urine during the control and treatment week, respectively	50
			Oral			
Methylparaben Butylparaben Isobutylparaben	Human	Healthy 31-year old volunteers, n=3 (1 woman and 2 men)	10 mg deuterated (D4-ring-labeled) paraben/dose, dissolved in ethanol and added to a cup of breakfast coffee or tea	Each subject ingested a dose of each paraben, a different paraben each time, with at least 2 weeks between exposures; the first urine samples were collected before exposure and then at 4 13-h intervals for 48 h after exposure for HPLC analysis; ring-deuterated standards included ethyl 4-hydroxybenzoate-2,3,5,6-d4, iso-butyl 4-hydroxybenzoate-2,3,5,6-d4, n-butyl 4-hydroxybenzoate-2,3,5,6-d4, and 4-hydroxybenzoic-2,3,5,6-d4 acid	Free and conjugated parabens and their known, non-specific metabolites, *4-Hydoxybenzoic Acid* and *p*-hydroxyhippuric acid, were detected in the urine samples; new oxidized metabolites with hydroxy groups on the alkyl side chain (3OH-n-butylparaben and 2OH-iso-butylparaben) and species with oxidative modifications on the aromatic ring were discovered; 17.4 %, 6.8 %, 5.6% of the doses of Methylparaben, Isobutylparaben and Butylparaben, respectively, were excreted in the urine; about 16% and 6% of Isobutylparaben and Butylparaben were excreted as 2OH-iso-butylparaben and 3OH-n-butylparaben, respectively; less than 1% was excreted as ring-hydroxylated metabolites; For all parabens tested, 4-Hydoxybenzoic Acid was the major metabolite (57.2% - 63.8%) and urinary *p*-hydroxyhippuric acid ranged from 3.0% - 7.2% of the doses; 80.5% - 85.3% of the doses were excreted as the metabolites detected in this study within 24 h after exposure	51

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

Table 11. Short-Term Toxicity Studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
				Animal			
				Dermal			
Isopropylparaben Isobutylparaben	Rat (Sprague-Dawley)	5-week old males and females, n=10/sex/ group, 13 groups	50, 100, 300, or 600 mg/kg bw/day Isopropylparaben, Isobutylparaben, or 100, 200, 600 and 1200 mg/kg bw/day of a 1:1 mixture of Isopropylparaben and Isobutylparaben, in 99% ethanol	28 days	Protocol followed current OECD TG 410 for short-term repeated dermal exposure studies; test material was topically applied to shaved dorsal skin and covered with a porous gauze dressing and non-irritating tape, 5 days/week; 8 hematological parameters were evaluated; brains, hearts, kidneys, the large lobe of livers, and sectioned dorsal skin were harvested for histological evaluation; hormone concentrations were measured by ELISA, including concentrations of T3, FSH, estradiol, insulin, T, and TSH	There were no significant changes in body and organ weights in any group; macroscopic and microscopic histopathological examinations revealed mild-to-moderate skin damage in female rats; NOAELs for Isobutylparaben and Isopropylparaben were 600 mg/kg bw/day, and 50 mg/kg bw/day, respectively; a LOAEL for hyperkeratosis of 50 mg/kg bw/day was estimated for the mixture; Analysis of serum concentrations showed that FSH was dose-dependently decreased in animals treated with ≥200 mg/kg bw/day of the mixture (i.e. ≥100 mg/kg bw/day each of Isopropylparaben and Isobutylparaben combined)	52
				Oral			
Propylparaben	Rat (Wistar)	Adult males, n=8/group, 3 groups	100 or 300 mg/kg bw/day, suspended in a few drops of Tween-80 (stock solution) and diluted in distilled water (vehicle)	4 weeks	At the end of the treatment period, blood was collected from the abdominal aorta, liver, kidneys, heart and testes were excised, organ to total body weight ratio was calculated, right lobe of the liver and the left testis were fixed for histological examination and homogenates of the remaining liver and testis were prepared ALT, AST, ALP, and LDH activities were analyzed using ELISA; TP, Alb and creatinine concentrations were measured using commercial assay kits; reduced GSH, lipid peroxides (as MDA) and total NO were determined in liver and testis homogenates by the colorimetric methods and CAT and SOD activities were determined; Serum free T and E2 concentrations were measured by ELISA	Statistically-significant effects included dose-dependent increase in relative liver weights, increases in serum ALT, AST, ALP and LDH activities, and reduced total serum protein and albumin (at both dosage rates) and serum globulin (at 300 mg/kg bw/day) concentrations; Serum urea concentrations and urea/creatinine ratios were statistically-significantly increased (at both dosage rates), as was the serum creatinine concentration (at 300 mg/kg bw/day); Statistically-significant decrease in GSH, CAT and SOD activities, and increase of lipid peroxidation and NO generation (at both dosage rates); Statistically-significant dose-dependent reduction in serum testosterone concentration and T/E2 ratio, and elevation in serum E2; Livers exhibited presence of dilated congested central and portal veins,	53

Table 11. Short-Term Toxicity Studies

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
						focal areas of dilated sinusoids, highly proliferated bile ducts with fibrotic reactions around them, expanded portal areas with edema, multifocal areas of necrotic hepatocytes with inflammatory cells infiltration and severe cytoplasmic vacuolization of hepatocytes (at both dosage rates); Testes exhibited evidence of severe spermatogenic arrest, seminiferous tubules occupied with ill-defined eosinophilic mass structure and giant cells in the lumen, detached spermatogenic lineage, edematous eosinophilic interstitial space with congested blood vessels and a mild loss of Leydig cells population	
Methylparaben	Rats (Wistar)	Females (146 ± 10 g bw), n=10/group	250 mg/kg bw/day, administered in the diet	10 days	Blood samples were collected from the retro-orbital sinuses of the animals on the 10th day of the experiment; plasma was analyzed for total MDA concentrations by HPLC and for 2,3-DHBA by LC-MS/MS	Serum MDA (lipid-peroxidase end-product) and 2,3-DHBA (marker of in vivo hydroxyl radical production) concentrations were statistically-significantly elevated compared with controls ($p<0.01$)	54
Butylparaben	Mouse (albino Swiss)	Adult female, n=50, n=10/group, 5 groups	13.33, 20 and 40 mg/kg bw/day, in olive oil by gavage	30 days	Animals were killed on 31st day by cervical dislocation, the liver was excised, a liver sample was homogenized and analyzed for MDA, catalase, GSH, GST, protein, TAA, SOD, GPx, and GR content; Lipid peroxidation in the liver tissue was measured by estimating MDA	All three dosage rates elevated MDA levels in the liver in a statistically-significant ($p < 0.05$), dose-dependent manner TAA levels were reduced by ($p < 0.05$) by 11.34%, 27.03%, and 41.02% at 13.33, 20 and 40 mg/kg bw/day, respectively; GSH levels were reduced by ($p < 0.05$) by 22.22%, 44.53% and 55.74% at 13.33, 20 and 40 mg/kg bw/day, respectively; Statistically-significant ($p < 0.05$), dose-dependent reductions in SOD, CAT, GPx, GR, and GST levels were noted	55

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

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

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben	Rat (Wistar)	Young adult, pregnant females, n=18/group	0, 10, 100, or 500 mg/kg bw/day in corn oil, by gavage	Dams were dosed once daily from GD7 to the day before expected birth (GD21) and again after birth from PND1 to PND22	Statistically-significant, dose-dependent reductions in anogenital distance in male and female neonates and ovary weight in prepubertal females was noted at 100 and 500 mg/kg bw/day; Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adults were statistically-significantly reduced at all dosage rates; Testicular CYP19a1 (aromatase) expression was reduced in prepubertal males, but not in adults, at all dosage rates; Prostate histology was altered (reduced epithelial area and the ratio between epithelium and lumen; increased incidence of large acini with cuboidal epithelium) in prepubertal rats only at 100 mg/kg; Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day In male offspring, sperm count was significantly reduced at all doses from 10 mg/kg/day, but non dose-response relationship was demonstrated between Butylparaben exposure and reduction of epidermal sperm concentrations.	56
Butylparaben	Rat (Wistar)	Pregnant females, n=7 or 8/group, 5 groups	0, 64, 160, 400, and 1000 mg/kg bw/day in corn oil, by gavage	Dams were dosed daily from GD7 to PND21	Average body weight of male offspring of the 1000 mg/kg bw/day group was statistically-significantly reduced on PND21 and PND90 ($p < 0.05$); Serum testosterone concentrations were statistically-significantly reduced on PND21 and PND90 ($p < 0.05$) in males of the 1000 mg/kg bw/day group and on PND21 in the 400 mg/kg bw/day group (36% reduction in the 1000 mg/kg bw/day group); Serum E2 concentrations in males of the 400 and 1000 bw/day groups on PND21, and the 1000 mg/kg bw/day group on PND90, were statistically-significantly ($p < 0.01$) higher than the control concentrations (up to 58% elevated on PND21); The expression of StAR, P450scc, SULT1E1, and AR in the testes was statistically-significantly reduced, at both the transcript and protein level, in males of the 400 and/or 1000 mg/kg bw/day groups; CYP19 and ERα expression was statistically-significantly increased and the methylation rate of the ERα promoter was statistically-significantly decreased in males of the 400 and/or 1000 mg/kg bw/day groups	57
Butylparaben	Rat (Wistar)	Pregnant females, n=7 or 8/group, 5 groups	0, 64, 160, 400 and 1000 mg/kg bw/day in corn oil, by gavage	Dams were dosed daily from GD7 to PND21	Weights of the testes in the male offspring were statistically significantly-reduced on PNDs 21 to 90 in the 400 and 1000 mg/kg bw/day groups, weights of the epididymides in these groups were statistically-significantly reduced at all monitoring intervals except PND35, and seminal vesicle weights were reduced on PND21 but increased by PND35; Serum T concentrations were statistically-significantly decreased in males of the 400 and/or 1000 mg/kg bw/day groups, especially on PND49 (>50% decrease in the 1000 mg/kg bw/day group); E2 concentrations were statistically-significantly elevated in males of the 400 and/or 1000 mg/kg bw/day groups, except on PND 180; Serum LH and FSH concentrations in the Butylparaben treated groups were lower on PNDs 21, 35 and 49 but elevated on PND90, compared to controls; Butylparaben reduced epididymal cauda sperm counts and daily sperm production in a dose-dependent manner; this difference was statistically significant in offspring in the 400 and 1000 mg/kg bw/day groups	58

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

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben	Rat (Sprague-Dawley)	3-week old males, n=8	Single 1000 mg/kg bw dosage in 5% ethanol/95% corn oil (vehicle), by gavage	Control animals received the same volume of vehicle (4 mL/kg bw); rats were then killed at 3, 6 and 24 h after dosing, and testes were collected and subjected to histopathological and immunohistochemical examinations	6 h after dosing, vimentin filaments showed shorter projections, concentration near the basal region and disappearance of the apical extensions toward the lumen of the tubules; Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment, there was marked loss of vimentin filaments expression in apical extensions; The staining intensity of actin and α-tubulin was weak in the testes of treated rats, compared with controls, and the α-tubulin staining pattern was characterized by long defined tracts extending along the axes of the Sertoli cells; Primary Sertoli cells exposed to 0. 1, 100, and 1000 nmol/mL Butylparaben for 6 or 24 h in vitro exhibited dose- and time-dependent increase in the numbers of cytoplasmic vacuoles and disruption of vimentin filaments	59
Methylparaben Ethylparaben Propylparaben Isopropylparaben Butylparaben Isobutylparaben	Rat (Sprague-Dawley)	Prepubertal (8-week-old) females, N=200, n=10/group, 20 groups	0, 62.5, 250 or 1000 mg/kg bw/day in corn oil (vehicle), by gavage	Prepubertal females were dosed daily with a paraben in corn oil from PND21 to PND40; EE was used as a positive control (1 mg/kg bw/day)	Treatment with Methylparaben (1000 mg/kg bw/day) or Isopropylparaben (250 or 1000 mg/kg bw/day) resulted in a statistically-significant delay in vaginal opening in prepubertal females ($p < 0.05$); in contrast, the positive control (EE) significantly accelerated the date of vaginal opening; In the 1000 mg/kg bw/day groups, there were statistically-significant ($p<0.05$) decreases in ovary weights (Methylparaben or Isopropylparaben) and kidney weights (Ethylparaben, or Isopropylparaben) and increases in adrenal gland weights (Methylparaben, Ethylparaben, or Propylparaben) and thyroid gland weights (Methylparaben); Liver weights increased at all dosage rates of Butylparaben ($p < 0.05$); Histological analysis of the ovaries indicated decrease in the number of corpora lutea, increase in the number of cystic follicles, and thinning of the follicular epithelium; Morphological studies of the uterus revealed myometrial hypertrophy after exposure to 1000 mg/kg bw/day Propylparaben or Isopropylparaben and in animals of all dose groups of Butylparaben and Isobutylparaben; In the 1000 mg/kg bw/day groups, serum estradiol concentrations were statistically-significantly reduced (Ethylparaben or Isopropylparaben) and prolactin concentrations were increased (Methylparaben); Serum concentrations of T4 were statistically-significant reduced after treatment with 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Propylparaben or Isopropylparaben, or 62.5 mg/kg bw/ Isobutylparaben, propyl- and Isopropylparaben; The parabens exhibited affinities for ERα and ERβ (IC_{50}s ranging from 2.07 x 10^{-6} to 5.55 x 10^{-5}) in the following order: Isobutylparaben>Butylparaben>Isopropylparaben=Propylparaben>Ethylparaben; IC_{50} for 17β-estradiol was approximately 3 x 10^{-9}, by comparison	60
Butylparaben	Rat (Wistar)	Young adult, pregnant females, n=8/group	0, 100 mg/kg bw/day (vehicle not specified), by gavage	Pregnant females were dosed daily from GD7 to GD21; fetuses were removed on PND21, blood from the fetuses of each litter were pooled (males and females separately) for measurement of plasma insulin, leptin, MCP1, IL-1B, PAI-1 active, IL6, and TNFα concentrations Livers, adrenals and testes were collected from GD21 males for histopathology examination, gene expression analysis, or hormone measurements (estradiol and testosterone)	Butylparaben reduced plasma leptin concentrations in male and female offspring ($p<0.01$)	61

Table 12. Oral 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	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	62
Propylparaben	Rat (Wistar–Crl:WI [Han])	Lactating females (n=36), each with a litter ≥5 male pups supplied on PND14, n=20 pups/group (10/subgroup)	0, 10, 100, 1000 mg/kg bw/day, 2% suspended in a 1% aqueous hydroxycellulose, by gavage	Juvenile male rats were dosed for 8 weeks starting on PND21	There was no evidence of an effect on the weight of the male reproductive organs, epididymal sperm parameters, hormone concentrations, or histopathology; The highest dosage rate tested (1000 mg/kg/day) was the NOAEL	63
Butylparaben	Rat (Sprague-Dawley)	7-week-old males, n=5/group, 4 groups	0, 10, 100 and 1000 mg kg in corn oil (vehicle), by gavage	Performed in accordance with OECD TG 407 for repeated 28-day oral toxicity studies; 24 h after the last dose, testes, tails and epididymal spermatozoa samples were collected, DNA was extracted, and the DNA samples from each group were pooled, digested (methylation-specific restricted restriction digestion), and analyzed by differential display random amplification of polymorphic DNA (RAPD)	Among 57 RAPD amplicons, six were methylation specific. Densitometric analysis of stained agarose gels revealed that five of these amplicons were elevated 1.4- to 3.8-fold in epididymal sperm DNA in treated vs. control animals, indicating an epigenetic effect on spermatogenic germ cells in adult rats	143
Methylparaben Butylparaben	Rat (Wistar –Crl:WI [BR])	Males, 22 days of age, n=16/group, 4 groups	0, 100, 1000 or 10,000 ppm in the diet	Rats were 22 days of age at the start of exposure, which was continued for 8 weeks; parameters evaluated included organ weights, histopathology of reproductive tissues, sperm production, motility, and morphology; reproductive hormone concentrations (LH, FSH, and T) were measured in blood samples from Butylparaben-treated rats and corresponding controls	Methylparaben exposure resulted in a statistically-significantly higher incidence of abnormal sperm in the 1000-ppm ($p \leq 0.01$) and 10,000-ppm ($p \leq 0.05$) exposure groups, mostly sperm with no head in 4% to 5% of sperm, vs. 2.3% in 100-ppm and control groups; 100-ppm Methylparaben in the diet corresponds to 11.2 ± 0.5 mg/kg bw/day; Hormone concentrations were comparable across groups and were not altered from controls, with the following exceptions: Testosterone concentration was statistically-significantly reduced in the 1000-ppm and 10,000-ppm Butylparaben-treated groups after 3 weeks of exposure – removing two rats with aberrantly high testosterone measurement from the control group resulted in a mean control values that were comparable to those of the other groups; T and FSH concentrations were statistically-significantly higher (by 72% and 53%, respectively) in the 10,000-ppm Butylparaben-treated group, compared with the control group; LH concentrations were statistically-significantly lower ($p \leq 0.01$) in the 1000-ppm (by 35%) and 10,000-ppm (by 30%) exposure groups, compared with controls, but	44

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

Test Substance(s)	Species/ Strain	Test Population-Sex	Dosage (Vehicle)	Procedure	Results	Reference
					only at the 5-week exposure point The authors concluded that none of the parameters evaluated for either paraben showed compound- or dosage-dependent adverse effects, and the NOAEC was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of 1141.1 ± 58.9 and 1087.6 ± 67.8 mg/kg/day for Methylparaben and Butylparaben, respectively	

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

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
			In Vitro			
Butylparaben	Mouse (strain not specified)	Murine NIH-3T3-L1 fibroblasts	0, 1, 3, 10, 30, and 100 µM in DMSO (<0.3%)	For the mPPARα/γ transactivation assay, cells were transfected with the luciferase reporter plasmid 4xUAS-TK and either gal4-DBD_mPPARaLBD or gal4-DBD_mPPARcLBD expression vectors; media containing Butylparaben was added and cells incubated for 22 h at 37°C; For analysis of the human PPAR, cells were transfected with expression plasmid for the ligand binding domain of the hPPARα or hPPARγ coupled to Gal4 and a plasmid containing an UAS linked luciferase reporter gene (UAS-TK-luc); For the adipocyte differentiation assay, confluent cells were exposed to induction cocktail for 3 days, the medium was then replaced with differentiation medium with 0.1% DMSO (vehicle) or Butylparaben and the medium changed every 2 days until day 6, when the plates were stained with ORO; rosiglitazone served as a positive control compound; Cytotoxicity was evaluated in parallel experiments not used for Oil Red staining, with resazurin for 3 h followed by measuring fluorescence; To quantify the concentrations of resistin, leptin, and adiponectin in the supernatant from the adipocyte differentiation assay using commercially-available assay kits	Weak activation of mPPARα was seen with the highest concentrations of Butylparaben; Butylparaben activated mPPARγ with a LOEC of 30 µM and a maximal 4-fold induction at 100 µM; The human data for Butylparaben (hPPARα and hPPARγ) were comparable to those obtained with mPPARα and mPPARγ; Butylparaben showed induction of lipid accumulation at 20 µM, and increased leptin, resistin and adiponectin release	66
Methylparaben Ethylparaben Propylparaben	Chinese hamster	CHO cells, AR-transfected	0, 12 concentrations within the range of 0.025 - 50 µM	Cells were transfected with the expression vector pSVAR0 and the MMTVLUC reporter plasmid; test compounds were added to the cells with or without 0.01	Only Isobutylparaben antagonized the AR; the effect was statistically significant at ≥ 25 µM; Butylparaben and Propylparaben inhibited the R1881-	67

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben Isobutylparaben				nM of the AR agonist R1881; The principle of concentration addition was applied to predict the effects caused by an equimolar (1:1:1:1:1) of the parabens; concentration-response relationship for the mixture was calculated using data fitted from the concentration-response curves of the individual compounds	induced response, but only at cytotoxic concentrations; The mixture was predicted to antagonize the AR at concentrations ≥ 2 µM	
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	144
Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, 10 µM, ethanol vehicle (0.1% final concentration)	BT-474 cells are HER2 negative and ERα-positive; MCF-7 cells are ERα-positive and HER2-negative; SKBR3 cells are HER2-positive and ERα-negative; All cells were grown in phenol red-free culture medium and incubated for 2 h (for RT-PCR and Western blot analysis) or from 1 to 3 h (for chromatin immunoprecipitation analysis), with and without Butylparaben, with and without the HER2 HRG at 27°C	Propylparaben and Butylparaben statistically-significantly, synergistically, elevated c-Myc mRNA expression in BT-474 cells in the presence of HRG; Butylparaben was selected for further study because it was most effective; In BT-474 cells, no increase in c-Myc protein concentrations was observed with Butylparaben or HRG alone; in the presence of HRG with 1 µM and 10 µM Butylparaben, the increase in c-Myc protein concentrations was similar to that induced by 0.01 µM E2 plus HRG; the increase was blocked by ER antagonists ICI 182,780, raloxifene, and tamoxifen; MCF-7 cells treated Butylparaben exhibited a similar enhancement of HRG-induced c-Myc protein expression; no synergistic increase in c-Myc protein concentrations was observed in SKBR3 cells Butylparaben increased the number of BT-474 cells entering S-phase (EC_{50}=0.551 µM); the effect was enhanced in the presence of HRG (EC_{50}=0.024 µM After 1-h treatment with HRG and Butylparaben together, maximal 8-fold enhancement of ERα binding to c-Myc enhancer sequence was observed in BT-474 cells; Butylparaben enhanced binding about 4-fold and HRG <2-fold, by comparison	68
Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, 10 nm, and 1 µM, dissolved in DMSO (vehicle)	Cells, stably transformed with MMTV-luciferase, were cultured in Leibovitz's L-15 medium with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin and pre-treated with androgen antagonist flutamide (5 µM) at 37°C; cells then incubated 24 h with and without test compound, and evaluated by means of a cell proliferation assay and an assay for glucocorticoid activity (luciferase-reporter gene)	EC_{50} for glucocorticoid-like activity was 1.75 mM for Butylparaben and 13.01 mM for Propylparaben; Butylparaben and Propylparaben tested separately induced glucocorticoid-like activity at 1 µM, but only Butylparaben induced activity (44% higher than control) at 10 nM	69
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	Butylparaben statistically-significantly enhanced the hydrocortisone-induced GR signal by 85%; Methylparaben, Ethylparaben, and Propylparaben did not; Without hydrocortisone but with flutamide,	70

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				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	Ethylparaben, Propylparaben, and Butylparaben increased GR activity by more than 50%, and Methylparaben by more than 20%	
Butylparaben	Human	T47D-KBluc human breast carcinoma cells (ERα and ERβ positive)	0, 3, 10, 30, 60, and 100 µM in DMSO vehicle	Cells were incubated in phenol red-free Dulbecco's Modified Eagle's F-12 containing 10% charcoal stripped FBS, with and without Butylparaben, in the presence or absence of E2 (20 pM), for 24 h at 37°C	Butylparaben exhibited estrogen agonism at all concentrations tested; maximum effect (24% greater than that of E2) was observed at 10 µM; Butylparaben exhibited estrogen antagonism at all concentrations tested in the presence of 30 pM E2; maximum effects at 10 and 30 µM; calculated IC_{50}=59.82 µM	71
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Human	MCF-7 human breast adenocarcinoma cells	Range of concentrations tested was not specified, ethanol vehicle	Cells prepared as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 5% (v/v) FCS, 10 mg/mL insulin, and 10-8 M E2 at 37°C; incubated with or without paraben or E2 for 7 or 14 days; cellular proliferation was measured using a Coulter counter EC_{100}, EC_{50}, LOEC, and lowest concentration which gave an increase in cell number statistically different (P<0.05) from the LOEC were reported	After 14 days of exposure, the EC_{50}s for cellular proliferation ranged from 0.4 - 40 µM, LOECs from 0.1 - 20 µM, and NOECs from 0.05 - 8 µM for the parabens; the parabens, in descending order of these values, were Isobutylparaben>Butylparaben> Propylparaben >Ethyl-paraben> Methylparaben; In comparison, corresponding values for E2 were EC_{50}=2 x 10^{-6} µM, LOEC=10^{-6} µM, and 1 x10^{-7} µM; A mixture of all 5 parabens, each at its 7-day NOEC, increased the number of cell doublings above that with any of the parabens tested individually, but lower than with E2	72
Propylparaben	Human	MCF-12A and MCF-10A non-transformed, immortalized breast epithelial cells (3D cultures)	10 µM in DMSO vehicle	An in vitro 3D model for breast glandular structure development, using breast epithelial MCF-12A cells cultured in a reconstituted basement membrane matrix (Matrigel); the cells are estrogen-receptor (ERα and ERβ) and GPER competent; cells were cultured, with or without Propylparaben, for 16 days in Matrigel at 37°C	ERα and ERβ were expressed at relatively high levels in MCF-12A cells; MCF-10A cells express no measurable levels of ERα and very low levels of ERβ; Both cell lines expressed the transmembrane GPER MCF-12A cells formed organized acini, with deposition of basement membrane and hollow lumen; treatment with E2 or Propylparaben resulted in deformed acini and filling of the acinar lumen; the ER-inhibitor (ICI 182,780) and/or GPER-inhibitor (G-15) Propylparaben inhibited the Propylparaben-induced effects on acini	73
Methylparaben	Human Mouse (FVB)	MCF-7 and MDA-MB-231 human breast adenocarcinoma cells; HCI-7-Luc2 ER+ PDX human breast tumor cells; Normal cells from murine mammary glands of 8-week-old FVB mice	10 nM in ethanol (vehicle control, 0.1%)	Cells were grown in accordance with standard protocols; mammospheres were established, treated with 0.1% ethanol, 10 nM E2, 10 nM Methylparaben, 1 µM tamoxifen or 100 nM fulvestrant on days 4 and 7, and imaged on day 10	10 nM E2 exposure stimulated the proliferation of MCF-7 cells 7-fold after 1 week of exposure; 10 nM Methyl-paraben did not have this effect, and also failed to increase expression (mRNA) of p52 (TFF1) or proges-terone receptor (canonical estrogen-responsive genes) MCF-7 mammospheres treated with Methylparaben exhibited increased expression of ALDH1 (marker of human mammary stem cells) and were larger than control and E2-treated mammospheres; HCI-7-Luc2 and normal murine mammospheres treated with 10 nM Methylparaben were also larger than controls; Methylparaben statistically-significantly increased NANOG, OCT4, and ALDH1 (all of which are stem cell markers) mRNA expression in both MCF-7 and HCI-7-Luc2 mammospheres; Methylparaben also upregulated NANOG protein expression in MCF-7 mammospheres; none of these effects were seen in MDA-MB-231	74

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					mammospheres; Neither tamoxifen nor fulvestrant inhibited effects of Methylparaben on MCF-7 mammospheres	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Mouse (strain not specified) Human	Murine 3T3-L1 fibroblasts Differentiated hADSCs	0, 1, 10, 100 µM in DMSO vehicle	Murine 3T3-L1 cells were grown in DMEM containing 10% calf serum at 37°C until they reached confluence; hADSCs were grown and differentiated according to the supplier's instructions; For the detection of early target genes, Butylparaben or DMSO was added to the media with or without dexamethasone or the differentiation cocktails (cortisone, methylisobutyxanthine, and insulin) For the studies of the antagonists of GR or PPARγ, cells were pretreated with the antagonists of PPARγ (GW9662 and BADGE) or GR (RU-486) or DMSO for 1 h before the cells were treated with Butylparaben or DMSO in the presence of the antagonist	Butylparaben in the presence of differentiation cocktail enhanced 3T3-L1 cell differentiation, as revealed by ORO-stained lipid accumulation, adipocyte morphologies and ORO absorbance; Parabens enhanced differentiation with potencies that increased with the length of the linear alkyl chain (Methylparaben < Ethylparaben < Propylparaben < Butylparaben), and the extension of the linear alkyl chain with an aromatic ring in Benzylparaben further augmented adipogenicity; 4-hydroxybenzoic acid or benzoic acid did not have these effects; In 3T3-L1 cells, the parabens also induced mRNA expression of adipocyte marker genes as well as adiponectin and leptin mRNA, in a concentration-related manner, and activated GR and/or PPARγ; no direct binding to, or modulation of, the ligand binding domain of GR was detected in competitor assays; 50 µM Butylparaben or Benzylparaben, in the presence of differentiation media promoted lipid accumulation in hADSCs as early as day 3 and throughout the differentiation process; on day 14, Benzylparaben showed the most potent adipogenic effects (upregulation of mRNA expression of adipocyte marker gene and lipid-filled adipocyte morphology); 1 µM Butylparaben had the strongest adipogenic effects of the parabens tested, whereas Ethylparaben, Propylparaben, and Benzylparaben had no effect at 1 or 10 µM)	75
Butylparaben	Mouse (F1 hybrid (C57BL/6j ×CBA/Caj) Human	Ovaries from immature 13-day-old female mice were used for follicle isolation; hGC were isolated from blood cells and follicular fluid	10 nM, 100 nM, 1 µM and 10 µM (1.9 ng/ml to 1.9 µg/ml) in DMSO vehicle	After 24 h of incubation to allow cell attachment, the medium was replaced by fresh equilibrated medium containing different concentrations of Butylparaben, DEHP or a mixture of both; The cells were treated with Butylparaben at different concentrations, for 24, 48, 72, or 96 h; Two control groups (control and DMSO) were included in each experiment which consisted of three independent cultures; Progesterone output was measured using commercial progesterone enzyme immunoassay kit	In follicle culture, DEHP and Butylparaben attenuate estradiol output but only when present together; Butylparaben attenuated DEHP induced reduction of progesterone concentrations in the spent media of hGC cultures; No effects on follicular development or survival were noted in the culture systems; DEHP and Butylparaben adversely affect steroidogenesis from the preantral stage onward and the effects of these chemicals are both stage-dependent and modified by co-exposure	81
Butylparaben Isobutylparaben	Human	MCF-7 and T47D human breast cancer cells	10 µM in ethanol or DMSO vehicle	MCF-7 and T47D cells were treated at 10 µM with Butylparaben, Isobutylparaben, 3-hydroxy n-butyl 4-hydroxybenzoate (3OH), and 2-hydroxy iso-butyl 4-hydroxybenzoate (2OH) for 2, 4, 6, or 18 h; Cell viability was measured by PrestoBlue assay; GREB1 expression was evaluated by Real-time PCR; ERE–luciferase reporter assay was performed to determine whether the estrogenic activity of the paraben metabolites is mediated by classical estrogen	The 3OH metabolite induced cellular proliferation with EC_{50} of 8.2 µM in MCF-7 cells; The EC_{50} for 3OH in T47D cells could not be reached; The 2OH metabolite induced proliferation with EC_{50} of 2.2 µM and 43.0 µM in MCF-7 and T47D cells, respectively; The EC_{50} for the parental Isobutylparaben and Butylparaben was 0.30 and 1.2 µM in MCF-7 cells, respectively;	77

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
				receptor mediated signaling; Computational docking studies were conducted to examine the ligand-binding domain interactions between paraben compounds and human ERα	The expression of GREB1 was induced by these compounds and blocked by co-administration of an ER antagonist (ICI 182, 780), confirming the ER-dependence of these effects; The metabolites promoted significant ER dependent transcriptional activity of an ERE-luciferase reporter construct at 10 and 20 µM for 2OH and 10 µM for 3OH; Molecular docking prediction studies showed that the paraben compounds exhibited the potential for favorable ligand-binding domain interactions with human ERα in a manner similar to known x-ray crystal structures of E2 in complex with ERα	
ANIMAL						
Oral						
Benzylparaben	Rat (Sprague-Dawley and Wistar)	Immature females, n=13 - 14/group	0, 0.0064, 0.032, 0.16, 0.8, 4, and 20 mg/kg bw/day by gavage, in peanut oil (vehicle)	Rats were exposed to Benzylparaben for 3 days, beginning on PND 21; on PND 24, the rats were weighed and killed, and uteri dissected and weighed	Relative uterine weights (ratios of uterine weights to final body weights) of Sprague-Dawley rats increased after treatment with ≥5 µg/kg bw/day E2, but Wistar rats given up to 100 µg/kg bw/day E2 showed no obvious effect; 400 µg/kg bw/day E2 increased relative uterine weight in Sprague-Dawley rats by 281% and in Wistar rats by 83%; Relative uterine weights were elevated in Sprague-Dawley rats after treatment with ≥0.16 mg/kg bw/day ($p<0.05$) in a dose-dependent manner; relative uterine weights increased by 3%, 7%, 19%, 24%, 27%, 31%, and 36% in the 0.0064, 0.032, 0.16, 0.8, 4, 20 and mg/kg bw/day groups, respectively The Wistar rats were not tested for sensitivity to Benzylparaben in this study	82
Methylparaben Ethylparaben	Rat (Sprague-Dawley)	Immature females (PND 20); n=6 - 9/ group (n=17 in one of the control groups)	0, 0.8, 4, and 20 mg/kg bw/day (20 mg/kg bw/day when tested with 10 mg/kg bw/day fulvestrant) in peanut oil, by gavage	Rats were exposed to a paraben for 3 days, beginning on PND 21; rats were then weighed and sacrificed, and uteri dissected and weighed, and relative uterine weights calculated, except for 1 group that was transferred on PND 23 to individual metabolic cages in which only pure water was available, ad libitum, and from which urine was collected for 24 h and analyzed for Methylparaben and Ethylparaben concentrations; Relative expressions of estrogen-responsive genes in the uteri were evaluated by quantitative real-time RT-PCR	LOELs for increased relative uterine weight after treatment with Methylparaben and Ethylparaben were 20 and 4 mg/kg bw/day, respectively; NOELs for Methylparaben and Ethylparaben were 4 and 0.8 mg/kg bw/day, respectively; The uterotrophic effects of 25 µg/kg bw/day E2 or 20 mg/kg be/day Methylparaben or Ethylparaben were antagonized by 10 mg/kg bw/day fulvestrant; Expression of icabp, itmap1, CaBP-9k, and/or Pgr biomarker genes were elevated in a concentration-dependent manner after treatment with 4 or 20 mg/kg bw/day Methylparaben or Ethylparaben; Mean urinary concentrations of the Methylparaben and Ethylparaben increased in a dose-dependent manner, from 491 to 17,635 ng/mL for Methyl-paraben and 376 to 11,906 ng/mL for Ethylparaben in rats that received 0.8 to 20 mg/kg/day Methylparaben or Ethylparaben	83
Ethylparaben Propylparaben	Mouse (C57BL/6J)	Ovariectomized females, 8 weeks of	0, 1000 mg/kg bw/day	Study was performed in compliance with OECD TG 440 (Uterotrophic Bioassay in Rodents); mice were dosed	Ethylparaben and Propylparaben were negative for estrogen agonism and antagonism	84

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
		age, n=6/group, 11 groups	in corn oil, by gavage	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		
Butylparaben	Rat (Sprague-Dawley)	3-week old males, n=8	0, 1000 mg/kg, single oral dosage in 5% ethanol/95% corn oil vehicle	Rats were killed 3, 6, or 24 h after administration of Butylparaben; testes were collected for histopathological examination, in situ terminal deoxynucleotidyl transferase-mediated digoxigenin-dUTP nick-end-labeling (TUNEL) assay, and analysis using transmission electron microscopy	Histopathologic examination revealed progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules and reduction and/or disappearance of tubular lumen 3 h after Butylparaben treatment; Sertoli cells and spermatogonia with few spermatocytes remained within the seminiferous tubules were observed at 6 h; thin seminiferous epithelia and wide tubular lumen were found at 24 h; TUNEL assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h, and declined at 24 h, though still substantially greater than in the controls; Apoptotic spermatogenic cells were found in semi-thin sections of the testes to be more frequently in treated rats, compared with controls; Apoptotic cells were rounded-up and sur-rounded by empty space, sometimes appearing to be separate from neighboring cells; transmission electron microscopy revealed condensed chromatin and shrinkage of cytoplasm and nucleus of apoptotic spermatocytes.	85
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	Female rats (8-week old), n=6/group, 8 groups	100 mg/kg/day in the diet	Rats were orally exposed to 100 mg/kg bw/day for 5 weeks; Ovarian follicle development and steroid synthesis were investigated through real-time PCR and histological analyses; A disruptor of ovarian small pre-antral follicle 4-vinylcyclohexene diepoxide (VCD, 40 mg/kg bw/day), was used to induce premature ovarian failure (POF)	Propylparaben and Butylparaben treatment prolonged diestrus phases and shortened the interval of the estrous cycle, whereas Methylparaben treatment did not; No effect on number of primary follicles, and secondary follicles showed a decrease in total number in all treated groups; Propylparaben and Butylparaben decreased mRNA level of folliculogenesis-related genes (*Foxl2*, *Kitl* and *Amh*); Parabens induced an increase in FSH levels in serum, which implied impairment of ovarian function	78
Methylparaben	Rat (Sprague-Dawley)	Female rats (n= 3-10/group, 12 groups)	0.105 mg/kg /day, by gavage	Rats were orally exposed across several key developmental stages including perinatal (GD1–GD20, n=10 or PND1–PND21, n=10), prepubertal (PND21–PND42, n=5) and pubertal (PND42-PND63, n=5) windows as well as long-term exposures from birth to lactation (PND1–PND146, n=3)	Perinatal Methylparaben exposure decreased amounts of adipose tissue and increased expansion of the ductal tree within the fat pad; Pubertal Methylparaben exposure elevated the amounts of glandular tissue, visible as a higher degree of branching relative to the total gland area; Long-term Methylparaben treatment from birth to lactation did not result in significant histological changes; In the pubertal window, expression alterations in 993	79

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					genes enriched in pathways including cholesterol synthesis and adipogenesis were observed	
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 prostate of the female gerbil showed additional changes such as stromal inflammatory infiltration, intraepithelial neoplasia foci, and an increase in AR-positive frequency	80
HUMAN						
Dermal						
Butylparaben	Human	Healthy Caucasian male volunteers, 21 to 36 years old (mean= 26 years old), n=26	2% (w/w) Butylparaben in cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate	Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week, followed by daily application of cream with test substances for 1 week; concentrations of the following hormones were measured in blood serum (as well as the serum concentrations of Butylparaben): FSH, LH, T, estradiol, inhibin B, TSH, FT4, T3, and T4	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	38

AR=Androgen receptor; CHO=Chinese hamster ovary; DEHP= di-(2-ethylhexyl) phthalate; DHT=5α-dihydrotestosterone; DMEM=Dulbecco's modified Eagle's medium; DMSO=Dimethyl sulfoxide; E2=17β-estradiol; EC_{100}=Lowest concentration from maximal stimulation of proliferation; EC_{50}=Concentration for half maximal stimulation of proliferation; E2: Estradiol; ER=Estrogen receptor; ERE=Estrogen-response element; FBS=Fetal bovine serum; FCS=Fetal calf serum; FSH=Follicle stimulating hormone; FT4=Free thyroxine; GD=gestation day; GPER=G-protein coupled estrogen receptor 1; GR=Glucocorticoid receptor; GREB1=Estrogen-inducible gene; hADSC=Human adipose-derived stem cells; HER2=Human epidermal growth factor receptor; hGC=Human granulosa cell; 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. Aggregate Exposure

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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	88
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Human	Human placentas collected from healthy mothers after delivery (singleton term pregnancies) at St. Hospital Joan de Déu (Barcelona), n=12	Aggregate exposures (undefined sources)	Placental tissue was obtained from the maternal side, each placenta sectioned transversally, and three fragments of about 1 cm^3 of tissue near the umbilical cord insertion were biopsied after removal of amniotic and chorionic layers; analytes were extracted from the samples and separated by a chromatographic procedure developed by the authors; MS/MS detection was performed in negative ESI under SRM mode for improved selectivity and sensitivity	Methylparaben, Butylparaben, and Benzylparaben were detected in all samples; The highest measured concentration was 11.77 ng Methylparaben/g tissue	89
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	Human ovarian tumor samples were obtained from Yong Loo Lin School of Medicine, National University of Singapore, n=30	Aggregate exposures (undefined sources)	15 ovarian malignant tissues and 15 benign tissues were analyzed; technique involves the simultaneous use of MASE and micro-solid SPE, in tandem with HPLC/UV analysis for the determination of parabens concentration; ovarian tissues were not spiked with parabens; the mass fractions of parabens present in human ovarian tissues were then calculated	-The tissue mass fractions of Methylparaben and Propylparaben were higher than Propylparaben and Butylparaben; -The tissue mass fractions of four parabens in all the ovarian cancer tissues are at least twice as much as those present in the benign tissues; -The method detection limits for parabens ranged from 0.005 to 0.0244 ng/g	90
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben Heptylparaben	Human	Human adipose fat samples collected from Wadsworth Center, New York City, n = 20	Aggregate exposures (undefined sources)	Human adipose fat samples were collected from volunteers who underwent liposuction surgery between 2003 and 2004; tissues were spiked with methanol solution containing isotope labeled internal standards and analyzed by HPLC-MS/MS for the presence of parabens as well as several environmental phenols and aromatic compounds	-Among the six parabens analyzed, Ethylparaben and Propylparaben were more frequently detected than the other parabens, at a detection frequency of 60% and 50%, and a GM concentration of 0.90 and 0.49 ng/g, respectively; -4-Hydroxybenzoic Acid was detected in almost all samples, at concentrations as high as 17,400 ng/g; -The GM concentration of the sum of six parabens and 4-Hydroxybenzoic Acid ($C_{\Sigma parabens}$) in adipose fat was 3420 ng/g; - Among the 20 samples analyzed, high $C_{\Sigma parabens}$ (>10^5 ng/g) were found in 5 females	91

Table 14. Aggregate Exposure

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					and 2 males, indicating high exposure to parabens by some individuals; -No gender-related difference in $C_{\Sigma parabens}$ was found, and the age related difference between the two age groups (18–33 yr and 34–58 yr) was equivocal; -Paraben concentrations in adipose fat samples of Caucasian volunteers (GM: 7050 ng/g) were higher than those of African Americans (GM: 3440 ng/g)	
Methylparaben Propylparaben	Human	Human serum samples from 5 male and 11 female donors at Tennessee Blood Services (n=16)	Aggregate exposures (undefined sources)	16 commercially available serum samples collected between 1998 and 2003 were purchased from Tennessee Blood Services in Memphis; To determine the concentrations of the free plus conjugated species of the parabens, the enzyme solution, containing β-glucuronidase/sulfatase in ammonium acetate buffer , and radio-labeled standards were added into the serum; Six phenols concentrations in the serum sample, including bisphenol A, benzophenone-3, triclosan, 2,5-dichlorophenol, Methylparaben and Propylparaben, were measured by on-line SPE coupled to HPLC -MS/MS	The mean paraben concentrations in serum are 42.6 µg/L and 7.4 µg/L for Methylparaben and Propylparaben, respectively; The free concentration of Methylparaben and Propylparaben in the serum is 2.2 µg/L and 0.5 µg/L, respectively, indicating that parabens that are not hydrolyzed to 4-Hydroxybenzoic Acid are rapidly conjugated; The conjugated species of Methylparaben and Propylparaben are more stable than their corresponding urinary conjugates	[92]
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	Human urine specimens from US National Health and Nutrition Examination Survey (NHANES), male and female participates ≥ 6 years of age	Aggregate exposures (undefined sources)	-Annual survey conducted by CDC between 2005 and 2014; -Three age groups (6-11years, 12-19 years, 20 years and older), total 13,076 subjects: 2005-2006, n= 2448; 2007-2008, n= 2604; 2009-2010, n= 2749; 2011-2012, n= 2489; 2013-2014, n= 2686; -NHANES includes household interviews, standardized physical examinations, and collection of urine specimens for parabens exposure examination via HPLC-MS/MS analysis; -Urine samples were treated to free conjugated paraben in urine, thus representing a total concentration	- The median urine concentration was similar across the two sampling periods of 2011-2012 and 2013-2014 for the three parabens with Methylparaben at much higher concentrations than Propylparaben and Butylparaben; - The median urine concentration of the three parabens was decreased in the 2011-2014 sampling period comparing to the 2005-2010 sampling period; - For the 2013–2014 sampling period, Methylparaben in urine was 48.1 µg/L (95th percentile: 819 µg/L), and Propylparaben in urine was 5.74 µg/L (95th percentile: 224 µg/L); - For Butylparaben, the median concentration in urine was below the limit of detection (0.1 µg/L) for all groups in the 2011–2014 reporting period; - In females, the median concentration of Ethylparaben in the 2013–2014 reporting period was 1.6 µg/L (95th percentile: 145 µg/L) while males were below the limit of detection (95th percentile: 34 µg/L); - The reported median concentration in male urine for Methylparaben (24.4 µg/L) and Propylparaben (1.7 µg/L) was lower than that for females (Methylparaben: 73.9 µg/L;	[93]

Table 14. Aggregate Exposure

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
					Propylparaben: 13.5 µg/L)	
Methylparaben Propylparaben Butylparaben	Human	Human urine samples from US NHANES program, male and female participates ≥ 20 years of age(men, n=1399; women, n= 1350)	Aggregate exposures (undefined sources)	-A PBPK model for Methylparaben, Propylparaben, and Butylparaben were developed which were parameterized through a combination of quantitative QSAR for tissue solubility and quantitative IVIVE for hydrolysis in portals of entry including intestine, skin, and liver; -The human paraben PBPK model was then used to estimate the plasma free paraben concentration in adults consistent with 95th percentile urine concentration reported in US NHANES program (2009 - 2010 collection period); - The model assume that 4-Hydroxybenzoic Acid and the conjugated metabolites were exclusively excreted in urine; - The EC10 used in this assessment were generated from two assays, ERLUX (reporter gene) and E-Screen (cell proliferation), which were used to assess estrogenicity of the parabens; - In vitro metabolic parameters (nmol/min/ mg microsomal protein) were converted to an intrinsic clearance (Cl_{int}) expressed in terms of L/h-mg protein; The Cl_{int} was then scaled to the whole tissue based on the amount of microsomal protein per gram of tissue; - An in vitro based cumulative MOS was calculated by comparing the effective concentrations from an in vitro assay of estrogenicity to the free plasma paraben concentrations predicted by the model to be associated with the 95th percentile urine concentrations reported in NHANES (2009–2010 collection period)	- 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	[115]

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; 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. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			Prospective Studies			
Methylparaben Propylparaben Butylparaben	245 women who completed ≥1 IVF cycle and provided ≥1 urine sample/IVF cycle between November 2004 and April 2012 at the Massachusetts General Hospital (MGH) Fertility Center	Subjects recruited from 11/2004 to 4/2012	- Subjects provided up to two spot urine samples per IVF cycle; first collected between Day 3 and Day 9 of the gonadotrophin phase, second collected on day of oocyte retrieval - Urinary concentrations of total parabens were measured by HPLC-MS/MS - Clinical information was abstracted from the patient electronic medical records - Serum concentrations of FSH and E2 were measured - Each subject was assigned an infertility diagnosis by a physician - Subjects underwent one of three controlled ovarian stimulation IVF treatment protocols, after completing a cycle of oral contraceptives - Embryologists determined the total number of oocytes retrieved per cycle and classified them - Oocytes underwent either conventional IVF or ICSI, and embryologists determined fertilization rate 17-20 h after insemination - Embryo quality was classified based on morphology and number of blastomeres, ranging from 1 (best) to 5 (worst) on day 2 and 3 - In women who underwent an embryo transfer, implantation was assessed and pregnancy was confirmed by ultrasound at 6 weeks - Live birth was defined as birth of a neonate on or after 24 weeks gestation - Exposures were categorized into quartiles of urinary concentrations; the lowest quartile used as the reference group - Associations between urinary concentrations and demographics and baseline reproductive characteristics were evaluated using Kruskal-Wallis and Chi-squared tests - Multivariable generalized linear mixed models were used to evaluate associations between concentrations and IVF outcomes - Poisson distributions and log link functions were specified for oocyte counts, and a binomial distributions and logit link functions for embryo quality, fertilization rates, and clinical outcomes (implantation, clinical pregnancy and live birth) - Potential confounders considered include factors previously related to IVF outcomes in this or other studies and factors associated with paraben exposure and IVF outcomes in this study - Final models were adjusted for age, BMI, race (white vs nonwhite), smoking status (never vs ever), and infertility diagnosis (male factor, female factor, unexplained) <u>Limitations</u> - Study design may not allow extrapolation of the findings to the general population - Misclassification of paraben exposure based on concentrations from spot urine samples is possible	Urinary paraben concentrations were not associated with IVF outcomes; Geometric means of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben were 133, 24 and 1.5 µg/L, respectively; The urinary concentrations were not associated with total or mature oocyte counts, proportion of high embryo quality, fertilization rates, implantation rates, clinical pregnancy, or live births	None of the ORs calculated for total oocyte yield, metaphase II oocyte yield, >1 best embryo quality, and fertilization rate in the 2nd, 3rd, and 4th quartiles of Methylparaben, Propylparaben, and Butylparaben urinary concentrations were statistically-significantly different from those of the 1st quartile, adjusted or unadjusted	98

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Propylparaben Butylparaben	400 men (18 - 55 year old) at the Massachusetts General Hospital Fertility Center	2004-2015	- This was a prospective cohort study, enrolled couples seeking fertility treatment ; - At each visit, men completed a questionnaire on PCPs use within the past 24h and at what time they last used each PCP prior to the collection of each urine sample; - PCPs included deodorants, shampoo, conditioner/crème rinse, hairspray/hair gel, combined other hair care products (including mousse, hair bleach, relaxer, perm, and straightener), shaving cream, aftershave, cologne/perfume, mouthwash, bar soap, liquid soap/body wash, hand sanitizer, hand/body lotion, and suntan/sunblock lotion; - Urine samples were collected at each men's visit. The analytical technique for quantification of the urinary biomarkers involved enzymatic deconjugation of the urinary metabolites, followed by solid phase extraction and HPLC-MS/MS analysis <u>Limitations:</u> - single-PCP approach may be susceptible to multiple testing statistical issues; - Butylparaben had a low detection frequency; - No information about frequency of PCP use, amount of product used, whether it was used with hot or cold water, parabens product content, or brand names of the PCPs	- This 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 a 254–1,333% increase in monoethyl phthalate and parabens concentrations; - Self-reported PCP use among men was associated with higher urinary concentrations of three parabens (Methylparaben, Propylparaben, and Butylparaben)		[99]

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben Benzylparaben	80 pregnant women (age 18 years or older) at the Ottawa Hospital, Canada	2009-2010	- Prior to 20 weeks of pregnancy, 80 women collected all their urine from two 24 h periods on a weekday and/or a weekend day as multiple spot urine samples; a subset of women (n = 31) who provided multiple spot urine samples (n = 542) collected over two 24-h periods; - Women were instructed to keep the urine cool at all times and samples were delivered to hospital within 36 h; - Breast milk samples were collected at the woman's home 2-3 months after delivery (n =56); - Women recorded the date and time of the sample collection, which breast they collected it from, the time since the last feed from that breast and the name of any creams, lotions, or cleansers used on their breast; - At the same time as the urine collection, women were asked to c to record their activities, food consumption, and personal care product use throughout the day; the personal care product content of the diaries were manually categorized into the 16 mutually exclusive categories; - Five parabens were measured on in urine and breast milk samples by HPLC-MS/MS analysis Limitations: - Small sample size and the high socioeconomic status of the participants, limiting the generalizability of our results to other populations; - Commonly used items such as hand soap, or misreported use of other product may not be recorded by some women; -Lacked information on the amount of product used by the participants and the paraben content of the products; - This work does not explored potential sources of exposure to parabens include food, dust sanitary wipes, paper products, and medications	- Women who used lotions in the past 24 h had significantly higher geometric mean paraben concentrations (80 - 110%) in their urine than women who reported no use in the past 24 h; -Women who used shampoo, conditioner, and cosmetics also showed 70.80% higher Butylparaben concentrations in their urine; - There was 100%, 72%, 96%, and 90% detection of Methylparaben, Butylparaben, Propylparaben, and Ethylparaben in urine respectively; Lower detection rates were seen for Isobutylparaben (39%) and Benzyl paraben (41%); - All parabens with >70% detection (Methylparaben, Ethylparaben, Butylparaben, and Propylparaben) were significantly and strongly correlated with each other with Spearman correlation coefficients ranging from 0.48 (Methylparaben and Ethylparaben) to 0.86 (Propylparaben and Methylparaben); -Breast milk samples had 82%, 66%, and 57% detection for Methylparaben, Propylparaben, and Ethylparaben; - There was <1% detection for Butylparaben, Benzylparaben and Isobutylparaben		[100]

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			Retrospective Studies			
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	185 pregnant women (18 to 45 years of age) recruited from Brooklyn's Prenatal Clinic and their singleton infants	Subjects recruited from 10/2007 to 12/2009	- Random "spot" urine specimens were provided once per participant during last 4 months of pregnancy - Convenience subset of the subjects were followed to delivery, when umbilical cord blood was collected - Maternal urinary concentrations were measured - Random subset of umbilical-blood plasma samples were analyzed for free and total parabens - Questionnaire was used to gather demographic - Neonate outcome data were from patient charts - Urinary biomarker concentrations were corrected for creatinine levels and were log-transformed - Non-detect values were treated as the MDL divided by the square root of 2 - Covariates were selected if they achieved $p < 0.05$ in Spearman correlations or Chi-square tests in relation to biomarker concentrations or birth outcomes - Measures of birth outcomes (body length, gestational age at birth, birth weight, and head circumference) were analyzed using linear models - Multiple linear regression analysis was used to evaluate concentration-outcome associations adjusted for maternal age, nativity, neonate gender, and alcohol and tobacco use; additional adjustments were made for confounders independently associated with outcomes or which changed the magnitude of effects by ≥ 5% -Relationships between concentrations and dichotomous outcomes were analyzed by logistic regression <u>Limitations</u>: - Maternal urine was used as a proxy for fetal exposure, except where neonate cord blood plasma was available - Timing of sampling may have biased results; product use contributing to exposure may differ over the course of the pregnancy - Multiple urine levels may be more appropriate to capture variability and characterize exposures - No correction was made for conducting multiple data comparisons - Small size and homogeneity of the participant population the limit generalizability of the results	In regression models adjusting for confounders, adverse exposure-outcome associations observed between Butylparaben concentrations and increased odds of PTB, decreased gestational age at birth and birth weight, and decreased body length (Propylparaben), and between Benzylparaben concentrations and protective effects on PTB ($p<0.05$). No associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated		101
				<u>Low Birth Weight and Maternal Urine Concentrations</u>	**<u>OR</u>**	
				Methylparaben	0.83 (0.37-1.87)	
				Ethylparaben	1.18 (0.74-1.89)	
				Propylparaben	0.92 (0.44-1.94)	
				Butylparaben	1.45 (0.88-2.39)	
				Benzylparaben	NA	
				<u>Low Birth Weight and Cord Blood Concentrations</u>		
				Methylparaben	NA	
				Ethylparaben	1.89 (0.62-5.81)	
				Propylparaben	1.52 (0.66-3.45)	
				Butylparaben	10.27 (0.68-156.07	
				Benzylparaben	0.18 (0.01-2.63)	
				Preterm Birth and Maternal Urine Concentrations		
				Methylparaben	0.78 (0.40-1.54	
				Ethylparaben	1.15 (0.78-1.69)	
				Propylparaben	1.27 (0.67-2.43)	
				Butylparaben	1.42 (0.93-2.16)	
				Benzylparabe	NA	
				<u>Preterm Birth and Cord Blood Concentrations</u>		
				Methylparaben	NA	
				Ethylparaben	2.65 (0.83-8.48)	
				Propylparaben	1.86 (0.84-4.08)	
				Butylparaben	**60.77 (2.60-1417.93)**	
				Benzylparabe	**0.03 (0.01-0.44)**	

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	520 mother-son pairs with complete data on prenatal (3 ultrasound measurement), neonatal (biometry), and postnatal growth up to 3 years of age (≥4 weight/height measurements and clinical exam), recruited before the end of gestation week 28 from Poitiers and Nancy University hospitals (France)	Subjects recruited from 4/2003 to 3/2006	- Biparietal diameter was measured by ultrasound during gestation weeks 12.6, 22, and 32.6 (on average) - Fetal head circumference, abdominal circumference, and femur length were assessed during the last 2 ultrasound examinations - Fetal weights were estimated from measures of abdominal circumferences, femur lengths, head circumferences, and biparietal diameter - Weight and length at birth were extracted from hospital records - Infants were weighed and measured at 1 and 3 years of age - Mothers were mailed questionnaires at 4, 8, 12, 24, and 36 months about the boys' weight and height measures - Jenss nonlinear model was used to evaluate growth and predict weight and height at 6, 12, 24, and 36 months - Head circumference was assessed within 4 days after birth and at 3 years - Abdominal circumference was measured at 3 years - Urine samples were collected between gestation weeks 22 and 29 - Total paraben concentration was calculated by summing molar concentrations of the 4 parabens - Non-detects were replaced by the lowest instrumental reading value divided by the square root of 2 - Concentrations were standardized for collection conditions, including creatinine concentrations - Cross-sectional analyses and linear regression models with a random effect variable corresponding to the mother-son pair were used to study associations between concentrations and growth parameters - Models for prenatal and postnatal growth were adjusted for maternal and paternal height, pre-pregnancy weight, maternal active and passive smoking during pregnancy, maternal education, recruitment center, and parity - Model for head circumference was also adjusted for number of days between birth and assessment of head circumference - Analyses of postnatal growth were additionally adjusted for breastfeeding duration - Effect estimates were reported for an increase by 1 IQR of ln-transformed standardized concentrations Limitations: - Use of only 1 urine sample to assess paraben concentrations increases the chances of exposure misclassification - Use of estimates of caloric intake (rather than specific food usually eaten) increases the chance of confounding by differences in eating behavior.	No statistically-significant associations were found between maternal urinary paraben concentrations during pregnancy and prenatal or postnatal growth of male newborns. However, maternal urinary concentrations during pregnancy appeared to be positively associated with body weights: Body Weight at Birth Methylparaben Ethylparaben Propylparaben Butylparaben Body Weight at 6 Months Methylparaben Ethylparaben Propylparaben Butylparaben Body Weight at 12 Months Methylparaben Ethylparaben Propylparaben Butylparaben Body Weight at 24 Months Methylparaben Ethylparaben Propylparaben Butylparaben Body Weight at 36 Months Methylparaben Ethylparaben Propylparaben Butylparaben β coefficients calculated for Ethylparaben and Butylparaben, body weights estimated at the 3rd ultrasound examination, were 13.00 (-13.1-39.1) and 23.5 (-3.96-50.9), respectively; coefficients for all other parameters were < 7.5 with CIs spanning across negative and positive values	**β Coefficient** 36.0 (-12.4-84.4) 49.9 (-2.21-102) 48.0 (-3.64-99.6) 50.1 (-5.69-106) 85.3 (-16.5-187) 17.8 (-92.9-129) 80.1 (-27.4-188) 55.8 (-62.0-174) 81.2 (-45.4-208) 2.60 (-135-140) 79.1 (-54.9-213) 54.5 (-91.1-200) 128 (-31.88-287) 45.3 (-128-219) 116 (-53.3-285) 111 (-71.2-294) 193 (-3.88-389) 113 (-101-327) 159 (-49.4-368) 179 (-45.3-404)	102

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	28 boys diagnosed with cryptorchidism and/or hypospadias at San Cecilio University Hospital of Granada: 19 cryptorchidism cases (n=9 unilateral, 6 bilateral), 12 hypospadias cases, 1 case with both disorders; 51 matched controls	Subjects recruited from 10/2000 to 7/2002	- This was a case-control study nested within a prospective birth cohort study of risk factors for male urogenital malformations - All boys in the cohort were examined at birth and those diagnosed with cryptorchidism and/or hypospadias were re-examined at 1month of age -Information on potential confounding variables related to parents, pregnancy/delivery and activities were gathered from structured interviews with the mother within 48 h after delivery - There was a larger proportion of mothers reporting historical (pre-pregnancy) use of oral contraceptives in the selected versus non-selected cases (21% vs. 53%, p=0.034), although not in the selected versus non-selected controls (37% vs.42%, p=0.686) - Placentas were collected immediately after delivery and analyzed by UPLC–MS/MS - Crude and adjusted ORs and corresponding 95% CIs were calculated by conditional logistic regression - Concentrations of parabens were used as independent variables and analyzed both as continuous variables and in tertiles, with the first tertile as the reference group - Concentrations below the LOQ were assigned a value of half of the LOQ - Potential confounding variables were selected if they were statistically-significantly associated with outcomes in bivariate analyses or changed the β coefficient by >20% in the multivariable analysis - Only maternal age and newborn birthweight had a substantial effect on results - In the bivariate analyses, differences between groups were tested with Pearson's chi-square test or Fisher's exact test, when appropriate <u>Limitations:</u> - 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	Methylparaben	**OR (unadjusted)**	103
				<0.4 ng/g	1.00	
				0.44-1.91 ng/g	1.00 (0.32-3.09)	
				1.96-11.69 ng/g	**3.18 (0.88-11.48)**	
				Concentration as continuous variable	1.17 (0.94-1.46)	
				Ethylparaben		
				<LOD	1.00	
				0.07-0.89 ng/g	0.29 (0.08=1.06)	
				0.91-5.49 ng/g	1.51 (0.44-5.15)	
				Concentration as continuous variable	1.07 (0.74-1.55)	
				Propylparaben		
				<LOD	1.00	
				0.06-1.15 ng/g	1.23 (0.30-5.04)	
				1.16-5.52 ng/g	**4.72 (1.08-20.65)**	
				Concentration as continuous variable	**1.90 (1.12-3.22)**	
				Butylparaben	**OR (adjusted)**	
				<0.08 ng/g	1.00	
				0.16-0.74 ng/g	2.29 (0.65-8.05)	
				0.79-1.60 ng/g	2.31 (0.72-7.46)	
				Concentration as continuous variable	2.27 (0.8-6.42)	
					OR (adjusted)	
				Methylparaben		
				<0.4 ng/g	1.00	
				0.44-1.91 ng/g	1.04 (0.33-3.26)	
				1.96-11.69 ng/g	3.24 (0.83-12.69)	
				Concentration as continuous variable	1.17 (0.93-1.48)	
				Ethylparaben		
				<LOD	1.00	
				0.07-0.89 ng/g	0.26 (0.07-1.00)	
				0.91-5.49 ng/g	1.25 (0.34-4.60)	
				Concentration as continuous variable	1.00 (0.68-1.47)	
				Propylparaben		
				<LOD	1.00	
				0.06-1.15 ng/g	1.39 (0.33-5.91)	
				1.16-5.52 ng/g	6.42 (1.16-35.47)	
				Concentration as continuous variable	2.16 (1.16-4.01)	
				Butylparaben		
				<0.08 ng/g	1.00	
				0.16-0.74 ng/g	2.26 (0.62-8.21)	
				0.79-1.60 ng/g	2.11 (0.62-7.16)	
				Concentration as continuous variable	2.07 (0.71-6.06)	

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	436 3-year old children recruited from Sheyang Maternal and Child Health Care Centre (China)	Subjects recruited between 7/2012 and 4/2013	- Questionnaire survey was administered to each child's caregiver by trained interviewers, covering sociodemographics, living environment and lifestyles - Pregnancy and maternal health information was obtained from medical records and questionnaires - Spot urine sample was collected from each child, and urinary paraben concentrations were measured by LVI-GC-MS/MS - EDI_{urine} of parabens was calculated based on urinary concentrations and a steady-state toxicokinetic model - Anthropometry measurements were compared with sex-specific WHO child growth standards, and age- and sex-standardized z scores were calculated - Generalized linear models were used to examine associations between SG-adjusted concentrations and body growth outcomes - Individual paraben concentrations and the $P_{parabens}$ were adjusted for SG - Analyses of quartiles of $P_{parabens}$ were conducted separately - Urinary concentrations were log transformed for univariate and multivariate analyses - Associations between concentrations and sociodemographic characteristics were examined using a Wilcoxon rank-sum or Kruskal-Wallis rank sum test - Log-transformed concentrations were assessed using Pearson correlation coefficients - Concentrations below LOD were substituted with LOD divided by the square root of two - Covariates considered included: maternal and paternal BMI, child's sex, maternal education, family income, habitation in town, suburb or countryside, feeding pattern, smoking status, time spent outdoors, sampling season, and birth outcome - Potential confounders that were separately include: urinary bisphenol A, triclosan, and benzophenone-3 concentrations Limitations: - Spot urine samples may cause exposure misclassification - Specific diet information was not sufficiently obtained and evaluated	Weight z Score (Boys) Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben ∑Parabens Height z Score (Boys) Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben ∑Parabens All β coefficients calculated for girls and all other β coefficients for boys were not statistically significant	**β Coefficient** 0.08 (-0.06-0.23) **0.16 (0.03-0.28)** 0.00 (-0.16-0.17) 0.12 (-0.09-0.32) -0.04 (-0.18-0.10) 0.17 (-0.04-0.39) 0.11 (-0.02-0.26) **0.15 (0.03-0.27)** 0.05 (-0.11-0.21) 0.14 (-0.06-0.34) 0.08 (-0.06-0.21 **0.23 (0.03-0.43)**	104
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 subsample of US NHANES participants n=185 adolescent males (ages 12 to 19) males, 171 adolescent females, 785 adult (ages ≥20) males, and 708 adult females	2007-2008	- Stratified multistage probability sample of civilian US population was surveyed via household interviews, physical exams, and collection of medical histories and biologic specimens. - Urinary parabens concentrations were measured - Spot urine samples were analyzed by HPLC-MS/MS - LOD values were estimated as 3 x standard deviation as concentrations approached zero - Serum thyroid measures included free and total T3 and T4, thyroglobulin, and TSH (or thyrotropin) - Potential confounders considered: age, sex, BMI, urinary creatinine levels, race/ethnicity, poverty income ratio , education, serum cotinine levels and alcohol intake	Adults, Total T4 (µg/dL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, ln-Free T3 (pg/mL) Methylparaben Ethylparaben Propylparaben Butylparaben	β Coefficient -0.04 (-0.12-0.03) ***-0.5 (-0.10 - -0.002)*** -0.19 (-0.46-0.07) ***-0.20 (-0.36 - -0.03)*** 0.005 (-0.01-0.000) ***-0.006 (-0.001- -0.0001)*** ***-0.02 (-0.04- -0.002)*** ***-0.02 (-0.03- -0.002)***	105

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Variables used as the basis for creation of sample weights, including race/ethnicity, PIR, and education, were not included in final models to avoid over-adjustment - Following ln-transformation of the remaining variables with log-normal distributions, Pearson correlations, one-way ANOVA, and t-tests were used to evaluate potential confounders - Covariates were adjusted for in the final models if there were statistically-significantly associated with one exposure or outcome variable based on a priori evidence or the analysis, and if they altered parameter estimates of the main effects by more than 10% - Final regression models included age, sex, BMI, and urinary creatinine - Concentrations of urinary parabens below the LOD were replaced with values equal to the LOD divided by the square root of two. - Parabens were analyzed on a creatinine-adjusted basis for univariate and bivariate analyses; unadjusted urinary concentrations were used in regression models with urinary creatinine included as a covariate - Final multivariate linear regression models included serum thyroid concentrations (continuous variable) as the dependent variable and an individual urinary Methylparaben and Propylparaben concentration (continuous) as a predictor, along with age (continuous), sex (dichotomous), BMI (continuous), and ln-transformed urinary creatinine (continuous) Limitations: - Causality cannot be established because NHANES is an observational, cross-sectional study - Exposures were evaluated based on spot urine measurements; - Spot urine samples served as the basis for estimating exposures, so time of sample collection could be a source of intra-individual variability and the concentrations may not accurately represent average body burdens	Adult Females, ln-Free T4 (ng/mL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, T4 (μg/dL) Methylparaben Ethylparaben Propylparaben Butylparaben All other β coefficients calculated were not statistically significant	 ***-0.01 (-003- -0.000)*** ***-0.01 (-0.02- -0.003)*** -0.02 (-0.05-0.01) ***-0.04 (-0.07- -0.004)*** -0.09 (-0.26-0.08) -0.08 (-0.20-0.05) -0.30 (-0.65-0.06) ***-0.36 (-0.57- -0.16)***	
Methylparaben Propylparaben Butylparaben	Female participants of a prospective fertility study at the MGH Fertility Center, undergoing infertility evaluation, n=109 to 142, depending parameter measured	2004-2010	- Subjects had at least one hormonal or ultrasonographic marker of ovarian reserve measured and contributed at least one urine sample - Clinical information was abstracted from medical records - Intravenous blood sample was drawn on the 3rd day of the menstrual cycle, and the serum was analyzed for FSH - AFC and OV were measured for both ovaries using transvaginal ultrasound - Each patient was given an infertility exam and diagnosis by a physician at the MGH Fertility Center - Demographic data were collected using a nurse-administered questionnaire at entry into the study - Convenience spot urine sample was collected at recruitment and at subsequent visits during infertility treatment cycles - Paraben concentrations were measured by HPLC-MS/MS - Distribution of exposures was summarized using the median, IQR, and range of urinary paraben concentrations	Methylparaben Tertile 1 (5.13-132 μg/L) Tertile 2 (145-377 μg/L) Tertile 3 (381-2,428 μg/L) p_{trend} =0.31 Propylparaben Tertile 1 (<LOD-25.2 μg/L) Tertile 2 (26.3-81.8 μg/L) Tertile 3 (87.8-727 μg/L) p_{trend} =0.07 Butylparaben Tertile 1 (<LOD-0.73 μg/L) Tertile 2 (0.75-5.12 μg/L)	**MPC in AFC** 0 (Reference) -6.8 (-23.5-13.7) -10.6 (-28.2-11.2) 0 (Reference) -5.0(-23.7-18.4) -16.3 (-30.8-1.3) 0 (Reference) -4.8 (-22.5-16.8)	106

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Urinary concentrations below LOD were assigned a value equal to the LOD divided by the square root of two - Concentrations were corrected for SG - Spearman's rank correlation coefficients (r_S) were calculated for markers of ovarian reserve, age, and BMI - Multivariable linear regression was used to estimate associations between within-person paraben concentrations (divided into tertiles) and day-3 FSH and OV; OV was ln-transformed before all regression analyses - Poisson regression was used to estimate associations between within-person paraben concentrations (tertiles) and AFC - Covariates considered included age at time of outcome and BMI determinations at study entry into the study - MPC in outcome from the lowest tertile of paraben concentrations was calculated for both OV and AFC - Secondary analysis combined concentrations of parabens using two methods: an EEQ factor approach, and summation of concentrations - Multivariable linear regression was used to evaluate association between EEQ (parabens) and Σ (parabens) with day-3 FSH and OV <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	Tertile 3 (5.44-177 µg/L) p_{trend} =0.86 All MPCs and p_{trens} calculated for AFC and OV were not statistically significant	-2.0 (-21.0-21.6)	
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 sub-sample of the US NHANES participants ≥6 years of age, n=860 (450 males, 410 females)	2005-2006	- Sociodemographic data, urinary paraben levels, total and specific IgE levels, respiratory disease and medical condition questionnaire data were included in the dataset - Urinary parabens levels were collected - Subject answered the following questions: Has a doctor or other health professional ever told you that you have asthma? In the past 12 months, have you had wheezing or whistling in your chest? - Atopic asthma was defined as having doctor-diagnosed asthma in addition to at least 1 positive aeroallergen-specific IgE level - Nonatopic asthma was defined as having doctor-diagnosed asthma with negative specific IgE test results - Atopic wheeze was defined as having a history of wheezing in the past 12 months in addition to at least 1 positive aeroallergen-specific IgE level - Nonatopic wheeze was defined as having a history of wheezing in the past 12 months with negative specific IgE test results	<u>Aeroallergen and Food Sensitization (males and females)</u> Methylparaben Tertile 1 Tertile 2 Tertile 3 P_{trend}=0.4 Propylparaben Tertile 1 Tertile 2 Tertile 3 **P_{trend}=0.04** Propylparaben Tertile 1	 **<u>OR (unadjusted)</u>** 1.0 (Reference) 1.11 (0.82-1.47) **1.74 (1.02-3.11)** 1 (Reference) 1.35 (1.00-1.82) 1.74 (0.98-3.08) **OR (adjusted)** 1.0 (Reference)	107

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			- Parabens were measured in urine samples by HPLC-MS/MS - Serum total IgE levels and aeroallergen-specific IgE levels were measured, including IgE specific for cat, dog, mouse, rat, Dermatophagoides, cockroach, ragweed, thistle, rye, Bermuda, oak, birch, *Alternaria* species, and *Aspergillus* species - Food-specific IgE levels measured were for milk, egg, peanut, and shrimp - Subjects were considered to have aeroallergen or food sensitization if the specific IgE level was ≥0.35 kU/L - Urinary paraben concentrations were divided into tertiles or dichotomized when 50% or fewer of the subjects had detectable levels (as was the case for Butylparaben) - Linear regression was used to determine whether mean urinary concentrations varied by race/ethnicity. - Logistic and linear regression were used to determine associations between paraben concentrations and food and aeroallergen sensitization, atopic and nonatopic asthma and wheeze, and total IgE levels - Test for trend was performed by using the variable for tertiles of the paraben concentrations - Multivariate models were adjusted for age, sex, race/ethnicity, urinary creatinine level, and PIR Limitations: -Data are drawn from a cross-sectional study, which introduces the possibility of reverse causation (i.e., subjects with allergy might use more products containing parabens} - Use of allergen sensitization as an outcome was limited by lack of clinical correlation of allergic disease - Urinary paraben levels were used as biomarkers of exposure, which might not reflect actual exposure	Tertile 2 Tertile 3 P_{trend}=0.2 Butylparaben Tertile 1 Tertile 2 p_{trend}=0.9 Nonatopic Asthma (males and females) Methylparaben Tertile 1 Tertile 2 Tertile 3 **p_{trend}=0.04** Nonatopic Wheeze (males and females) Methylparaben Tertile 1 Tertile 2 Tertile 3 p_{trend}=0.47 In addition, the OR and p_{trend} calculated for Propylparaben concentrations and aeroallergen and food sensitization in males were statistically significant The ORs and p_{trend}s calculated for all other comparisons were not statistically significant	**1.51 (1.15-1.99)** **2.04 (1.12-3.74)** 1 (Reference) **1.55 (1.02-2.33)** **OR (adjusted)** 1.0 (Reference) 0.43 (0.47-3.73) **0.25 (0.07-0.90)** 1 0.51 (0.18-1.46) **0.23 (0.05-0.99)**	
Methylparaben Propylparaben Butylparaben	194 male partners (18 to 55 years old; mean = 36.7 years of age) of subfertile couples seeking treatment from the Vincent Memorial Obstetrics and Gynecology Service, Andrology Laboratory, Massachusetts	2000-2004	- A single spot urine sample was collected on day of each subject's clinic visit; 2nd and 3rd samples were collected from a subset of men at subsequent visits - Concentrations of total (free + conjugated) parabens were measured in urine samples by HPLC-MS/MS, - One nonfasting blood sample was drawn on the same day and time as the first urine sample - Serum testosterone, E2, sex-hormone-binding globulin, inhibin B,	Comet Tail % Butylparaben <0.2 µg/L 0.2-0.6 µ/L >0.6 µg/L **P_{trend}=0.03**	**β Coefficient (adjusted)** 0 6.81 (-1.80-15.4) 8.23 (-0.41-16.9)	108

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
	General Hospital (MGH)		FSH, LH, prolactin, free thyroxine (T4), total triiodothyronine (T3), and TSH were measured - Free androgen index (FAI), testosterone:LH ratio, FSH:inhibin B and E2:testosterone ratios were calculated - Semen quality parameters and motion characteristics were measured: sperm concentration, motility, and motion parameters - Total sperm count was calculated and sperm morphology was assessed - Sperm damage was assessed by comet assay: comet extent, tail distributed moment (TDM), and percent DNA located in the tail (Tail%) were determined - Multivariable linear regression was used to explore relationships between urinary paraben concentrations and hormone levels, semen quality parameters, and sperm DNA damage measures - Distribution of sperm count, sperm concentration, FSH, LH, SHBG, prolactin, TSH, all calculated hormone ratios, and paraben concentrations were ln-transformed for statistical analyses - Paraben concentrations < LOD were assigned values of LOD/2 - Inclusion of covariates in the multivariable models was based on statistical and biologic considerations - Age and BMI were modeled as continuous variables; abstinence period was treated as an ordinal categorical variable - Race, smoking status, and timing of the clinic visit by season and time of day were considered for inclusion as dichotomous variables - Covariates with $p < 0.2$ in their relationship with one or more paraben or ≥ 1 outcome measure in preliminary bivariate analyses were included in a “full” model - Covariates with $p > 0.15$ in full models for all measures within the three sets of outcomes (hormone levels, semen quality, sperm DNA damage) were removed from the final models <u>Limitations</u>: - Urine samples were collected weeks or months after, rather than before, serum and semen samples were collected - Only a single blood or semen sample was available for assessment of hormone levels, semen quality, and sperm DNA damage - Cross-sectional design restricts the ability to draw conclusions about causal relationships - Relatively small sample size provided low statistical power	No other comparisons were statistically significant in this study		

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			Cross-sectional Studies			
Methylparaben Ethylparaben Propylparaben Butylparaben	A nationally representative US sample of 3,529 adults from the National Health and Nutrition Examination Survey	2009–2012	- 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 limit of detection (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 Limitations: - The data was not collected with the specific intent of examining predictors of exposure; - "Always" estimates of sunscreen and mouthwash reflected use over the last day; however "Sometimes" users may not have had any use during the relevant window of interest; - Have no information on month of questionnaire and sample collection, while sunscreen exposure route was likely to be associated with seasonal variation; - Only examine these two types of products, leaving the potential for residual confounding from other personal care product use; - The questionnaire data did not inform amount of mouthwash or sunscreen applied at each use or brand	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		109

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings			OR, β, or MPC (95% C.I.)*		Reference
Methylparaben Ethylparaben Propylparaben Butylparaben, Isobutylparaben	315 men who attended the infertility clinic for diagnostic purposes in Lodz, Poland	2008-2011	- Semen samples were analyzed for sperm concentration, motility, and motion parameters using a computer-aided semen analysis (CASA) (Hamilton-Thorne Version 10HTM-IVOS); - Three principal parameters for the vigor and pattern of sperm motion were examined: straight-line velocity, curvilinear velocity, and linearity; - Sperm morphology was quantified using strict Kruger criteria to classify men as having normal or below normal morphology; - Sperm chromatin structure assay was performed using flow cytometry to assess sperm DNA damage; - Levels of follicle-stimulating hormone, testosterone, and estradiol were determined in human plasma using a Chemiluminescent Microparticle Immunoassay	- The statistically significant associations were found between urinary parabens concentrations and an increase the percentage of sperm with abnormal morphology and percentage of sperm with high DNA stainability; - Neither categories of urinary concentrations of parabens nor continuous concentrations of parabens were associated with the level of reproductive hormones; - Urinary concentrations of Methylparaben and Propylparaben were not related to any of the examined semen quality parameters, sperm DNA damage, or the level of reproductive hormones					[110]
			Limitations: - A single urine sample was used to assess parabens exposure, to describe the level of reproductive hormones, and to assess semen quality; - Temporal reliability was less for concentrations of urinary metabolites of parabens than for phthalate; - As conducted among men recruited through an infertility clinic, the study is limited to generalize the results to the general population; - As a large number of analyses were performed, some of the observations could be chance findings due to multiple testing			Percentile of Exposure	β Coefficient (adjusted)	*P*	
				Ethylparaben					
					Morphology	≤ 25th	Reference		
						>75th	1.97 (0.05-12.16)	0.048	
				Butylparaben					
					Morphology	≤ 25th	Reference		
						>75th	9.51 (0.80-18.21)	0.03	
				Isobutylparaben					
					High DNA stainability	≤ 25th	Reference		
						>75th	*3.52 (1.02-16.03)*	*0.03*	

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	215 healthy unselected young university students (18–23 years old) in Southern Spain (Murcia Region).	2010-2011	- All men provided a urine, blood and semen sample on a single day; - Urinary paraben concentrations were measured by DLLME and UHPLC-MS/MS; - Semen quality was evaluated by measuring volume, sperm concentration, total sperm count, motility and morphology following WHO guidelines; - Serum samples were analyzed for reproductive hormones, including follicle-stimulating hormone, luteinizing hormone, testosterone, inhibin B and estradiol using immunoassays; - Associations between urinary concentrations of parabens and semen quality parameters and reproductive hormone levels were examined using linear regression, adjusting for potential covariates Limitations: - As with all observational studies, causal inference is limited. Residual confounding should always be considered and low statistical power might have played a role in the null findings; - Both urinary parabens and our outcomes were based on a single blood serum, urine or semen sample; - Exposure measurement error or misclassification cannot be ruled out	- Taking into account important covariates, urinary concentrations of parabens or their molar sum were not significantly associated with any semen parameters or any of the reproductive hormone levels; - 94% of the men had detectable urinary concentrations of parabens	Relative to men in the lowest quartile of sum of urinary paraben concentrations, the adjusted difference (95% CI) of sperm count for men in the 2nd, 3rd, and 4th quartiles were 4.1% (-37.1-45.3), -1.6% (-41.9-38.8), and -9.8% (-52.5-32.8), respectively (P-trend = 0.55)	111
Methylparaben Ethyl paraben Propylparaben Butylparaben	100 Latina girls (14-18 years old) living in Salinas, California	2016	- A community-based intervention study to determine whether using personal care products whose labels stated they did not contain these chemicals for 3 days could lower urinary concentrations of parabens; - Pre- and post-intervention urine samples were analyzed for parabens using HPLC-MS/MS Limitations: - Not able to test the replacement products to ensure that they did not contain the chemicals of concern, therefore unable to identify the sources of the increased Ethylparaben and Butylparaben exposure; - Small sample size, study participants were all Latina and mostly low-income and their personal care product use patterns may differ from than the general US population; - 3-day intervention period may not be long enough to observe larger decreases in urinary metabolite concentrations ; - Replacement products were not tested to ensure that they did not contain the chemicals of concern, thereby the sources of the increased Ethylparaben and Butylparaben exposure were not identified	- Methylparaben and Propylparaben concentrations decreased by 43.9% (95% CI: –61.3, –18.8) and 45.4% (95% CI: –63.7, –17.9, respectively; - The GM of Methylparaben decreased from 77.4 µg/L to 43.2 µg/L; - The proportion of girls with detectable concentrations of Methylparaben decreased non significantly from 93% to 87%, and decreases in concentrations were observed in 61% of girls; - The GM of Propylparaben decreased from 22.6 µg/L to 12.3 µg/L, with decreases observed in 63% of girls; - The proportion of girls with detectable concentrations of Propylparaben also decreased between pre- and post-intervention (90% vs 87%), but not significantly; - Unexpectedly, Ethylparaben and Butylparaben concentrations both increased over the course of the intervention period, with Butyl paraben increasing by 101.7% (95% CI: 35.5, 203.2) and Ethylparaben increasing by a nonsignificant 47.3% (95% CI: –0.7, 118.4)		112
Experimental Epidemiological Study						

Table 15. Epidemiological studies of parabens

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methyparaben Propylparaben	18 females (21-25 years old) from the Federal University of Alfenas-MG located in Minas Gerais, Brazil,	2015	- In phase 1, the women used paraben-containing products according to their routine - In phase 2, the women used donated lipstick containing Methylparaben and Propylparaben for 5 days in conjunction with the routine use of paraben-containing products - In phase 3, the women routinely used paraben-containing products while abstaining from lipstick for five days, and blood (15mL) was collected for HPLC-MS/MS analysis Limitations: - A large degree of variability in habits was observed among the individuals; - Non-parametric tests were used to further analyze the data because large inter-individual variability in Methylparaben and Propylparaben serum concentrations was observed	- In phase 2, total paraben levels were significantly higher than phases 1 and 3; - The median concentration ± average deviation was 2.14 ng/mL ± 3.24 ng/mL in phase 2, comparing to 1.06 ng/mL ± 0.80 ng/mL in phase 1 and 1.27 ng/mL ± 0.79 ng/mL in phase 3; - Statistically significant difference was demonstrated between serum parabens in women who used lipstick containing Methylparaben and Propylparaben (p = 0.0005 and 0.0016, respectively); - A strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202)		[94]

* **Bolded text** was used to highlight statistically significant increases; ***Italicized text*** was used to highlight statistically significant decreases
AFC=Anthral follicle count; ANOVA=Analysis of variance procedures; BMI=Body mass index; 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; $P_{parabens}$=Sum molar concentrations of the parabens; PIR=Poverty income ratio; PTB=Preterm birth; SART= Society for Assisted Reproductive Technology; SG=Specific gravity; UPLC-MS/MS=Ultra-high-performance liquid chromatography-tandem mass spectrometry; WHO=World Health Organization

Table 16. Margins of safety for parabens based on an NOAEL of 160 mg/kg/day derived from rat oral study

Exposed population	Paraben exposure	Dermal Absorption Estimate	MOS
Adult	Butylparaben or other single paraben	50%	270
Adult	Multiple parabens	50%	135
Infant	Butylparaben or other single paraben	50%	952
Infant	Multiple parabens	50%	476
Adult	Butylparaben or other single paraben	3.7% *	3652
Adult	Multiple parabens	3.7%*	1826
Infant	Butylparaben or other single paraben	3.7%*	12870
Infant	Multiple parabens	3.7%*	6435

*: SCCS assumption of dermal absorption rate of un-metabolized Butylparaben in humans

Table 17. Margins of safety for parabens in cosmetics as a function of exposed population and single versus multiple paraben usage.[2]

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

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2018 FDA Frequency of Use data: Parabens

Methylparaben

01A - Baby Shampoos	99763	3
01B - Baby Lotions, Oils, Powders, and Creams	99763	20
01C - Other Baby Products	99763	14
02A - Bath Oils, Tablets, and Salts	99763	15
02B - Bubble Baths	99763	17
02D - Other Bath Preparations	99763	26
03A - Eyebrow Pencil	99763	60
03B - Eyeliner	99763	428
03C - Eye Shadow	99763	668
03D - Eye Lotion	99763	131
03E - Eye Makeup Remover	99763	47
03F - Mascara	99763	328
03G - Other Eye Makeup Preparations	99763	175
04A - Cologne and Toilet waters	99763	42
04B - Perfumes	99763	13
04C - Powders (dusting and talcum, excluding aftershave talc)	99763	72
04E - Other Fragrance Preparation	99763	20
05A - Hair Conditioner	99763	410
05B - Hair Spray (aerosol fixatives)	99763	7
05C - Hair Straighteners	99763	19
05D - Permanent Waves	99763	5
05E - Rinses (non-coloring)	99763	11
05F - Shampoos (non-coloring)	99763	325
05G - Tonics, Dressings, and Other Hair Grooming Aids	99763	339
05H - Wave Sets	99763	9
05I - Other Hair Preparations	99763	347

06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	99763	158
06B - Hair Tints	99763	11
06C - Hair Rinses (coloring)	99763	10
06D - Hair Shampoos (coloring)	99763	11
06E - Hair Color Sprays (aerosol)	99763	3
06F - Hair Lighteners with Color	99763	2
06G - Hair Bleaches	99763	19
06H - Other Hair Coloring Preparation	99763	56
07A - Blushers (all types)	99763	195
07B - Face Powders	99763	293
07C - Foundations	99763	294
07D - Leg and Body Paints	99763	27
07E - Lipstick	99763	297
07F - Makeup Bases	99763	51
07G - Rouges	99763	5
07H - Makeup Fixatives	99763	8
07I - Other Makeup Preparations	99763	183
08A - Basecoats and Undercoats	99763	3
08B - Cuticle Softeners	99763	17
08C - Nail Creams and Lotions	99763	5
08E - Nail Polish and Enamel	99763	17
08F - Nail Polish and Enamel Removers	99763	5
08G - Other Manicuring Preparations	99763	21
09A - Dentifrices	99763	10
09B - Mouthwashes and Breath Fresheners	99763	4
09C - Other Oral Hygiene Products	99763	4
10A - Bath Soaps and Detergents	99763	271
10B - Deodorants (underarm)	99763	21
10C - Douches	99763	1
10E - Other Personal Cleanliness Products	99763	193

11A - Aftershave Lotion	99763	86
11D - Preshave Lotions (all types)	99763	2
11E - Shaving Cream	99763	31
11F - Shaving Soap	99763	1
11G - Other Shaving Preparation Products	99763	45
12A - Cleansing	99763	506
12B - Depilatories	99763	8
12C - Face and Neck (exc shave)	99763	877
12D - Body and Hand (exc shave)	99763	984
12E - Foot Powders and Sprays	99763	4
12F - Moisturizing	99763	2206
12G - Night	99763	207
12H - Paste Masks (mud packs)	99763	203
12I - Skin Fresheners	99763	88
12J - Other Skin Care Preps	99763	476
13A - Suntan Gels, Creams, and Liquids	99763	34
13B - Indoor Tanning Preparations	99763	105
13C - Other Suntan Preparations	99763	17

Potassium methylparaben

No reported uses

Sodium Methylparaben

02B - Bubble Baths	5026620	8
02D - Other Bath Preparations	5026620	1
03B - Eyeliner	5026620	12
03C - Eye Shadow	5026620	3
03D - Eye Lotion	5026620	3
03E - Eye Makeup Remover	5026620	4
03F - Mascara	5026620	9

03G - Other Eye Makeup Preparations	5026620	14
04E - Other Fragrance Preparation	5026620	1
05A - Hair Conditioner	5026620	7
05B - Hair Spray (aerosol fixatives)	5026620	1
05D - Permanent Waves	5026620	1
05F - Shampoos (non-coloring)	5026620	42
05G - Tonics, Dressings, and Other Hair Grooming Aids	5026620	9
05H - Wave Sets	5026620	1
05I - Other Hair Preparations	5026620	11
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	5026620	65
06B - Hair Tints	5026620	1
06C - Hair Rinses (coloring)	5026620	2
06D - Hair Shampoos (coloring)	5026620	4
06F - Hair Lighteners with Color	5026620	2
06H - Other Hair Coloring Preparation	5026620	1
07C - Foundations	5026620	1
07I - Other Makeup Preparations	5026620	10
10A - Bath Soaps and Detergents	5026620	9
10C - Douches	5026620	3
10E - Other Personal Cleanliness Products	5026620	2
11E - Shaving Cream	5026620	1
12A - Cleansing	5026620	30
12B - Depilatories	5026620	2
12C - Face and Neck (exc shave)	5026620	55
12D - Body and Hand (exc shave)	5026620	18
12E - Foot Powders and Sprays	5026620	3
12F - Moisturizing	5026620	21
12G - Night	5026620	5
12H - Paste Masks (mud packs)	5026620	11
12I - Skin Fresheners	5026620	4

12J - Other Skin Care Preps	5026620	22
13B - Indoor Tanning Preparations	5026620	2

Ethylparaben

01A - Baby Shampoos	120478	1
01B - Baby Lotions, Oils, Powders, and Creams	120478	12
01C - Other Baby Products	120478	2
02A - Bath Oils, Tablets, and Salts	120478	4
02B - Bubble Baths	120478	6
02D - Other Bath Preparations	120478	21
03A - Eyebrow Pencil	120478	13
03B - Eyeliner	120478	73
03C - Eye Shadow	120478	154
03D - Eye Lotion	120478	67
03E - Eye Makeup Remover	120478	18
03F - Mascara	120478	168
03G - Other Eye Makeup Preparations	120478	85
04B - Perfumes	120478	1
04C - Powders (dusting and talcum, excluding aftershave talc)	120478	10
04E - Other Fragrance Preparation	120478	9
05A - Hair Conditioner	120478	54
05B - Hair Spray (aerosol fixatives)	120478	3
05C - Hair Straighteners	120478	5
05E - Rinses (non-coloring)	120478	2
05F - Shampoos (non-coloring)	120478	161
05G - Tonics, Dressings, and Other Hair Grooming Aids	120478	71
05H - Wave Sets	120478	3
05I - Other Hair Preparations	120478	134
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	120478	90

06B - Hair Tints	120478	1
06D - Hair Shampoos (coloring)	120478	5
06F - Hair Lighteners with Color	120478	2
06H - Other Hair Coloring Preparation	120478	17
07A - Blushers (all types)	120478	31
07B - Face Powders	120478	63
07C - Foundations	120478	112
07D - Leg and Body Paints	120478	3
07E - Lipstick	120478	69
07F - Makeup Bases	120478	23
07G - Rouges	120478	36
07H - Makeup Fixatives	120478	1
07I - Other Makeup Preparations	120478	54
08A - Basecoats and Undercoats	120478	1
08B - Cuticle Softeners	120478	12
08C - Nail Creams and Lotions	120478	2
08E - Nail Polish and Enamel	120478	11
08F - Nail Polish and Enamel Removers	120478	2
08G - Other Manicuring Preparations	120478	12
09B - Mouthwashes and Breath Fresheners	120478	1
10A - Bath Soaps and Detergents	120478	126
10B - Deodorants (underarm)	120478	10
10C - Douches	120478	1
10E - Other Personal Cleanliness Products	120478	82
11A - Aftershave Lotion	120478	34
11D - Preshave Lotions (all types)	120478	1
11E - Shaving Cream	120478	9
11F - Shaving Soap	120478	1
11G - Other Shaving Preparation Products	120478	17
12A - Cleansing	120478	219
12B - Depilatories	120478	6

12C - Face and Neck (exc shave)	120478	414
12D - Body and Hand (exc shave)	120478	360
12E - Foot Powders and Sprays	120478	4
12F - Moisturizing	120478	470
12G - Night	120478	119
12H - Paste Masks (mud packs)	120478	79
12I - Skin Fresheners	120478	21
12J - Other Skin Care Preps	120478	181
13A - Suntan Gels, Creams, and Liquids	120478	18
13B - Indoor Tanning Preparations	120478	55
13C - Other Suntan Preparations	120478	8

Potassium Ethylparaben

No reported uses

Sodium Ethylparaben

03D - Eye Lotion	35285688	SODIUM ETHYLPARABEN	1
03F - Mascara	35285688	SODIUM ETHYLPARABEN	2
03G - Other Eye Makeup Preparations	35285688	SODIUM ETHYLPARABEN	8
07C - Foundations	35285688	SODIUM ETHYLPARABEN	1
07I - Other Makeup Preparations	35285688	SODIUM ETHYLPARABEN	1
10C - Douches	35285688	SODIUM ETHYLPARABEN	2
12C - Face and Neck (exc shave)	35285688	SODIUM ETHYLPARABEN	4
12D - Body and Hand (exc shave)	35285688	SODIUM ETHYLPARABEN	2
12F - Moisturizing	35285688	SODIUM ETHYLPARABEN	3
12I - Skin Fresheners	35285688	SODIUM ETHYLPARABEN	1
12J - Other Skin Care Preps	35285688	SODIUM ETHYLPARABEN	4

Isopropylparaben

02A - Bath Oils, Tablets, and Salts	4191735	1

03B - Eyeliner	4191735	6
03C - Eye Shadow	4191735	20
03D - Eye Lotion	4191735	3
03F - Mascara	4191735	19
03G - Other Eye Makeup Preparations	4191735	3
04A - Cologne and Toilet waters	4191735	1
04B - Perfumes	4191735	1
05A - Hair Conditioner	4191735	13
05F - Shampoos (non-coloring)	4191735	3
05G - Tonics, Dressings, and Other Hair Grooming Aids	4191735	5
07A - Blushers (all types)	4191735	15
07B - Face Powders	4191735	5
07C - Foundations	4191735	6
07E - Lipstick	4191735	31
07F - Makeup Bases	4191735	1
07G - Rouges	4191735	2
07I - Other Makeup Preparations	4191735	12
08B - Cuticle Softeners	4191735	2
08C - Nail Creams and Lotions	4191735	1
08G - Other Manicuring Preparations	4191735	3
10A - Bath Soaps and Detergents	4191735	3
10E - Other Personal Cleanliness Products	4191735	21
11A - Aftershave Lotion	4191735	1
12A - Cleansing	4191735	4
12B - Depilatories	4191735	1
12C - Face and Neck (exc shave)	4191735	3
12D - Body and Hand (exc shave)	4191735	17
12F - Moisturizing	4191735	68
12G - Night	4191735	2
12H - Paste Masks (mud packs)	4191735	1
12J - Other Skin Care Preps	4191735	6

13B - Indoor Tanning Preparations	4191735	3

Sodium Isopropylparaben

No reported uses

Propylparaben

01A - Baby Shampoos	94133	3
01B - Baby Lotions, Oils, Powders, and Creams	94133	21
01C - Other Baby Products	94133	12
02A - Bath Oils, Tablets, and Salts	94133	20
02B - Bubble Baths	94133	14
02D - Other Bath Preparations	94133	23
03A - Eyebrow Pencil	94133	75
03B - Eyeliner	94133	425
03C - Eye Shadow	94133	607
03D - Eye Lotion	94133	80
03E - Eye Makeup Remover	94133	29
03F - Mascara	94133	249
03G - Other Eye Makeup Preparations	94133	135
04A - Cologne and Toilet waters	94133	2
04B - Perfumes	94133	5
04C - Powders (dusting and talcum, excluding aftershave talc)	94133	48
04E - Other Fragrance Preparation	94133	24
05A - Hair Conditioner	94133	188
05B - Hair Spray (aerosol fixatives)	94133	3
05C - Hair Straighteners	94133	12
05D - Permanent Waves	94133	2
05E - Rinses (non-coloring)	94133	7
05F - Shampoos (non-coloring)	94133	182
05G - Tonics, Dressings, and Other Hair Grooming Aids	94133	195

05H - Wave Sets	94133	5
05I - Other Hair Preparations	94133	140
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	94133	119
06B - Hair Tints	94133	10
06C - Hair Rinses (coloring)	94133	6
06D - Hair Shampoos (coloring)	94133	9
06F - Hair Lighteners with Color	94133	2
06G - Hair Bleaches	94133	1
06H - Other Hair Coloring Preparation	94133	27
07A - Blushers (all types)	94133	160
07B - Face Powders	94133	237
07C - Foundations	94133	215
07D - Leg and Body Paints	94133	20
07E - Lipstick	94133	594
07F - Makeup Bases	94133	29
07G - Rouges	94133	4
07H - Makeup Fixatives	94133	4
07I - Other Makeup Preparations	94133	191
08A - Basecoats and Undercoats	94133	3
08B - Cuticle Softeners	94133	19
08C - Nail Creams and Lotions	94133	4
08E - Nail Polish and Enamel	94133	14
08F - Nail Polish and Enamel Removers	94133	2
08G - Other Manicuring Preparations	94133	17
09A - Dentifrices	94133	4
09B - Mouthwashes and Breath Fresheners	94133	1
09C - Other Oral Hygiene Products	94133	2
10A - Bath Soaps and Detergents	94133	206
10B - Deodorants (underarm)	94133	14
10C - Douches	94133	1

10E - Other Personal Cleanliness Products	94133	140
11A - Aftershave Lotion	94133	45
11D - Preshave Lotions (all types)	94133	2
11E - Shaving Cream	94133	26
11F - Shaving Soap	94133	1
11G - Other Shaving Preparation Products	94133	33
12A - Cleansing	94133	326
12B - Depilatories	94133	18
12C - Face and Neck (exc shave)	94133	543
12D - Body and Hand (exc shave)	94133	778
12E - Foot Powders and Sprays	94133	2
12F - Moisturizing	94133	1765
12G - Night	94133	134
12H - Paste Masks (mud packs)	94133	133
12I - Skin Fresheners	94133	45
12J - Other Skin Care Preps	94133	365
13A - Suntan Gels, Creams, and Liquids	94133	35
13B - Indoor Tanning Preparations	94133	73
13C - Other Suntan Preparations	94133	12

Potassium Propylparaben

No reported uses

Sodium Propylparaben

01C - Other Baby Products	35285699	1
02B - Bubble Baths	35285699	3
02D - Other Bath Preparations	35285699	1
03B - Eyeliner	35285699	2
03C - Eye Shadow	35285699	3
03D - Eye Lotion	35285699	2

03F - Mascara	35285699	5
03G - Other Eye Makeup Preparations	35285699	8
05F - Shampoos (non-coloring)	35285699	2
05I - Other Hair Preparations	35285699	1
06C - Hair Rinses (coloring)	35285699	1
07C - Foundations	35285699	1
07I - Other Makeup Preparations	35285699	5
10A - Bath Soaps and Detergents	35285699	3
10C - Douches	35285699	3
12A - Cleansing	35285699	11
12C - Face and Neck (exc shave)	35285699	27
12D - Body and Hand (exc shave)	35285699	13
12E - Foot Powders and Sprays	35285699	3
12F - Moisturizing	35285699	9
12G - Night	35285699	3
12H - Paste Masks (mud packs)	35285699	10
12I - Skin Fresheners	35285699	1
12J - Other Skin Care Preps	35285699	18
13B - Indoor Tanning Preparations	35285699	1

Isobutylparaben

01A - Baby Shampoos	4247023	1
01B - Baby Lotions, Oils, Powders, and Creams	4247023	2
01C - Other Baby Products	4247023	2
02A - Bath Oils, Tablets, and Salts	4247023	3
02B - Bubble Baths	4247023	3
02D - Other Bath Preparations	4247023	19
03A - Eyebrow Pencil	4247023	5
03B - Eyeliner	4247023	36
03C - Eye Shadow	4247023	41

03D - Eye Lotion	4247023	25
03E - Eye Makeup Remover	4247023	9
03F - Mascara	4247023	76
03G - Other Eye Makeup Preparations	4247023	41
04A - Cologne and Toilet waters	4247023	1
04B - Perfumes	4247023	2
04C - Powders (dusting and talcum, excluding aftershave talc)	4247023	4
04E - Other Fragrance Preparation	4247023	4
05A - Hair Conditioner	4247023	24
05E - Rinses (non-coloring)	4247023	1
05F - Shampoos (non-coloring)	4247023	56
05G - Tonics, Dressings, and Other Hair Grooming Aids	4247023	22
05H - Wave Sets	4247023	1
05I - Other Hair Preparations	4247023	33
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	4247023	23
06D - Hair Shampoos (coloring)	4247023	4
06H - Other Hair Coloring Preparation	4247023	15
07A - Blushers (all types)	4247023	23
07B - Face Powders	4247023	18
07C - Foundations	4247023	54
07D - Leg and Body Paints	4247023	2
07E - Lipstick	4247023	69
07F - Makeup Bases	4247023	5
07G - Rouges	4247023	2
07H - Makeup Fixatives	4247023	1
07I - Other Makeup Preparations	4247023	41
08A - Basecoats and Undercoats	4247023	1
08B - Cuticle Softeners	4247023	12
08C - Nail Creams and Lotions	4247023	2
08E - Nail Polish and Enamel	4247023	7

08F - Nail Polish and Enamel Removers	4247023	2
08G - Other Manicuring Preparations	4247023	13
10A - Bath Soaps and Detergents	4247023	91
10B - Deodorants (underarm)	4247023	5
10C - Douches	4247023	1
10E - Other Personal Cleanliness Products	4247023	79
11A - Aftershave Lotion	4247023	20
11D - Preshave Lotions (all types)	4247023	1
11E - Shaving Cream	4247023	2
11F - Shaving Soap	4247023	1
11G - Other Shaving Preparation Products	4247023	11
12A - Cleansing	4247023	99
12B - Depilatories	4247023	3
12C - Face and Neck (exc shave)	4247023	240
12D - Body and Hand (exc shave)	4247023	210
12E - Foot Powders and Sprays	4247023	2
12F - Moisturizing	4247023	260
12G - Night	4247023	47
12H - Paste Masks (mud packs)	4247023	41
12I - Skin Fresheners	4247023	10
12J - Other Skin Care Preps	4247023	114
13A - Suntan Gels, Creams, and Liquids	4247023	7
13B - Indoor Tanning Preparations	4247023	32
13C - Other Suntan Preparations	4247023	3

Sodium Isobutylparaben

12I - Skin Fresheners	84930154	SODIUM ISOBUTYLPARABEN	1

Butylparaben

01A - Baby Shampoos	94268	2
01B - Baby Lotions, Oils, Powders, and Creams	94268	6
01C - Other Baby Products	94268	3
02A - Bath Oils, Tablets, and Salts	94268	4
02B - Bubble Baths	94268	4
02D - Other Bath Preparations	94268	15
03A - Eyebrow Pencil	94268	51
03B - Eyeliner	94268	326
03C - Eye Shadow	94268	211
03D - Eye Lotion	94268	43
03E - Eye Makeup Remover	94268	21
03F - Mascara	94268	103
03G - Other Eye Makeup Preparations	94268	67
04A - Cologne and Toilet waters	94268	1
04B - Perfumes	94268	2
04C - Powders (dusting and talcum, excluding aftershave talc)	94268	14
04E - Other Fragrance Preparation	94268	10
05A - Hair Conditioner	94268	50
05C - Hair Straighteners	94268	5
05E - Rinses (non-coloring)	94268	4
05F - Shampoos (non-coloring)	94268	118
05G - Tonics, Dressings, and Other Hair Grooming Aids	94268	43
05H - Wave Sets	94268	2
05I - Other Hair Preparations	94268	59
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	94268	23
06B - Hair Tints	94268	2
06D - Hair Shampoos (coloring)	94268	5
06H - Other Hair Coloring Preparation	94268	15
07A - Blushers (all types)	94268	81
07B - Face Powders	94268	118

07C - Foundations	94268	104
07D - Leg and Body Paints	94268	7
07E - Lipstick	94268	281
07F - Makeup Bases	94268	12
07G - Rouges	94268	37
07H - Makeup Fixatives	94268	1
07I - Other Makeup Preparations	94268	86
08A - Basecoats and Undercoats	94268	2
08B - Cuticle Softeners	94268	14
08C - Nail Creams and Lotions	94268	3
08E - Nail Polish and Enamel	94268	8
08F - Nail Polish and Enamel Removers	94268	2
08G - Other Manicuring Preparations	94268	13
10A - Bath Soaps and Detergents	94268	124
10B - Deodorants (underarm)	94268	8
10C - Douches	94268	1
10E - Other Personal Cleanliness Products	94268	102
11A - Aftershave Lotion	94268	24
11D - Preshave Lotions (all types)	94268	1
11E - Shaving Cream	94268	9
11F - Shaving Soap	94268	1
11G - Other Shaving Preparation Products	94268	13
12A - Cleansing	94268	175
12B - Depilatories	94268	4
12C - Face and Neck (exc shave)	94268	346
12D - Body and Hand (exc shave)	94268	308
12E - Foot Powders and Sprays	94268	2
12F - Moisturizing	94268	427
12G - Night	94268	65
12H - Paste Masks (mud packs)	94268	72
12I - Skin Fresheners	94268	16

12J - Other Skin Care Preps	94268	161
13A - Suntan Gels, Creams, and Liquids	94268	24
13B - Indoor Tanning Preparations	94268	45
13C - Other Suntan Preparations	94268	9

Potassium Butylparaben

No reported uses

Sodium Butylparaben

12I - Skin Fresheners	36457202	1

Benzylparaben

No reported uses

Calcium Paraben

No reported uses

Potassium Paraben

No reported uses

Sodium Paraben

No reported uses

4-hydroxybenzoic acid

No reported uses

Assessing the reproductive and developmental toxicity of parabens

Presentation to CIR

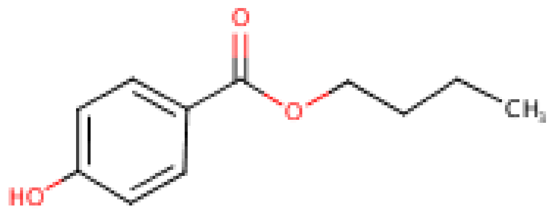
March 5, 2018

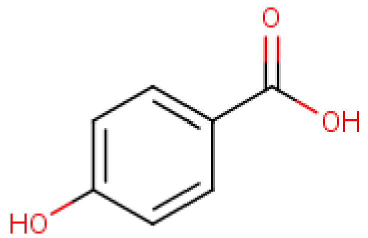
Overview

- Mode of action
- Metabolism
- Toxicity and risk

Mode of Action

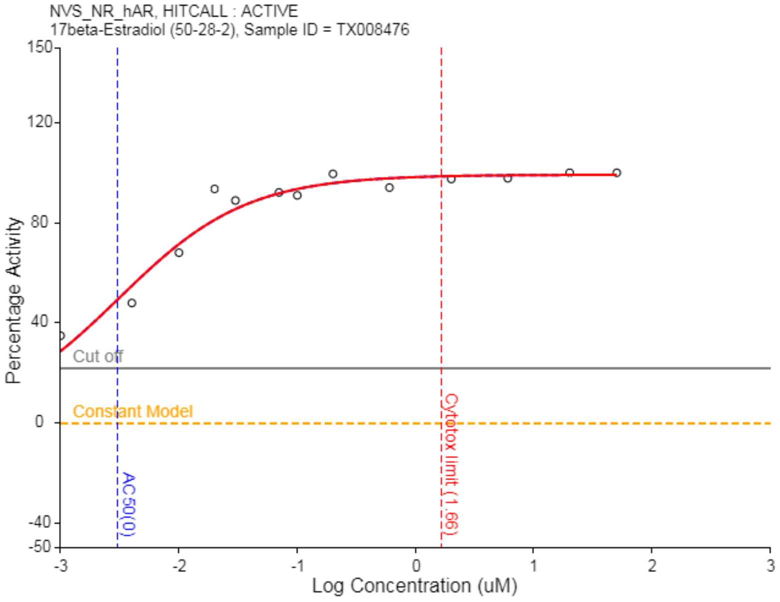
- Weak estrogen receptor agonist
  - In vitro displacement of estradiol from both isoforms of ER, transcription of E2-responsive genes
  - Butylparaben is 10,000-100,000x less potent than estradiol, methylparaben 1,000,000, and propyl and ethyl are in between
  - No activity for p-hydroxybenzoic acid

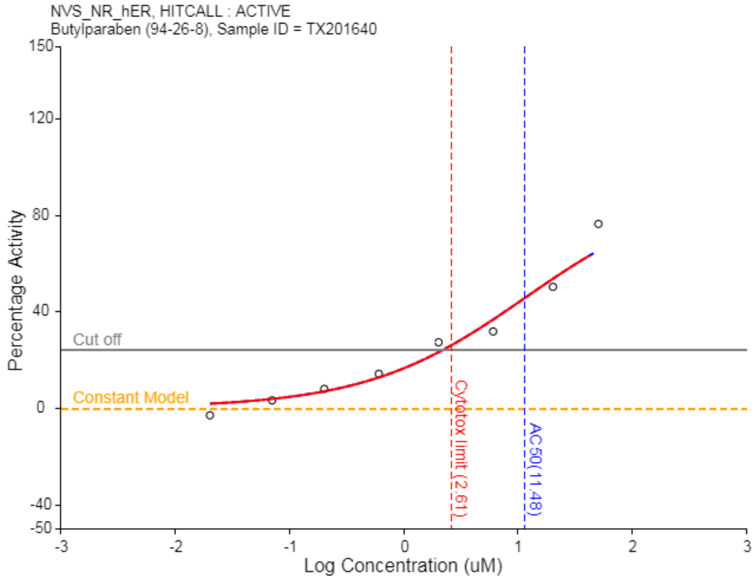




EDSP21 results

Chemical	# of positive estrogenicity results (out of 18)
17-beta-estradiol	17
Butyl paraben	15
Propyl paraben	14
Ethyl paraben	11
Methyl paraben	5
p-hydroxybenzoic acid	2

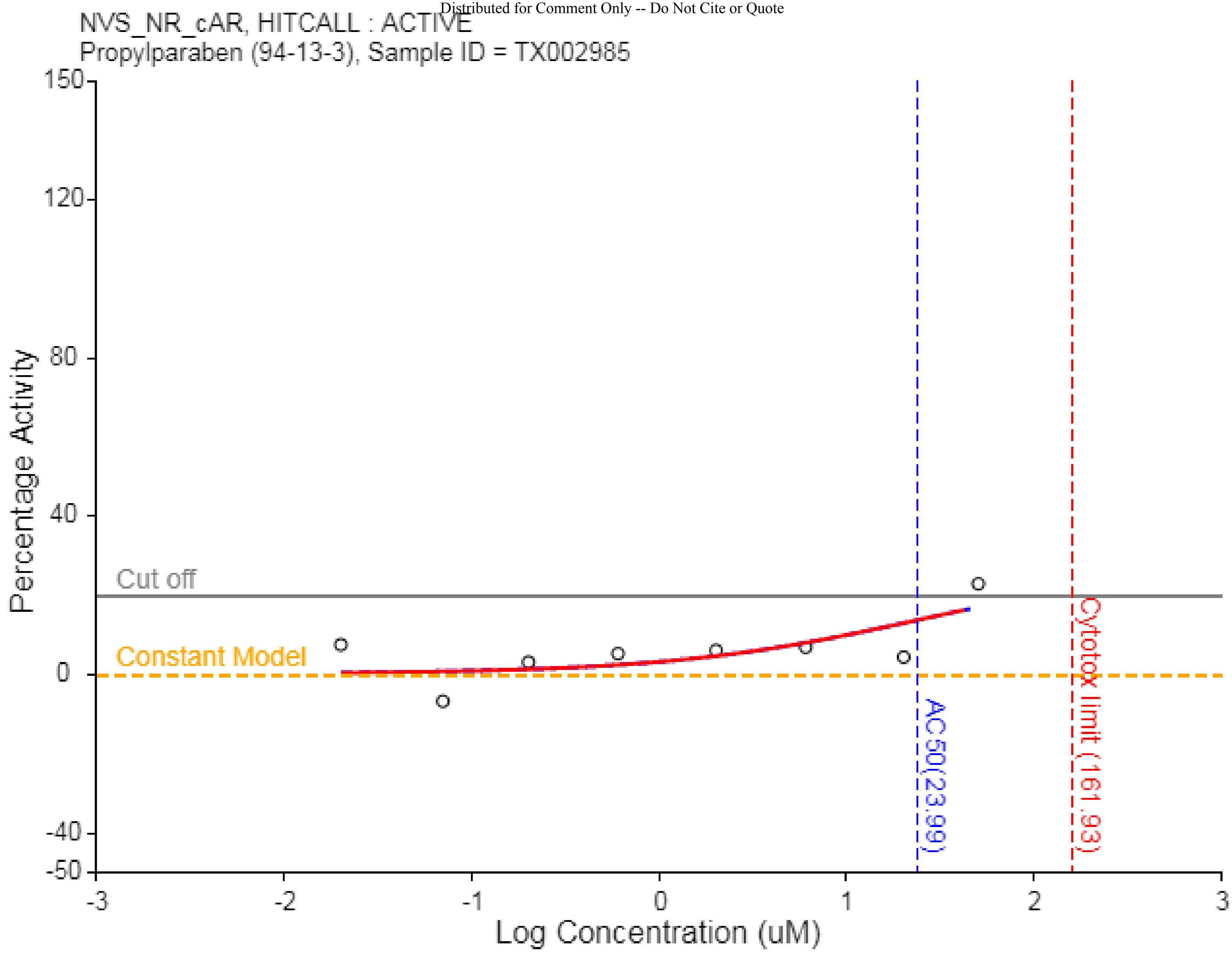




Are parabens anti-androgens?

- Positive results in one or two in vitro reporter gene assays
- EDSP21 results

Chemical	# of positives
Butylparaben	0/10
Propylparaben	1/9
Ethylparaben	0/11
Methylparaben	0/8


NVS_NR_cAR, HITCALL : ACTIVE
Propylparaben (94-13-3), Sample ID = TX002985
Percentage Activity
150
120
80
40
0
-40
-50
Cut off
Constant Model
AC50(23.99)
Cytotox limit (161.93)
-3
-2
-1
0
1
2
3
Log Concentration (uM)

Effects of an estrogen agonist in vivo on male rat offspring

- Decreased body weight gain (probably as a result of decreased T)
- Decreased epididymis weight
- Decreased circulating T and LH (no effect on FSH)
- Decreased epididymal sperm number
- Slight decrease in normal sperm morphology (97% vs 99%)
- No effect on sperm motility

- From Cook et al. (1998) Tox. Sci. 44: 155-168, a one-generation study with 17-beta-estradiol

Effects of anti-androgens in vivo on male rat offspring development

- Malformations of the reproductive tract
  - Undescended testes
  - Urethral malformations, including hypospadias
  - Small prostate, seminal vesicles
- Marked decreases in anogenital distance (40-60% in male pups early, 10% in adult offspring)
- Later puberty
- Decreased serum T
- Decreased epididymal sperm concentration
- Areola/nipple retention

Metabolism

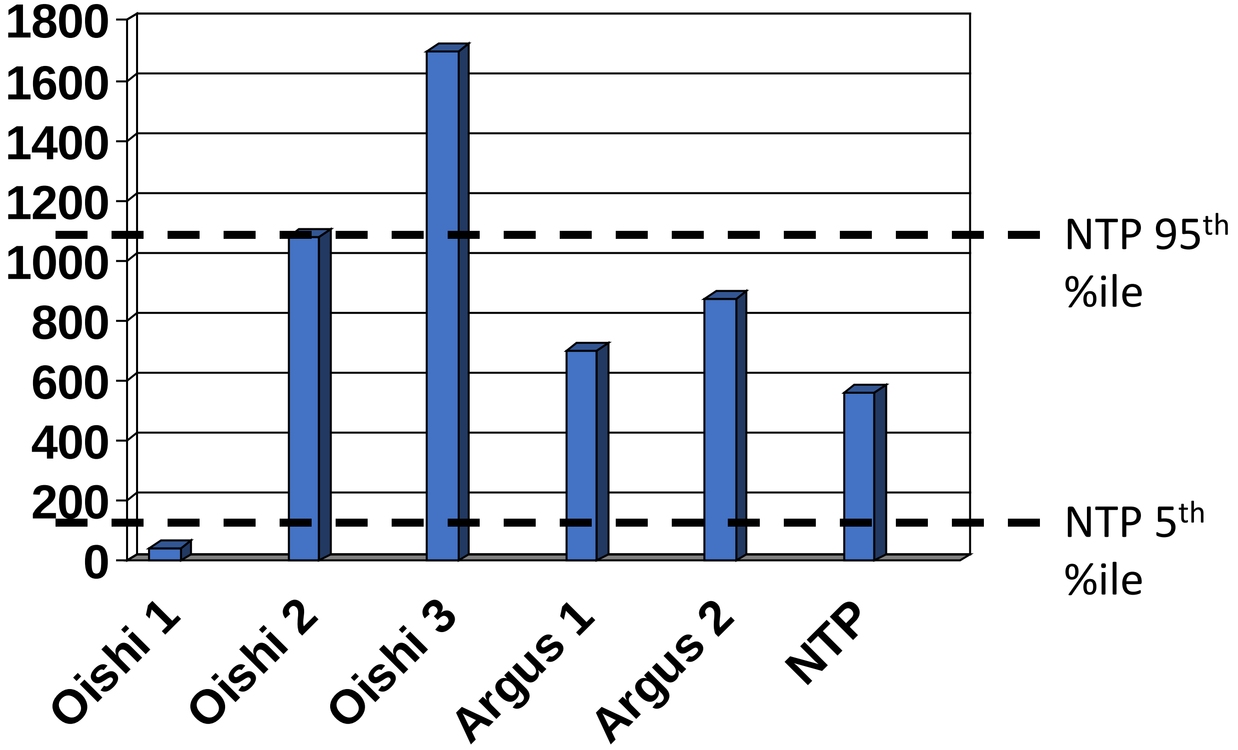
- Parabens are rapidly hydrolyzed at portals of entry (dermal and oral)
- Products are p-hydroxybenzoic acid and short-chain alcohols
- Clinical studies measuring absorption show only very small percentage of paraben in plasma (approx. 2% of administered dose)

- Therefore, studies using sc injection might be useful for understanding the hazard, but not the risk of parabens

Biological effects and toxicity

- Estrogenicity in vivo?
  - Uterotrophic studies show a lack of effect from oral exposures (up to 1200 mg/kg/day BP), a blunted effect from sc exposures (400-1200 mg/kg/day BP)
- Toxicity
  - No developmental toxicity in a guideline study up to 1000 mg/kg/day by gavage
  - Some reports of effects on male reproductive development when exposure was early postnatal (Oishi)
  - Failure to replicate in a GLP study (Hoberman et al.)
  - Oishi’s results were inconsistent with historical data for the affected parameters

Control Sperm Concentration



CIR Questions: 1

- Is epididymal sperm concentration a relevant DART endpoint for defining a NOAEL?
- Yes. Epididymal sperm concentration is highly correlated with sperm count, and a decrease in sperm count would increase the risk of infertility. Like any individual measurement, epididymal sperm concentration should be viewed in the context of the weight of evidence. A lack of effect on testicular or epididymal histology would tend to decrease the validity of the effect.

- NB: The NOAEL for sc injection would not be a relevant point of departure for risk assessment because it circumvents portal of entry metabolism

CIR Questions: 2

- Is anogenital distance a relevant DART endpoint on which to base a NOAEL?
- No. AGD on its own should be considered to be a biomarker of effect and not an adverse outcome.

Recent animal studies

- Garcia et al (2017): butylparaben, sc exposure, young male rats
- Taxvig et al (2008): butylparaben or ethylparaben, sc, gestational
- Zhang et al (2014): butylparaben, oral, gestational and early postnatal
- Boberg et al (2016): butylparaben, oral, gestational and early postnatal

- Manservisi et al (2015): a very low dose of methylparaben, two-gen study: high rate of pup mortality in every group, not consistent with any other study
- Gazin et al (2013): propylparaben to juvenile male rats, no effects on repro parameters up to 1000 mg/kg/day oral

Garcia et al (2017)

- 6 week old male rats
- Sc injection, 3 x per week, for 57 days
- Two control groups: vehicle and untreated
  - Statistical comparisons appear to have been done vs the untreated control, even though there were big differences between this and the vehicle control

Garcia et al (2017)

Parameter	Untreated	Control	150 mg/kg	300 mg/kg	600 mg/kg
Prostate weight (g/kg bw)	1.53 (0.32)	1.90 (0.24)	1.98 (0.26)*	1.86 (0.27)	2.25 (0.24)
Epididymal sperm conc. (million/ml)	400 (26)	300 (146)	174 (85)*	149 (56)*	205 (56)*
Testicular spermatid conc. (million/ml)	21.5 (36.2)	15.2 (10.6)	14.7 (16.1)	21.0 (79.3)	13.4 (15.1)*
% progressively motile sperm	60 (8)	52 (9)	48 (7)*	46 (8)*	47 (7)*
% normal sperm	75 (7)	72 (5)	67 (6)*	55 (5)*	50 (6)*

Charles River historical control ranges:
Motility: 57-80%
Normal sperm: 86-98%

Taxvig et al (2008)

- Ethylparaben (400 mg/kg/day) or butylparaben (200 or 400 mg/kg/day), sc, GD 7-21
- No effects on AGD or other parameters in fetuses, including sex steroid levels
- Effects on some adrenal steroid synthesis gene expression in females but not males

Boberg et al (2016)

- Butylparaben, oral gavage, GD7 – PND 22
- 13-17 litters per group
- 10, 100, 500 mg/kg/day
- Effects on AGD, male and female, two higher dose levels (around 10%, not obviously dose-related)
- No effect on areola/ nipple retention
- Decreased ventral prostate weight and seminal vesicle weight on PND 80-90 in high dose group (vs. increased prostate weight in Garcia et al)
- Effects on prostate and mammary gland histology at higher dose levels, not dose-responsive

Zhang et al (2014)

- Butylparaben, oral, GD 7- PND 21, 64, 160, 400, 1000 mg/kg/day
- Only 7-8 litters per group
- AGD decreased in males at two higher dose levels, PND 1 and 21 (approx. 10%) but data not normalized to body mass
- 3-4 day delay in preputial separation at two high higher dose levels, but body weight at PPS was the same across groups
- Decreased serum T and LH over different ages at high dose
- Decreased epididymal sperm concentration and testis spermatid concentration at two higher dose levels
    - However, all values, including control appear to be far below historical control range
- Reported effects on histology at two higher dose levels, but suboptimal tissue preparation

Human studies

- Meeker et al (2011)
- Adoamnei et al (2018)
- Nassan et al (2017)
- Jurewicz et al (2017)

Meeker et al 2011

- Semen collection in patients at an infertility clinic and urinary measurements of various parabens and bisphenol A
- No relationship between any chemicals and semen or hormone parameters
- No interquartile effects (e.g., lowest quartile vs highest quartile) in sperm DNA damage for butylparaben but a significant trend test (p=0.03) across quartiles
- Authors make no definitive conclusions

Adoamnei et al (2018)

- Controlled study in university students (presumed fertile)
- Measurement of serum hormones, semen parameters, and urinary paraben levels
- No association between paraben levels and any measured parameter

Jurewicz et al (2017)

- 315 men visiting an infertility clinic, sperm conc. 15-300 million/ml
- Semen parameters, sperm DNA stability, serum hormones
- Urinary paraben measurement
- Frequency of detection

MP	EP	PP	BP	iBP
99	42	89	11	16

Jurewicz et al (2017)

- When paraben was below LOD, a value of LOD/2 was used
- As a consequence, the only statistics for EP, BP and isobutylP were for a group where the authors acknowledge that >75% of samples had no detectable paraben
- Significant p-value for
  - sperm morphology for EP and BP, but not PP
  - serum T for BP
  - High DNA stability for isobutylP
- 121 statistical comparisons, 4 had p values of $p<0.05$

Nassan et al (2017)

- Biomonitoring study
- Ability to detect urinary metabolites of parabens and monoethyl phthalate 6 hours after use of personal care products

CIR Questions: 3

- Are there reasons to elevate or discount any of the DART data?
  - Studies using sc injection are of interest as support, but not appropriate for risk assessment
  - Mode of action data are important in weighing consistency of data
    - Strong evidence that some parabens are weakly estrogenic
    - Preponderance of evidence that parabens are not anti-androgens
    - Taxvig et al suggest an effect on steroid synthesis, but results are not strong
  - Effects of estradiol:
    - Decreased body weight gain (probably because of decreased T)
    - Decreased epididymis weight
    - Decreased epididymal sperm concentration
    - Decreased T and LH
    - Slight effect on sperm morphology

Summary of recent animal studies

- Effects on AGD
  - Yes for Boberg and Zhang (but only 10%, not dose-responsive)
  - No for Taxvig
- Effect on serum T, LH
  - Yes for Zhang at 1000 mg/kg/day
- Effects on epididymal sperm concentration
  - Yes for Boberg (10, 100 and 500 mg/kg/day, no dose-response)
  - Yes for Zhang (400 and 1000 mkd, NOAEL = 160 mkd) (data outside HCD)
  - No for Hoberman up to 1000 mkd (but different dosing period)
- Effects on testicular spermatid concentration
  - Yes for Zhang (400 and 1000 mkd, NOAEL = 160)
  - No for Hoberman

CIR Questions: 4

- What is an appropriate DART NOAEL to use to calculate MOS?

Dosage (mkd)	10	64	100	160	400	500	1000
AGD	-	-	+	-	+	+	+
Serum T, LH		-		-	-		+
Epid. sperm conc.	+	-	+	-	+	+	+
Testis sperm conc.		-		-	+		+
Histology	-	-	+	-	+	+	+

Red = not dose-responsive

Conclusions

- Lots of conflicting data
- Mode of action studies: weak in vitro estrogens
- Metabolism: high level of hydrolysis at portals of entry
    - Explains lack of an in vivo estrogenic effect (uterotrophic assay) by oral or dermal route
- Two oral studies (Boberg, Zhang) report effects using a prenatal/perinatal dosing paradigm not used by others, some of which are consistent with an estrogen mechanism
    - Is there a downregulation of esterase activity during pregnancy/lactation in the rat?
- 400 mkd is a pragmatic LOAEL, 160 mkd NOAEL, from which to calculate MOS for butylparaben. Assuming an estrogenic mechanism, this would be adequately protective for propyl, ethyl and methylparaben

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Alexandra Kowcz
Industry Liaison to the CIR Expert Panel

DATE: March 6, 2018

SUBJECT: Draft Report: Safety Assessment of Parabens as Used in Cosmetics (draft prepared for the March 5-6, 2018 CIR Expert Panel Meeting)

The Council respectfully submits the following comments on the draft report, Safety Assessment of Parabens as Used in Cosmetics.

The first section includes comments submitted on the June 2017 draft that still need to be addressed.

Key Issues

Although NHANES data are mentioned in the epidemiology section, more information about their findings, e.g., 95th percentile values, numbers of subjects, should be included in the Aggregate Exposure section. The most recent data tables are available at https://www.cdc.gov/exposurereport/.

The following paper, which uses the NHANES data (2009-2010 collection period) and a PBPK model to calculate margins of safety for Methyl-, Propyl- and Butylparaben also needs to be added to the CIR report.

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

Dr. Campbell's presentation at the 2015 Council Safety Seminar is available at: http://eservices.personalcarecouncil.org/Science/15SS/paperlesssites/safety/campbellpresentation.pdf

The following exposure study should also be added to the CIR report (found at https://ehp.niehs.nih.gov/wp-content/uploads/124/10/ehp.1510514.alt.pdf):

Harley KG, Kogut K, Madrigal DS, et al. 2016. Reducing phthalate, paraben, and phenol exposure from personal care products in adolescent girls: Findings from the HERMOSA Intervention study. *EHP* 124(10): 1600-1607.

The Discussion of the 2008 report suggests that "infant exposure to parabens via breast-feeding was unlikely." The following recent study on parabens in breast milk should be added to the CIR report (this paper is on the I drive in the parabens folder [provided to CIR March 28, 2017]).

Fisher M, MacPherson S, Braun JM, et al. 2017. Paraben concentrations in maternal urine and breast milk and its association with personal care product use. *Environ Sci Technol* 51(7): 4009-4017.

Reviewing the search strategy for the paraben CIR report, suggests that perhaps a search on parabens and exposure should be completed. In addition to being part of the NHANES biomonitoring study, biomonitoring studies in California and Canada (perhaps other locations) also include parabens. It may also be helpful to complete a search on parabens and cosmetics.

Cosmetic Use, Summary - In addition to the SCCP opinions, the EU regulations for parabens should be presented in the Cosmetic Use section.

Additional Considerations

Introduction - As stated elsewhere in the report, two ingredients are reported to function as "fragrance ingredients" not "fragrances" as stated in the Introduction. Although these ingredients may be used in fragrance, it is unlikely that they impart a fragrance to the product.

Cosmetic Use - Concentrations of use were reported for the FDA product category face powders. It is not known if the products are "loose" or compact powders. Therefore, the word "loose" needs to be deleted from: "These ingredients are reportedly used in ~~loose~~ powder products...."

Cosmetic Use - The NICNAS assessment is presented twice in this section.

Dermal Penetration, old report summary - How was the extent of metabolism different between rodent and human skin?

Dermal Penetration, *In Vitro*, Summary - The summary of reference 38 says that "increasing the ethanol concentration" increased retention of parabens in the dermis. Was the ethanol concentration increased in the receptor solution or the dosing vehicle?

Aggregate Exposure - Please state the number of subjects included in the paraben breast tissue study.

Carcinogenicity, 1984 summary - Please provide some indication of the doses used in the studies described.

Table 9 - Please identify what was used as the receptor fluid in the study described in reference 37.

Table 10, *in vitro*, reference 48 - Based on the Sample Type/Test Population-Sex column, hepatocytes from one man and one woman were used. Therefore, in the results column, it is not appropriate to state "hepatocytes from males and females" when discussing the results for humans.

New Comments

ADME, *In Vitro*, Cell-Free Systems - The "system" used to biotransform Butylparaben (reference 44) should be stated.

DART, Oral Exposure - The doses used in each study are not clear. For example, this section states: "Liver weights increased at all dosage rates of Butylparaben," - but the doses of Butylparaben used are not stated.

Epidemiology Studies - The studies described under the Prospective Studies subheading do not appear to be prospective studies. With perhaps the exception of reference 88, the other studies are cross-sectional studies (similar to Adoamnei et al. 2018 study provided to the CIR Expert Panel) in which parabens and an outcome were measured at a specific point in time. This slide from the CDC explains different types of epidemiology studies https://www.fda.gov/ohrms/dockets/ac/02/briefing/3839s1_12_alter/sld002.htm.

Post-meeting Comments

The information Dr. Daston presented on the EDSP results should be added to the CIR report (found at https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-21st-century)

The aggregate exposure paper mentioned by Dr. Daston by Cowan-Ellsberry and Robison (2009) still needs to be added to the CIR report. This paper was previously provided to CIR on 8/2/2017 and is found on the I drive in the 2016_12_Parabens folder.