Safety Assessment of Parabens as Used in Cosmetics

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The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A.
This report was prepared by Ivan J. Boyer, Senior Toxicologist and Lillian C. Becker, Scientific Analyst/Writer.

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MEMORANDUM

To: CIR Expert Panel and Liaisons

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Senior Toxicologist

Lillian C. Becker, M.S.
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Date: May 19, 2017

Subject: Re-review of Parabens

Attached is the re-review of 20 parabens as used in cosmetics. These parabens are reported to function in cosmetics primarily as preservatives. In 2016, Sodium Methylparaben (which had not been reviewed by the Panel) was included in the CIR 2017 Priority List due to the large number of uses reported in the FDA's VCRP database. The Expert Panel agreed that it would be appropriate to group this ingredient, and some additional parabens and paraben salts, with the previously reviewed ingredients, Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben, for review. Accordingly, this report is a re-review, not a draft report, which the Panel has already agreed to reopen to 1) add the new ingredients, and 2) to assess updates that may have occurred in the safety profiles of the original seven ingredients. [*parabe062017Rep*]

In 2008, a safety assessment of parabens was published with the conclusion that seven parabens were safe in the present practices and concentrations. This report combined parabens that had been reviewed separately in previous reports, which were published in 1984, 1986, and 1995. For the Panel's convenience, the data from these reports are summarized within the attached report, *in italics*, in the appropriate sections. The Discussions from these reports are also included in the report. All of these reports are included in this packet for the Panel's information. [*parabe062017Prev_1, 2, 3, 4*] The minutes and transcripts of the Panel's discussions on these reports are also included in this packet. [*parabe062017Tran*]

An exhaustive search was conducted for new data on the safety of parabens; the most relevant data are summarized in this report. A few short-term but no new acute, subchronic or chronic toxicity studies were discovered. Nine new epidemiology studies explored the possibility of associations between markers of paraben exposure and adverse health outcomes, including 3 prospective and 6 retrospective studies. Exposures to Methylparaben, Propylparaben and Butylparaben were evaluated in all of these studies. In addition, exposures to Ethylparaben and Benzylparaben were considered in 6 and 2 of the studies, respectively. 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, focused on the statistically-significant results reported, and the details are provided in Table 14.

Dermal penetration, toxicokinetics, short-term toxicity, DART, endocrine-activity, and epidemiology studies are also briefly summarized in the body of the report, and details are provided in tables. On the other hand, 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 in the current draft of the report. Please note that the objective of some of the most recent animal toxicity studies was to investigate the potential for exposure to parabens at relatively low doses to cause adverse effects.

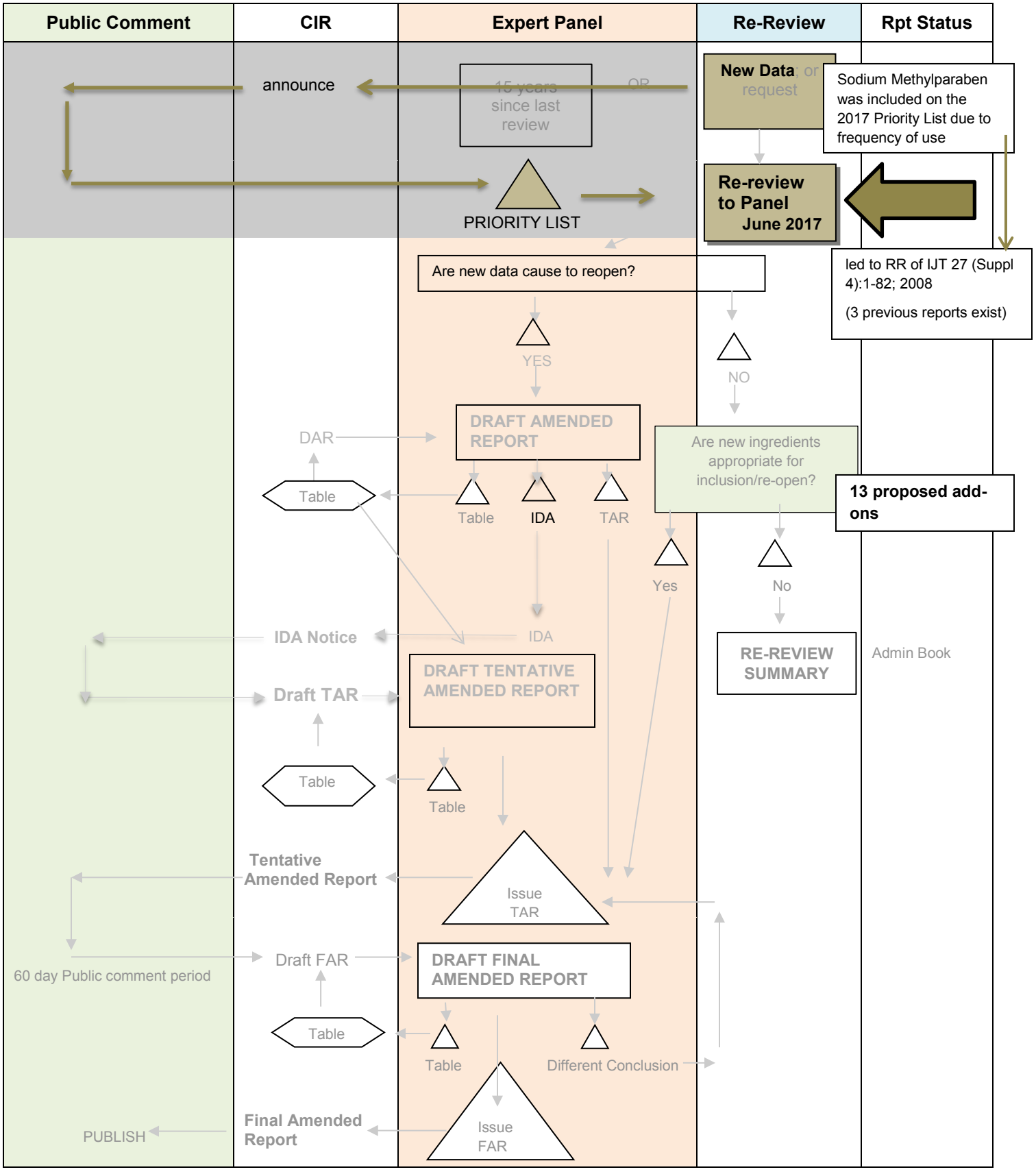
The Council has submitted concentration of use data that have been incorporated into the report. [*parabe062017Data1,2*] Most of the parabens that were previously in use have increased in the number of uses, and the concentrations of use are similar to those reported previously. No other unpublished data has been submitted.

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 come to a conclusion of safety and develop the basis for the Discussion. Thereby, the Panel should issue a Tentative Amended Report. However, if information (and/or added ingredients) raise new questions for the Panel that are not answered by the available data, then an Insufficient Data Announcement, with a list of data needs, should be issued.

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Parabens

MEETING June 2017



*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

History – 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**	

April 2017 – Panel examines new information. The Panel will confirm the current conclusion of safe as used or reopen the report for cause. The Panel will also decide if the report should be opened to add the new ingredients.

Parabens Data Profile for ***June*, 2017**. Writers – Ivan Boyer, Lill Becker																					
	ADME			Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Dermal Penetration	Log K_{ow}	Use	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
Sodium Methylparaben			X																		
Calcium Paraben																					
Potassium Butyparaben																					
Potassium Ethylparaben																					
Potassium Methylparaben																					
Potassium Paraben																					
Potassium Propylparaben																					
Sodium Butylparaben			X																		
Sodium Ethylparaben		X	X																		
Sodium Isobutylparaben			X																		
Sodium Isopropylparaben																					
Sodium Paraben			X																		
Sodium Propylparaben			X																		
RE-REVIEW:																					
Methylparaben	O N	X	O N	O	O	O	O N	O		O	N	O	O	N	O	O	N	O N	O	O I	O N
Ethylparaben	O N	X	O N	O			O			O		O		N	O	O	N	O N	O	I	
Propylparaben	N	X	O N	O	O		O N	O		O		O	O	N	O	O	N	O N	O N	O I	O
Isopropylparaben			O N					N						N			N	N	O		
Butylparaben	N		O N	O	O		O N					O	O	N	O	O	N	O N	O N	I	O
Isobutylparaben			O N				O	N						N			N	O N	O	O	
Benzylparaben	N		N	O			O			O		O		N			N				

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

Search Strategy for Parabens

- PubMed – January 12, 2017
  - 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 "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") 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")

    531 hits, reduced to 236 references of interest based on careful reading of the abstracts

- Scifinder – January 13, 2014
  - 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; 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

    20 hits

    Get References - Adverse Effect, including toxicity; Biological study: 13,433 hits

    Refine by:

    Acute toxicity; 51 hits
    Repeated dose toxicity; 3 hits
    Subacute toxicity; 3 hits
    Short-term toxicity; 2 hits
    Subchronic toxicity; 5 hits
    Chronic toxicity; 16 hits
    Adverse health effects; 13 hits
    Allergy; 138 hits
    Anaphylaxis; 12 hits
    Asthma; 7 hits
    Hypersensitivity; 38 hits
    Sensitization; 396 hits
    Cancer; 156 hits
    Carcinogenicity; 156 hits
    Cocarcinogenicity; 2 hits
    Tumor promotion; 4 hits
    Tumor progression; 0 hits
    Case report; 338 hits
    Case study; 338 hits

Clinical trial; 13 hits
Multicenter study; 2 hits
Clastogenicity, 2 hits
Genotoxicity; 15 hits
Mutagenicity; 41 hits
Comedogenicity; 0 hits
Cytotoxicity; 140 hits
Dermal absorption; 20 hits
Dermal penetration; 9 hits
Dermal irritation; 8 hits
Dermal effects; 108 hits
Dermal pigmentation; 0 hits
Developmental toxicity; 66 hits
Reproductive toxicity; 49 hits
Endocrine toxicity; 42 hits
Endocrine activity; 61 hits
Endocrine disruption; 199 hits
Epidemiology; 16 hots
Hepatotoxicity; 8 hits
Renal toxicity; 4 hits
4-Hydroxybenzoic Acid; 617 hits
Inhalation toxicity; 6 hits
Respiratory effects; 27 hits
In vitro toxicity; 31 hits
In vitro test; 637 hits
Neurotoxicity; 0 hits
Ocular effects; 132 hits
Oral exposure; 2 hits
Penetration enhancer; 51 hits
Phototoxicity; 4 hits
Photosensitivity; 1 hit
Risk assessment; 85 hits
Safety assessment; 21 hits
Toxicokinetics; 518 hits
Pharmacokinetics; 89 hits

Combined: 2,186 hits (after duplicates removed), total; reduced to 500, all years, based on careful reading of the abstracts

- Consolidated and eliminated duplicates in PubMed and SciFinder search results
  - 353 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: 62 references

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 Anouncement 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.

NEW DATA/SCCS OPINION
March 2012
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?

MS. 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.
MS. 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.
MS. 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.

Day Two

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 --

MS. BRESLAWEC: That is a drug.

DR. HILL: But not mouthwash?

MS. BRESLAWEC: The relevant use here is deodorant.

DR. HILL: Is what? Is deodorant?

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

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

MS. 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.

MS. 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 --

MS. 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.

MS. BRESLAWEC: Many of them are IP studies.

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

MS. 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.

MS. 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.

MS. 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?

MS. 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.

Day Two

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.

Safety Assessment of Parabens as Used in Cosmetics

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The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A.
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INTRODUCTION

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

In 2016, Sodium Methylparaben was included in the Cosmetic Ingredient Review (CIR) 2017 Priority List due to the number of uses reported in the U.S. Food and Drug Administration's (FDA) Voluntary Cosmetic Registration Program (VCRP) database.[2] It is appropriate to group this ingredient with additional paraben phenolic acids and their salts and carboxylic acids, some of which have already been reviewed by the CIR Expert Panel (Panel).

In 1984, a report on the safety assessment by the CIR Panel of four paraben phenolic acid cosmetic ingredients (e.g., **Methylparaben, Ethylparaben, Propylparaben**, and **Butylparaben**) was published with the conclusion that these cosmetic ingredients were safe as cosmetic ingredients in the present practices of use (Table 2).[3] In 1986, a report on the safety of **Benzylparaben** was published with the conclusion that the available data are insufficient to support the safety of this ingredient.[4] In 1995, a report on **Isobutylparaben** and **Isopropylparaben** was published with a conclusion of safe as cosmetic ingredients in the present practices of use.[5]

In 2008, an amended report on the safety of paraben phenolic acid cosmetic ingredients, that included the previously reviewed parabens from all three reports, was published; the Expert Panel found these seven cosmetic ingredients to be safe in the present practices and concentrations.[6] The Panel determined margins of safety for dermal exposure of these ingredients that ranged from 840 for multiple paraben exposure in adults to 5952 for a single paraben exposure in infants.

Additional paraben cosmetic ingredients (the salts and carboxylic acids of parabens) have been added to the current report. The 20 parabens included in this safety assessment are:

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 (*p*-hydroxybenzoic acid) is a cosmetic ingredient that is a metabolite of parabens.[1,3] This fragrance ingredient has not been reviewed by the CIR Panel (as the safety assessment of fragrances are not the purview of CIR).

Pertinent data were discovered in the European Chemicals Agency (ECHA) database.[7-13] 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).[14-22]

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

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

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

The objective of some of the most recent animal toxicity studies was to investigate the potential for exposure to parabens to cause adverse effects at relatively low doses.

CHEMISTRY

Definition and Structure

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

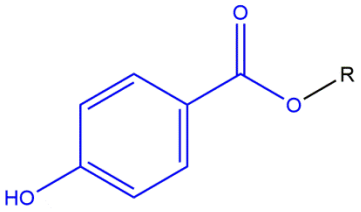


Figure 1a. Paraben phenolic acids: a generic structure wherein R is an alkyl group from 1 to 4 carbons long, or is benzyl.

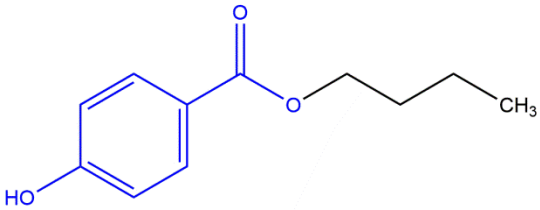


Figure 1b. Paraben phenolic acids: an example, Butylparaben (wherein R from the generic structure in Figure 1a, is an alkyl group 4 carbons long).

The salts of these phenolic acids are being added to this re-review of parabens. The phenolic proton is the most acidic in those parabens with an ester functional group, and the salt forms of these parabens share this same core structure (Figure 2).

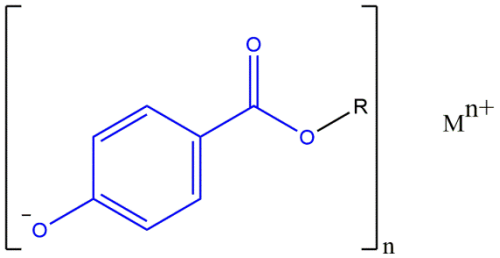


Figure 2a. Paraben phenolic salts: generic structure wherein R is an alkyl group from 1 to 4 carbons long and M is sodium or potassium.

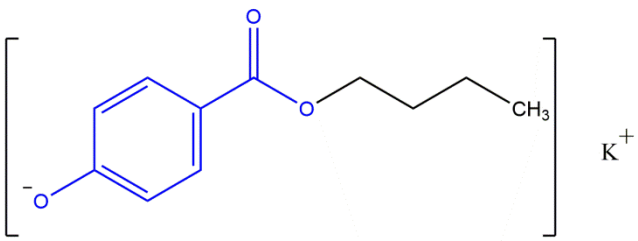


Figure 2b. Paraben phenolic salts: an example, Potassium Butylparaben (wherein R, from the generic structure in Figure 2a, is an alkyl group 4 carbons long and M is potassium).

Also added to this re-review, are the carboxylic acids of parabens (i.e., not esters). The carboxylic proton is the most acidic in those parabens without an ester functional group, and the salt forms of these parabens share this same core structure (Figure 3).

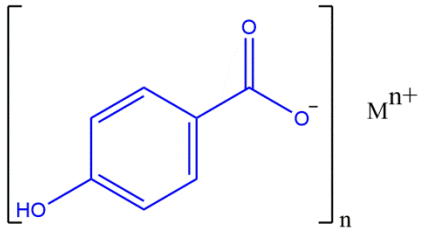


Figure 3a. Paraben carboxylic salts: a generic structure wherein M is sodium, potassium, or calcium.

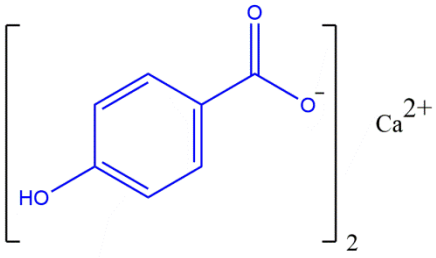


Figure 3b. Paraben carboxylic salts: an example, Calcium Paraben (wherein M, from the generic structure in Figure 3a, is calcium and n is 2).

Physical and Chemical Properties

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

Parabens form small colorless crystals or white crystalline powders with practically no odor or taste.[6] 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).[7,8,10,11]

Parabens are stable against hydrolysis during autoclaving and resist saponification.[23]

Method of Manufacture

Paraben phenolic acids (and salts) are prepared by esterifying *p*-hydroxybenzoic acid (PHBA) with the corresponding alcohol in the presence of an acid catalyst, such as sulfuric acid, and an excess of the specific alcohol.[6] The acid is then neutralized with caustic soda, and the product is crystallized by cooling, centrifuged, washed, dried under vacuum, milled, and blended. Benzylparaben can also be prepared by reacting benzyl chloride with sodium *p*-hydrobenzoic acid. Paraben carboxylic salts may be prepared by deprotonating PHBA with an appropriate alkaline salt (e.g., sodium hydroxide could be used to prepare Sodium Paraben).[24]

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the FDA and the cosmetic industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's VCRP database. Use concentration data are submitted by the cosmetic industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentration by product category.

According to VCRP survey data received in 2017, Methylparaben was reported to be used in 13,797 formulations (10,784 in leave-on formulations, 2889 in rinse-off formulation, and 124 in bath formulations); this is an increase from 8786 formulations reported in 2006 (Table 5 and Table 6).[6,25] Propylparaben had the next highest number of reported uses at 10,642 (8668 in leave-on formulations, 1875 rinse-off formulations, and 99 in bath formulations); this was an increase from 7118 formulations reported in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to none.

The results of the concentration of use survey conducted by the Council 2016 indicate Methylparaben had the highest reported maximum concentration of use; it is used at up to 0.9% in shampoos.[6,26] The highest maximum concentration of use reported for products resulting in leave-on dermal exposure is Ethylparaben in eye shadows at 0.65%. In 2006, Methylparaben had the highest reported maximum concentration of use at 1% in lipsticks. The maximum concentrations of use of the previously reviewed parabens have remained under 1% and the patterns of use are similar to those reported in the previous safety assessment.

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

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

Several of the parabens with reported uses are used in products that can be ingested (e.g., Methylparaben at up to 0.35% in lipstick), used near the eye (e.g., Methylparaben at up to 0.8% in mascara), come in contact with mucous membranes (e.g., Methylparaben at up to 0.5% in bath oils, tablets and salts), or in baby products (e.g., Methylparaben at up to 0.4% in baby lotions, oils and creams).

Some of the parabens were reported to be used in cosmetic sprays (including hair sprays, hair color sprays, skin care products, moisturizing products, suntan products, deodorants, and other propellant and pump spray products) and could possibly be inhaled. These ingredients are reportedly used at concentrations up to 0.41% in spray products (e.g., Methylparaben in the category or 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.[27-30] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[27,29] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[27] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Some of the parabens were reported to be used in dusting powders and face powders, and could possibly be inhaled. These ingredients are reportedly used in loose powder products (e.g., Ethylparaben in face powders at up to 0.5%). Conservative estimates of inhalation exposures to respirable particles during the use of loose-powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[31-33]

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

The SCCP of the EU has published several opinions on parabens over the last few years (Table 8).[16-22] The current SCCP opinion is:

> "The use of butylparaben and propylparaben as preservatives in finished cosmetic products are safe to the consumer, as long as the sum of their individual concentrations does not exceed 0.19%... With regard to methylparaben and ethylparaben, the previous opinion, stating that the use at the maximum authorized concentrations can be considered safe, remains unchanged… Limited to no information was submitted for the safety evaluation of isopropyl-, isobutyl-… Therefore, for these compounds, the human risk cannot be evaluated. The same is true for Benzylparaben…"[20,22]

National Industrial Chemicals Notification and Assessment Scheme (NICNAS) published the following conclusion:

> "Current risk management measures are considered adequate to protect public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory.
>
> The available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak oestrogenic activity, but there are no established adverse

outcome pathways for this effect. Should further information on adverse outcome pathways in mammals associated with weak oestrogenic activity become available, further assessment of these chemicals at Tier III could be required."[34]

Non-Cosmetic

2008

The European Food Safety Authority opinion cited reduction in daily sperm production in juvenile male rats fed Propylparaben at 10 mg/kg/day as the lowest observable adverse effect dose and contrasted these findings with the absence of effect for Methylparaben and Ethylparaben at doses up to 1000 mg/kg day-1.[6] The opinion restated the acceptable daily intake (ADI) of 0 to 10 mg/kg day-1 for the sum of Methylparaben and Ethylparaben. The opinion stated that Propylparaben should not be included in the ADI, and failed to recommend an alternative ADI because of the lack of a clear No-observed-adverse-effect-level (NOAEL).

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

An evaluation by the JECFA determined that the acceptable daily intake (ADI) of the sum of the Ethylparaben and Methylparaben to 0-10 mg/kg.[35] In view of the adverse effects in male rats, Propylparaben was excluded from the group ADI for the parabens used in food.[36]

TOXICOKINETIC STUDIES

Dermal Penetration

2008

Parabens in cosmetic formulations applied to skin penetrate the stratum corneum in inverse relation to the ester chain length.[6] Carboxylesterases present in keratinocytes hydrolyze parabens in the skin. The extent of the breakdown to PHBA is different between rodent and human skin. In vitro studies also indicate a difference in the extent of hydrolysis to PHBA, depending on whether viable whole skin or dermatomed human skin is used, with the former having a larger extent of hydrolysis. Chemicals that disrupt the stratum corneum may increase the skin penetration of Methylparaben and possibly Ethylparaben, but do not affect the penetration of parabens with longer ester chains.

In Vitro

In vitro dermal penetration studies are presented in Table 9.

In Franz-type diffusion cells, 2.3%-3.3% of the applied concentration (0.1%) of Methylparaben penetrated porcine skin (fresh or after stored frozen) in 4 h.[37] 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.[38] Increasing the ethanol concentration or the exposure duration increased the retention of the parabens in the dermis, compared to the epidermis. Binary combinations of the parabens reduced their permeation rates, which was attributed by the authors to high retention in the epidermis and dermis. Penetration of parabens in 3 commercial facial cream through rabbit ear skin ranged from 20%-60%, after 8 h in Franz-type diffusion cells, increasing (Propylparaben < Ethylparaben < Methylparaben) with increasing water solubility of the paraben, regardless of the formulation tested.[39] Retention varied widely in the epidermis and dermis depending on the formulation. Permeability coefficients estimated for Methylparaben, Propylparaben and Butylparaben in human cadaver skin (0.37 to 0.91 cm/h x 10^{-4}) and mouse skin (1.17 to 1.76 cm/h x 10^{-4}) were similar regardless of concentration tested (0.1%-2%).[40] Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin. The authors state that the results show that parabens may be classified as moderate penetrants. Penetration was inversely proportional to the lipophilicity of the parabens tested (0.057% for Methylparaben to 0.007% for Butylparaben), and increased with repeated applications.[41]

Human

Butylparaben

Dermal penetration was studied in 26 healthy Caucasian male volunteers, 21 to 36 years old, after application of 2% (w/w) Butylparaben in Essex cream, which also contained 2% diethyl phthalate and 2% dibutyl phthalate.[42] Daily whole-body topical application of 2 mg/cm^2 of the cream formulation without the test substances for 1 week (control week) were

followed by daily application of the cream with the test substances for 1 week. Concentrations of Butylparaben were measured in blood serum samples. Butylparaben serum concentrations were undetectable in most samples during the control week, with maximum concentrations not exceeding 1.0 µg/L. Butylparaben concentrations increased rapidly (mean peak concentration=135 ± 11 µg/L in 3 h) after the first application of cream containing the 3 test compounds. Twenty-four hours after the first application, but before the following application, the mean serum concentration was 18 ± 3 µg/L. Butylparaben could be detected in most serum samples collected throughout the second week of this study.

Penetration Enhancement

In Vitro

Methylparaben

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

Absorption, Distribution, Metabolism, and Excretion (ADME)

1984

Parabens are quickly absorbed from the blood and gastrointestinal tract, hydrolyzed to p-hydroxybenzoic acid, conjugated, and the conjugate excreted in the urine.[3] Data obtained from chronic administration studies indicate that parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Varying amounts of parabens are passed in the feces depending upon which paraben is administered and the size of the dose. Little or no unchanged paraben is excreted in the urine. Most of an administered dose can be recovered within 5 to 72 hours as p-hydroxybenzoic acid or its conjugates. Parabens appear to be rapidly absorbed through intact skin.

1986

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

1995

When male rabbits were administered either 800 mg/kg or 400 mg/kg of Isobutylparaben via a stomach tube, 77-85% of the ingredient was recovered as a form of p-hydroxybenzoic acid; 20% was not recovered.[5]

2008

Ingested parabens are quickly absorbed from the gastrointestinal tract, hydrolyzed to p-hydroxybenzoic acid, conjugated, and the conjugate excreted in the urine.[6] Data obtained from chronic administration studies indicate that parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Varying amounts of parabens are passed in the feces depending upon which paraben is administered and the size of the dose. Little or no unchanged paraben is excreted in the urine.

In Vitro

CELL-FREE SYSTEMS

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

Methylparaben, Ethylparaben, and Propylparaben did not exhibit binding affinity for α-fetoprotein (AFP).[44] On the other hand, the 50% inhibitory concentration (IC_{50}) of Benzylparaben was 0.012 µM. Butylparaben was biotransformed to *p*-hydroxybenzoic acid with maximum rate at saturating concentration (V_{max}) of 8.8 nmol/min/mg protein.[45]

Methylparaben and Ethylparaben were stable in human plasma, but Propylparaben, Butylparaben and Benzylparaben concentrations decreased by 50% within 24 h.[46] 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.[47] 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).[48] This trend was also observed for human skin microsomes (HSM), but at much lower rates. Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the paraben. In contrast to human tissue fractions, all rat tissue fractions tested hydrolyzed the parabens at rates that increased as the ester chain length increased. Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin.

CELL CULTURES

Cell culture ADME studies are presented in Table 10.

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference. [49] Butylparaben was extensively hydrolyzed to *p*-hydroxybenzoic acid as the major metabolite for both sexes and species. The other metabolite observed in the human hepatocytes was 4-hydroxyhippuric acid.

Animal

DERMAL EXPOSURE

Animal dermal ADME studies are presented in Table 10.

In rats exposed to a single dermal dosage of 100 mg/kg bw radiolabeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥693 and ≥614 ng eq/g in males and females, respectively) occurred within 8 h post-application, and blood concentrations decreased until the last quantifiable concentration within 24 h.[50] Most of the dosage (≥46.4%) was not absorbed, and less than 25.8% was found in the urine. Urinary excretion was the main route of elimination. Radioactivity was eliminated rapidly in the urine with averages ≥11.9% recovered in the first 48 h. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of radiolabeled Butylparaben was absorbed 72 h following application to the skin in rats.[49] Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in liver.

ORAL EXPOSURE

Oral ADME studies are presented in Table 10.

In rats exposed to a single oral dosage of 100 mg/kg bw radiolabeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥11432 and ≥21040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h.[50] Radioactivity was eliminated rapidly, with averages ≥69.6% recovered in the urine during the first 24 h. Radioactivity was excreted predominantly in urine in rats orally exposed to a single 10, 100, or 100 mg/kg bw/day dosage of radiolabeled Butylparaben.[49] The rate of urinary excretion was similar across all dosages, with ≥66% recovered in the first 24 h in males. Female rats excreted more Butylparaben in urine in the first 4 h after exposure, but there was no sex difference in the total dose excreted within 24 h. In general, tissue levels at 24 h were considerably higher in female rats. Metabolites detected in urine included newly discovered metabolites arising from ring hydroxylation followed by glucuronidation and sulfation.

Human

DERMAL EXPOSURE

Human dermal ADME studies are presented in Table 10.

All 26 healthy male volunteers showed increased excretion of Butylparaben following daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben. [51] Mean total Butylparaben excreted in urine during exposure was 2.6 ± 0.1 mg/24 h. The concentrations peaked in the urine 8-12 h after application. Free and conjugated parabens and their major, non-specific metabolites (*p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid) were detected in the urine samples of three subjects 24 h after an oral dose of deuterated Methylparaben, Butylparaben, and Isobutylparaben.[52] Minor metabolites discovered had hydroxy groups on the alkyl side chain or oxidative modifications on the aromatic ring.

AGGREGATE EXPOSURE

Aggregate exposure ADME studies are presented in Table 10.

One or more of 5 parabens (Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Isobutylparaben) was detected in 99% of breast tissue samples collected from women with breast cancer, and all 5 were detected in 60% of the samples.[53] 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.[54] The highest measured concentration was 11.77 ng Methylparaben/g tissue.

TOXICOLOGICAL STUDIES

Acute Dose Toxicity

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

1984

Acute toxicity studies in animals indicate that parabens are practically nontoxic by various routes of administration.[3]

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.[4]

1995

Isobutylparaben had a subcutaneous LD_{50} of 2,600 mg/kg in mice.[5]

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.[5]

Dermal

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

There were no significant changes in body and organ weights in any group when rats were dermally exposed to up to 600 mg/kg bw/day Isopropylparaben or Isobutylparaben for 28 days.[55] Macroscopic and microscopic examinations revealed mild-to-moderate skin damage in female rats. No-observed-adverse-effect-levels (NOAEL) for Isobutylparaben and Isopropylparaben were 600 mg/kg bw/day, and 50 mg/kg bw/day, respectively.

Oral

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

At 100 and 300 mg/kg bw/day Propylparaben administered orally, rats exhibited statistically-significant increases in relative liver weights, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, serum urea concentrations, lipid peroxidation and nitric oxide (NO) generation, and 17β-estradiol (E2) concentrations.[56] 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.[57] 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.[58]

Subchronic Toxicity Studies

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

1984

Subchronic… oral studies indicate that parabens are practically nontoxic.[3]

Chronic Toxicity Studies

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

1984

…[C]hronic oral studies indicate that parabens are practically nontoxic.[3]
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.[5] *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.[6] 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.[3]

2008

Methylparaben was nonteratogenic in rabbits, rats, mice, and hamsters, and Ethylparaben was nonteratogenic in rats.[6] Parabens, even at levels that produce maternal toxicity, do not produce terata in animal studies. One study examined the developmental toxicity of Butylparaben in rats and reported no effect on development up to an oral dose of 1000 mg/kg day- 1, even with some maternal toxicity at that dose. The maternal toxicity NOAEL dose was 100 mg/kg day-1.

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

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

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

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

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

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

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

Dermal Exposure

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

Oral Exposure

Oral DART studies are summarized in Table 12.

Statistically-significant, dose-dependent reductions in anogenital distance and ovary weights were observed in offspring of female rats exposed orally to 100 or 500 mg/kg bw/day Butylparaben from gestation day (GD) 7-GD21.[59] 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.[60] 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.[61] 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.[62] Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

Prepubertal female rats exposed orally to 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Isopropylparaben on PND21 to PND40 exhibited statistically-significant delays in vaginal opening.[63] 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.[64]

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.[65] 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.[66]

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.[45] Measurements of hormone concentrations were generally not altered, except that testosterone (T) and follicle-stimulating hormone (FSH) concentrations were higher in the 10,000-ppm Butylparaben-treated group, compared with the control group. The authors concluded that the NOAEC was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of about 1140 and 1100 mg/kg/day for Methylparaben and Butylparaben, respectively.

GENOTOXICITY STUDIES

1984

Numerous mutagenicity studies, including the Ames Test, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the parabens are nonmutagenic.[3]

1995

Chinese hamster fibroblast cell lines treated with 0.03% Isobutylparaben had no chromosomal aberrations after 48 h.[5]

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

2008

Numerous genotoxicity studies, including Ames testing, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the parabens are generally nonmutagenic, although Ethylparaben and Methylparaben were judged to induce significant chromosomal aberrations (11.0% and 15.0% increases, respectively) in an in vitro assay using Chinese Hamster ovary cells.[6]

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.[67] Alterations in cell proliferation were assessed by microscopic analysis of the mitotic index and by flow cytometry. Cells treated with 500 µM Propylparaben were analyzed for cell-cycle distribution. Induction of DNA double-strand breaks (DSBs) was examined by indirect immunofluorescence against the phosphorylated form of the variant histone γ-H2AX. Possible oxidative DNA damage was evaluated by immunocytochemical analysis of 8-hydroxydeoxyguanosine (8-OHdG). Statistically-significant, dose-dependent decrease in percentage of mitotic cells was observed across the concentrations tested (4-fold decrease at 500 µM, compared with control). Flow-cytometric analysis of DNA content revealed that the decline was attributable mainly to cell-cycle arrest at the G0/G1 phase. Immuno-detection techniques revealed statistically-significant induction of DNA DSBs (2-fold compared to control) at 500 µM increase in 8-OhdG staining at all concentrations tested (maximum intensity at 500 µM).

Propylparaben
Butylparaben

Chinese hamster ovary (CHO-K1) cells were grown, and incubated for 1 or 3 h with 0, 0.5, 1, 1.5, 2, or 2.5 µM Propylparaben or 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mM or 0, 0.1, 0.25, 0.5, or 0.75 µM Butylparaben (depending on the test), at 37°C in Ham's F-12 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL).[68] Sister chromatid exchange (SCE), chromosome aberration (CA), and DNA strand break (comet) assays were performed. Statistically-significantly elevated indices of DNA fragmentation were observed in cells incubated for 1 h with Butylparaben (≥0.4 µM) or Propylparaben (≥1 µM). Butylparaben, in particular, showed comparatively high incidences of fragmentation. Statistically-significantly elevated SCEs/cell were observed in cells incubated with Butylparaben (0.75 µM) or Propylparaben (≥1.5 µM) for 3 h. Statistically-significantly elevated CAs/cell were observed in cells incubated with Butylparaben (0.75 µM) or Propylparaben (≥1 µM) for 3 h.

In Vivo

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

CARCINOGENICITY STUDIES

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

1984

Methylparaben was noncarcinogenic when injected subcutaneously in mice or rats when administered intravaginally in rats and was not co-carcinogenic when injected subcutaneously in mice.[3] *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.[5]

In Vitro

Methylparaben
Propylparaben
Butylparaben

MCF-10A non-transformed, immortalized human breast epithelial cells were exposed to 500 µM Methylparaben, 10 µM Propylparaben or Butylparaben in semi-solid 2% methylcellulose suspension culture, or 1 µM Methylparaben or 0.1 µM Propylparaben or Butylparaben in monolayer culture.[69] Ethanol served as the vehicle. The cells were grown in suspension culture (non-adherent conditions) to assess colony growth after a 17-day incubation period. Cells were grown in monolayer culture (adherent conditions) to assess cellular proliferation after a 7-day incubation period. In suspension culture, MCF-10A cells produced very few colonies and only of a small size. The presence of 500 µM Methylparaben or 10 µM Propylparaben or Butylparaben resulted in greater numbers of colonies per dish ($p<0.05$) and greater average colony sizes ($p<0.001$) compared with controls. Average colony sizes of cells grown with a paraben were comparable to those of cells grown with 17β-estradiol (70 nM). Concentration-response experiments showed that maximal numbers of colonies were formed at 100 µM Methylparaben or 1 µM Propylparaben or Butylparaben. Control experiments showed that the parabens did not influence the growth of MCF-10A cells under adherent conditions (i.e., monolayer cultures).

Methylparaben

Human high-risk donor breast epithelial cells (HRBECs) were collected from the unaffected contralateral breasts of women undergoing breast surgery with a personal or family history of breast cancer, atypical neoplastic histopathology and/or high mammographic density.[70] 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. [70] Some cells exposed to 10 µM 4-hydroxy tamoxifen (OHT) or 1, 10 or 100 nM rapamycin for 24 h before functional analysis. Methylparaben substantially reduced the fraction of OHT-induced apoptotic cells in a concentration-dependent manner ($p=0.001$) at all three concentrations: 57.82% ± 6.77% at 1 µM, 55.93% ± 10.54% at 100 nM, and 28.14% ± 11.3% at 10 nM. Methylparaben induced a detectable decline in endogenously accumulated reactive oxygen species (ROS) in all cell cultures. In early passage HRBECs, average reduction in ROS by Methylparaben treatment was 38% ($p < 0.02$), without an evident concentration-response relationship. Prior exposure to Methylparaben resulted in a concentration-dependent, complete-to-partial evasion from the G1-phase arrest induced by OHT, and concurrent increase in the S-phase fraction. In contrast, the growth inhibitory effects of OHT were not reversed by a combination of luteal-phase serum concentrations of E2 and progesterone. The maintenance of S-phase in OHT-treated cells, like apoptosis evasion, was correlated with increasing concentrations of Methylparaben ($p<0.001$).

OTHER RELEVANT STUDIES

Endocrine Activity

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

Weak activation of mPPARα was seen in murine NIH-3T3-L1 cells at the highest concentrations of Butylparaben tested (100 µM).[71] Butylparaben activated murine peroxisome proliferator-activated receptor (mPPAR)γ with a lowest observed effect concentration (LOEC) of 30 µM and a maximal (4-fold) induction at 100 µM. The human data for Butylparaben (hPPARα and hPPARγ) were comparable to those obtained with mPPARα and mPPARγ.

Isobutylparaben antagonized the androgen receptor (AR) in Chinese Hamster ovary cells. The effect was statistically significant at ≥ 25 µM.[72] 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).[73] The EC_{50} for glucocorticoid-like activity in MDA-kb2 cells was 1.75 mM for Butylparaben and 13.01 mM for Propylparaben.[74] 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.[75]

Butylparaben exhibited estrogen agonism at all concentrations tested in T47D-KBluc cells.[76] The maximum effect was observed at 10 µM.

The EC_{50}s for stimulating proliferation of MCF-7 cells ranged from 0.4-40 µM, LOECs from 0.1-20 µM, and NOECs from 0.05-8 µM for the parabens tested.[77] 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.[78] 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.[79] 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.[80] In the presence of differentiation media, 50 µM Butylparaben or Benzylparaben promoted lipid accumulation in human adipose-derived stem cells (hADSCs) as early as day 3 and throughout the differentiation process. Butylparaben had the strongest adipogenic effects of the parabens tested, whereas other parabens had no effect at 1 or 10 µM.

Relative uterine weights were elevated in immature Sprague-Dawley rats after treatment with ≥0.16 mg/kg bw/day Benzylparaben on PND21-PND23.[81] LOELs for increased relative uterine weight after treatment of immature female rats with Methylparaben or Ethylparaben on PND21-PND23 were 20 and 4 mg/kg bw/day, respectively.[82] No observed effects levels (NOEL) for Methylparaben and Ethylparaben were 4 and 0.8 mg/kg bw/day, respectively. Ethylparaben and Propylparaben were negative for estrogen agonism and antagonism in ovariectomized female mice exposed to 1000 mg/kg bw/day by gavage for 7 days.[83] Histopathologic examination revealed progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules and reduction and/or disappearance of tubular lumen 3 h after a single 1000 mg/kg bw dosage of Butylparaben.[84] Transferase uridyl nick end labeling (TUNEL) assays revealed a substantial increase in the number of apoptotic spermatogenic cells in the treated rats; the effect was maximal at 6 h.

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

DERMAL IRRITATION AND SENSITIZATION STUDIES

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

1984

Methylparaben (100% and 10%), Propylparaben (10%), and Ethylparaben (100% and 10%) were, at most, mildly irritating when applied to rabbit skin.[3]

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.[4]

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

2008

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

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

In Vitro

Methylparaben
Ethylparaben
Propylparaben
Isopropylparaben
Butylparaben
Isobutylparaben
Benzylparaben

The parabens were tested individually for irritancy and sensitization potential in co-cultured human keratinocyte and peripheral blood mononuclear cells (PBMCs).[85] The keratinocytes were isolated from skin received as residual material from plastic surgery; PBMCs were enriched from buffy coats by density centrifugation. The cells were co-cultured in serum-free KGM-2 on 12-well cell culture plates. The co-culture was incubated for 48 h with or without a paraben. The concentrations tested were not specified, but likely ranged around 1-1000 µM, in dimethyl sulfoxide (DMSO; vehicle). Fluorescence-activated cells sorting (FACS) was used to identify and characterize dendritic cell-related cells (DC-rcs). Categorization of compounds as potential irritants and sensitizers was based on EC_{50}s calculated from concentration-response data for cell death (irritancy) and CD86-expression (sensitization), compared with vehicle controls. Substances with EC_{50} for cell death ≤ 50 µM were considered to be irritating, EC_{50} ranging from 50-1000 µM weakly irritating, and substances that did not reach the 50% threshold for cytotoxicity, or for which EC_{50}>1,000 µM, were considered non-irritating. Substances with EC_{50} for CD86-expression ≤ 12.5 µM were categorized as extreme sensitizers, >12.5 µM-<50 µM as strong sensitizers, >50 µM-<100 µM as moderate sensitizers, and >100 EC_{50} as non-sensitizers. Methylparaben and Ethylparaben showed no potential for irritation in this test. Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben appeared to be weak irritants. The sensitization potential of the parabens tested was correlated with side-chain length: Methylparaben, Ethylparaben, Propylparaben, and Isopropylparaben were classified as weak sensitizers; and Butylparaben, Isobutylparaben, and Benzylparaben were strong sensitizers in this study.

Photosensitization/Phototoxicity

1984

Photocontact sensitization and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methyl-,

Propyl-, and/or Butylparaben gave no evidence for significant photoreactivity.[3]

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.[86] The cells were grown and incubated, with or without Methylparaben, for 6 or 24 h in DMEM supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine, and 100 U/mL penicillin/streptomycin at 37°C. Methylparaben-treated and -untreated cells were exposed to UVB (15 or 30 mJ/cm^2) after replacing the medium with PBS. The UVB source was a bank of six fluorescent sunlamps with an emission spectrum of 275-375 nm, mainly in the UVB range, peaking at 305 nm, and including a small amount of UVA and UVC. After irradiation, the cells were incubated in culture medium without Methylparaben for various durations. Methylparaben statistically-significantly reduced cell viability within 6 h at 0.3% and within 24 h at 0.03%. Fluorescent microscopy using a fluorescent micro-plate reader revealed little evidence of reactive oxygen species (ROS) or nitric oxide (NO) production after Methylparaben exposure. UVB irradiation at 30 mJ/cm^2 (but not at 15 mJ/cm^2) induced small amounts of late apoptosis and necrosis. Methylparaben statistically-significantly elevated ($p<0.5$) UVB-induced cell death, as evaluated by immunocytochemistry and flow cytometry; the propidium iodide (PI) index increased 3- and 7-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 15 mJ/cm2, and 2- and 3-fold after treatment with 0.003% and 0.03% Methylparaben, respectively, at 30 mJ/cm2. Methylparaben at both concentrations elevated ($p<0.05$) measurements of ROS and NO production and lipid peroxidation, and activated NFκB and AP-1 in UVB-irradiated cells.

OCULAR IRRITATION STUDIES

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

1984

Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits.[3]

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.[4]

2008

There were no adverse reactions to 0.1 g of Benzylparaben.[6]

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.[87] 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.[3]

EPIDEMIOLOGICAL STUDIES

Primary Studies

PROSPECTIVE STUDIES

Prospective epidemiological studies are summarized in Table 14.

Preterm birth (PTB) was associated with umbilical cord blood concentrations of Butylparaben (OR=60.77; CI=2.60-1419.93) and Benzylparaben (OR=0.03, CI=0.01-0.44).[88] Linear regression analysis indicated an association between maternal urinary concentrations and decreased gestational age and body length in newborns. No statistically-significant associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated. In another prospective study, in vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility.[89] 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.[90]

RETROSPECTIVE STUDIES

Retrospective epidemiological studies are summarized in Table 14.

The incidence of cryptorchidism and/or hypospadias, combined, was associated with placental concentrations of Methylparaben ≥1.96 ng/g (OR=3.18; CI=0.88-11.48) and Propylparaben concentrations ≥1.16 ng/g (OR=4.72; CI=1.08-20.65).[91] Linear regression analyses indicated an association between urinary Ethylparaben concentrations in 3-year old children and their body weights and heights.[92] The latter parameter was also associated with calculated estimates of aggregate exposures to parabens, including Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben. All regression coefficients calculated for girls and all other coefficients for boys were not statistically significant.

Linear regression analyses of data from the U.S. National Health and Nutrition Examination Survey (NHANES) program indicated an association between reduced serum thyroxine (T4) concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben.[93]

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).[94]

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).[95] 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.[96] The exception was a trend for increased Tail% in comet assays of sperm DNA with increasing Butylparaben concentrations.

SUMMARY OF NEW DATA

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

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

According to VCRP survey data received in 2017, Methylparaben was reported to be used in 13,797 formulations; this is an increase from 8786 formulations in 2006. Propylparaben had the next highest number of reported uses at 10,642; this was an increase from 7118 formulations in 2006. All of the other previously reviewed parabens in this safety assessment increased in the number of reported uses since 2006 with the exception of Benzylparaben, which dropped from 1 reported use to none.

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

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

In Franz-type diffusion cells, 2.3%-3.3% of the applied concentration (0.1%) of Methylparaben penetrated porcine skin (fresh or after stored frozen) in 4 h.[37] 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.[38] Increasing the ethanol concentration or the exposure duration increased the retention of the parabens in the

dermis, compared epidermis. Binary combinations of the parabens reduced their permeation rates, which was attributed by the authors to high retention in the epidermis and dermis. Penetration of parabens in three commercial facial cream through rabbit ear skin ranged from 20% to 60%, after 8 h in Franz-type diffusion cells, increasing (Propylparaben < Ethylparaben < Methylparaben) with increasing water solubility of the paraben, regardless of the formulation tested.[39] Retention varied widely in the epidermis and dermis depending on the formulation. Permeability coefficients estimated for Methylparaben, Propylparaben and Butylparaben in human cadaver skin (0.37 to 0.91 cm/h x 10^{-4}) and mouse skin (1.17 to 1.76 cm/h x 10^{-4}) were similar regardless of concentration tested (0.1%-2%).[40] Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin. The authors state that the results show that parabens may be classified as moderate penetrants. Penetration was inversely proportional to the lipophilicity of the parabens tested (0.057% for Methylparaben to 0.007% for Butylparaben), and increased with repeated applications.

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

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

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

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

Butylparaben was rapidly cleared in hepatocytes from rats, and was cleared more slowly in hepatocytes from humans, with little or no sex difference. Butylparaben was extensively hydrolyzed to *p*-hydroxybenzoic acid as the major metabolite for both sexes and species. The other metabolite observed in the human hepatocytes was 4-hydroxyhippuric acid. Methylparaben, Ethylparaben, Propylparaben and Butylparaben were hydrolyzed by RLM and HLM in in vitro tests. In contrast to RLM, HLM showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length of the paraben tested. Human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes.

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

In rats exposed to a single dermal dosage of 100 mg/kg bw radiolabeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥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. Urinary excretion was the main route of elimination. Radioactivity was eliminated rapidly in the urine with averages ≥11.9% recovered in the first 48 h. About 52% and 8% of a single 10 or 100 mg/kg bw dosage, respectively, of radiolabeled Butylparaben was absorbed 72 h following application to the skin in rats. Urine was the primary route of elimination. Tissues contained about 4.3% of the 10 mg/kg dosage. The kidneys contained about twice the concentration of residues found in liver.

In rats exposed to a single oral dosage of 100 mg/kg bw radiolabeled Methylparaben, Propylparaben, or Butylparaben, C_{max} (≥11432 and ≥21040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and

blood concentrations decreased until the last quantifiable concentration at 12 h. Radioactivity was eliminated rapidly, with averages ≥69.6% recovered in the urine during the first 24 h. Radioactivity was excreted predominantly in urine in rats orally exposed to a single 10, 100, or 100 mg/kg bw/day dosage of radiolabeled Butylparaben. The rate of urinary excretion was similar across all dosages, with ≥66% recovered in the first 24 h in males. Female rats excreted more Butylparaben in urine in the first 4 h after exposure, but there was no sex difference in the total dosage excreted within 24 h. In general, tissue levels at 24 h were considerably higher in female rats. Metabolites detected in urine included newly discovered metabolites arising from ring hydroxylation followed by glucuronidation and sulfation.

All 26 healthy male volunteers showed increased excretion of Butylparaben following daily whole-body topical application of a cream formulation containing 2% (w/w) Butylparaben. Mean total Butylparaben excreted in urine during exposure was 2.6 ± 0.1 mg/24 h. The concentrations peaked in the urine 8 to 12 h after application. Free and conjugated parabens and their major, non-specific metabolites (*p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid) were detected in the urine samples of 3 subjects 24 h after an oral dose of deuterated Methylparaben, Butylparaben, and Isobutylparaben. Minor metabolites discovered had hydroxy groups on the alkyl side chain or oxidative modifications on the aromatic ring.

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

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

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

At 100 and 300 mg/kg bw/day Propylparaben administered orally, rats exhibited statistically-significant increases in relative liver weights, serum ALT, AST, ALP and LDH activities, serum urea concentrations, lipid peroxidation and NO generation, and E2 concentrations. Statistically-significant decreases in total serum protein and albumin, GSH, CAT and SOD activities, serum testosterone concentrations, and T/E2 ratios, were also reported. Livers of affected rats exhibited dilated congested central and portal veins, highly proliferated bile ducts with fibrotic reactions, and multifocal areas of necrotic hepatocytes, and testes exhibited evidence of severe spermatogenic arrest, among other effects.

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

Malondiadehyde levels were elevated in the liver in a statistically-significant, dose-dependent manner, among other effects, in mice orally exposed to 1.33-40 mg/kg bw/day Butylparaben for 30 days.

Statistically-significant, dose-dependent reductions in anogenital distance and ovary weights were observed in offspring of female rats exposed orally to 100 or 500 mg/kg bw/day Butylparaben from GD7 to GD21. Epididymal sperm counts and the expression of the Sertoli/Leydig cell marker Nr5a1 in adult male offspring were statistically-significantly reduced at 10 mg/kg bw/day or more. Adult prostate weights were statistically significantly reduced at 500 mg/kg bw/day. CYP19 and ERα expression was statistically-significantly increased, and the expression of StAR, P450scc, SULT1E1, and AR in the testes and methylation rate of the ERα promoter were statistically-significantly reduced, in male offspring of female rats exposed to 400 or 1000 mg/kg bw/day Butylparaben from GD7 to GD21. Weights of the testes, epididymal cauda sperm counts, and daily sperm production in male offspring were statistically significantly-reduced in the 400 and 1000 mg/kg bw/day groups of rats orally exposed to Butylparaben on GD7 to PND21. Vimentin filaments showed shorter projections, concentration near the basal region, and disappearance of the apical extensions toward the lumen of the seminiferous tubules in 3-week old rats 6 h after a single 1000 mg/kg bw oral dosage of Butylparaben. Spermatogenic cells were detached from Sertoli cells and sloughed into the lumen 24 h after treatment.

Prepubertal female rats exposed orally to 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Isopropylparaben on PND21 to PND40 exhibited statistically-significant delays in vaginal opening. In the 1000 mg/kg bw/day groups, there were statistically-significant decreases in the weights of the ovaries (Methylparaben or Isopropylparaben) and kidneys (Ethylparaben or Isopropylparaben), and increases in the weights of the adrenal glands (Methylparaben, Ethylparaben, or Propylparaben) and thyroid glands (Methylparaben). Liver weights increased at all dosage rates of Butylparaben. Morphological studies of the uterus revealed myometrial hypertrophy after exposure to 1000 mg/kg bw/day Propylparaben or Isopropylparaben and in animals of all dose groups of Butylparaben and Isobutylparaben. Among the effects on serum hormone concentrations, estradiol concentrations were statistically-significantly reduced (Ethylparaben or Isopropylparaben) and prolactin concentrations were increased (Methylparaben) in the 1000 mg/kg bw/day groups. Reduced plasma leptin concentrations were observed in male and female offspring of young adult female rats exposed orally to 100 mg/kg bw/day Butylparaben.

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

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

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

In prospective studies, PTB was associated with umbilical cord blood concentrations of Butylparaben (OR=60.77; CI=2.60-1419.93) and Benzylparaben (OR=0.03, CI=0.01-0.44). Linear regression analysis indicated an association between maternal urinary concentrations and decreased gestational age and body length in newborns. No statistically-significant associations were observed between Methylparaben or Ethylparaben concentrations and the outcomes evaluated. In another prospective study, in vitro fertilization outcomes were not associated with urinary Methylparaben, Propylparaben, or Butylparaben concentrations of women undergoing treatments for infertility. No statistically-significant associations were found between prenatal or postnatal growth of male newborns and maternal urinary paraben concentrations of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben.

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

Linear regression analyses of data from the U.S. NHANES program indicated an association between reduced serum T4 concentrations and urinary concentrations of Methylparaben, Ethylparaben, Propylparaben and Butylparaben.

MPC and the results of statistical tests for trends were not statistically significant in a study of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben in women undergoing infertility evaluation and OV or AFC.

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

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

PREVIOUS DISCUSSIONS

1984

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben[3]

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[4]

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

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

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

1995

Isobutylparaben and Isopropylparaben[5]

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[6]

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

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

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

The Expert Panel considered that the most important new data available for assessing the safety of parabens as used in cosmetics are those data generally in the category of endocrine disruption, but which include male reproductive toxicity and various estrogenic activity studies. The Expert Panel believes that the available data demonstrate that parabens

are, at most, weakly estrogenic. For example, the binding efficiency of parabens with estrogen receptors is around 4 orders of magnitude lower than estradiol.

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

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

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

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

For adults, the relevant calculations are:

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

Systemic dose (multiple parabens) = 17.76 g/ day of product
x 0.8% use concentration ÷ 60 - kg person
x 50% absorption x 1000 mg/kg = 1.18mg/kg day^{-1}

For infants, the relevant calculations are:

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

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

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

DISCUSSION

[*To be developed*]

CONCLUSION

[*To be developed*]

TABLES

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

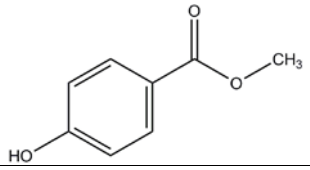
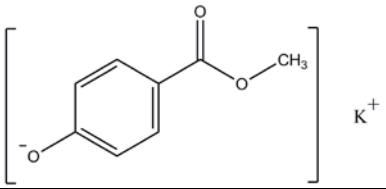
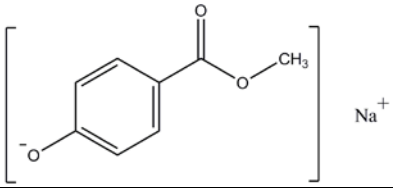
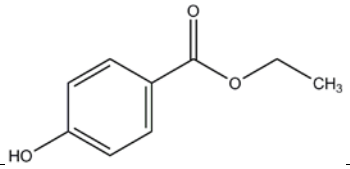
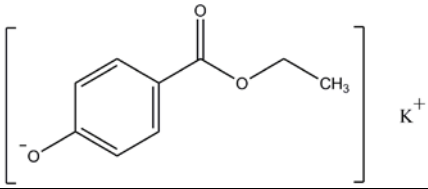
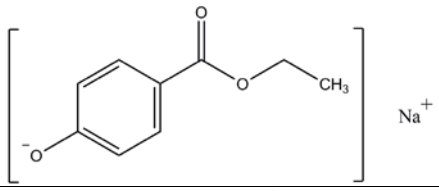
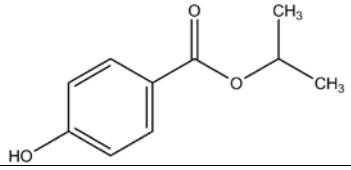
Ingredient CAS No.	Definition & Structure	Function
Parabens and Paraben Salts		
Methylparaben 99-76-3	Methylparaben is the ester of methyl alcohol and p-hydroxybenzoic acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Methylparaben 26112-07-2	Potassium Methylparaben is the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

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

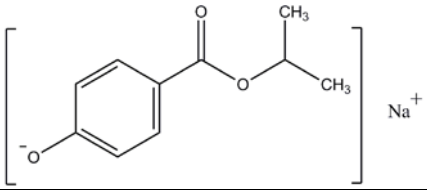
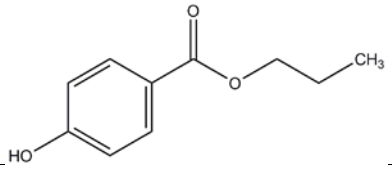
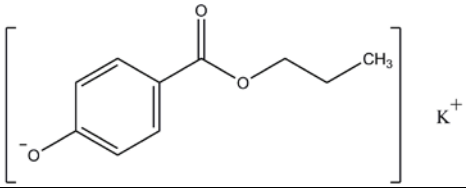
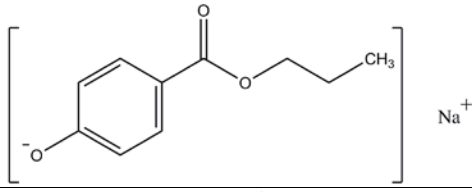
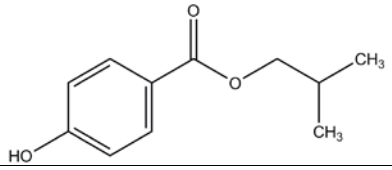
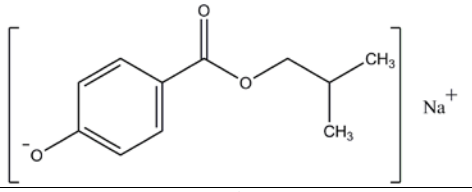
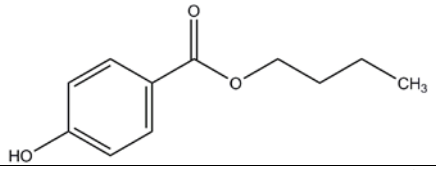
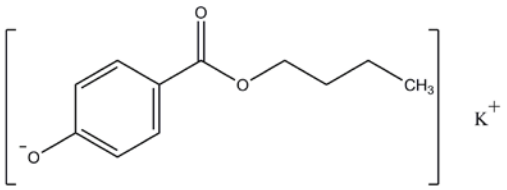
Ingredient CAS No.	Definition & Structure	Function
Sodium Isopropylparaben	Sodium Isopropylparaben is the sodium salt of Isopropylparaben: 	Preservative
Propylparaben 94-13-3	Propylparaben is the ester of n-propyl alcohol and *p*-hydroxybenzoic acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Propylparaben 84930-16-5	Potassium Propylparaben is the potassium salt of Propylparaben that conforms to the formula: 	Preservative
Sodium Propylparaben 35285-69-9	Sodium Propylparaben is the sodium salt of Propylparaben that conforms to the formula: 	Preservative
Isobutylparaben 4247-02-3	Isobutylparaben is the ester of isobutyl alcohol and *p*-hydroxybenzoic acid. It conforms to the formula: 	Preservative
Sodium Isobutylparaben 84930-15-4	Sodium Isobutylparaben is the sodium salt of Isobutylparaben: 	Preservative
Butylparaben 94-26-8	Butylparaben is the ester of butyl alcohol and *p*-hydroxybenzoic acid. It conforms to the formula: 	Fragrance ingredient, preservative
Potassium Butylparaben 38566-94-8	Potassium Butylparaben is the potassium salt of Butylparaben that conforms to the formula: 	Preservative

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

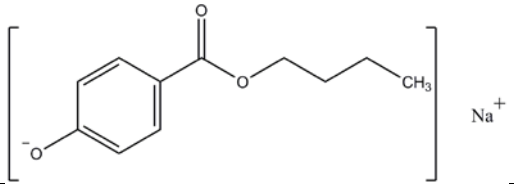
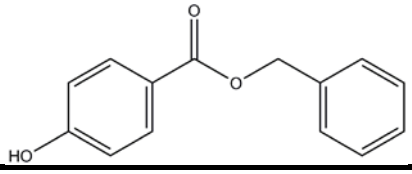
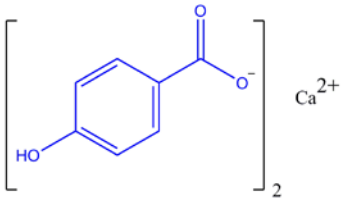
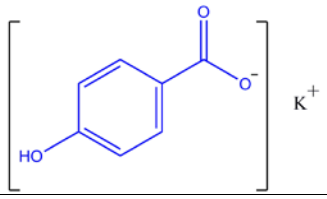
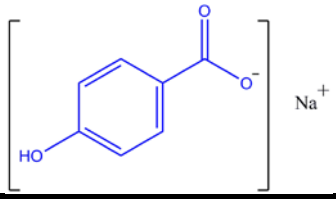
Ingredient CAS No.	Definition & Structure	Function
Sodium Butylparaben 36457-20-2	Sodium Butylparaben is the sodium salt of Butylparaben that conforms to the formula: 	Preservative
Benzylparaben 94-18-8	Benzylparaben is the ester of benzyl alcohol and *p*-hydroxybenzoic acid. It conforms to the formula: 	Preservative
Paraben Carboxylic Salts (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

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

Parabens	Conclusion	Reference
Methylparaben, Ethylparaben, Propylparaben, and Butylparaben	Safe as cosmetic ingredients in the present practices of use	1984[3]
Benzylparaben	Available data are insufficient to support the safety	1986[4]
Isobutylparaben and Isopropylparaben	Safe as cosmetic ingredients in the present practices of use	1995[5]
Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Benzylparaben, Isopropylparaben, and Isobutylparaben	Safe in the present practices and concentrations	2008[6]

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Sodium Methylparaben		
Physical Form	Crystalline solid	[7]
Color	White	[7]
Molecular Weight g/mol	174.131	[97]
Density @ 20°C	1.42	[7]
Melting Point °C	313	[7]
Water Solubility g/L @ 20°C & pH 11.4	>10.0	[7]
log P_{ow}	-0.63	[7]
Disassociation constants		
pKa @ 23°C	8.4	[7]
Calcium Paraben		
Molecular Weight g/mol	314.306	[98]
Potassium Butylparaben		
Molecular Weight g/mol	232.32	[99]
Potassium Ethylparaben		
Molecular Weight g/mol	204.266	[100]
Potassium Methylparaben		
Molecular Weight g/mol	190.239	[101]
Potassium Paraben		
Molecular Weight g/mol	176.212	[102]
Potassium Propylparaben		
Molecular Weight g/mol	218.293	[103]
Sodium Butylparaben		
Molecular Weight g/mol	216.212	[104]
Sodium Ethylparaben		
Physical Form	Solid, powder	[10]
Color	White	[10]
Molecular Weight g/mol	188.157	[34]
Density g/cm^3 @ 20°C	1.34	[10]
Melting Point °C	268	[10]
Water Solubility g/L @ 23°C & pH 10.4	>1000	[10]
log K_{ow}	-0.14	[10]
Sodium Isobutylparaben		
Molecular Weight g/mol	216.212	[105]
Sodium Paraben		
Molecular Weight g/mol	160.104	[106]
Sodium Propylparaben		
Physical Form	Solid, powder	[11]
Color	White	[11]
Molecular Weight g/mol	202.185	[107]
Density @ 20°C	1.24	[11]
@ 25°C	1.24	[11]
Vapor pressure mmHg @ 20°C	<0.001	[11]
Melting Point °C	302	[11]
Boiling Point °C	310 (decomp)	[11]
Water Solubility g/L @ 23°C	>100	[11]
log P_{ow}	0.27	[11]

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Methylparaben		
Physical Form	Powder	23
	Liquid	23
Color	White or colorless	23
Odor	Characteristic	23
Molecular Weight g/mol	152.16	6
Density g/cm^3 @ 137.2°C	1.1208	108
@ 20°C	1.209±0.06 est.	a
Vapor pressure mmHg @ 25°C	2.37x10^{-4}	23
Melting Point °C	131	6
	125-128	6
Boiling Point °C	270-280	6
	265	109
	140-141	110
Water Solubility g/L @ 25°C	2.50x10^3	23
	Slightly soluble	6
Other Solubility		
Alcohol	Very soluble	6
Benzene	Slightly soluble	6
Ether	Very soluble	6
Glycerin	Slightly soluble	6
log K_{ow}	1.93	39
Disassociation constants (pKa, pKb)		
pK_a	8.17	6
@ 25°C	8.31±0.13 est.	a
Ethylparaben		
Physical Form	Crystals or powder	111
Color	Colorless or white	111
Molecular Weight g/mol	166.18	6
Density @ 20°C	1.291	8
Vapor pressure mmHg @ 25°C	9.29x10^{-5}	111
Melting Point °C	116-118	6
	115-118	6
Boiling Point °C	297-298	6
Water Solubility g/L @ 25°C	0.885	111
Other Solubility		
Alcohol	Very soluble	6
Ether	Very soluble	6
Glycerin	Slightly soluble	6
log K_{ow}	2.47	8,111
	2.27	39
Disassociation constants (pKa, pKb)		
pK_a	8.22	6
	8.34	111

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Propylparaben		
Physical Form	Crystal or powder	112
Color	Colorless or white	112
Odor	Odorless or faint	112
Molecular Weight g/mol	180.21	6
Density	1.0630	6
	1.28	112
Vapor pressure mmHg @ 25°C	$5.55x10^{-4}$ est.[b]	112
Melting Point °C	96.2-98	6
	95-98	6
Boiling Point °C	294	109
	271	112
Water Solubility g/L	0.0500	112
	Insoluble	6
Other Solubility		
Alcohol	Soluble	6
Ether	Soluble	6
log K_{ow}	2.34	9
	2.81	39
Disassociation constants (pKa, pKb)		
pK_a	8.35	6
Isopropylparaben		
Molecular Weight g/mol	180.22	6
Melting Point °C	96-97	113
Boiling Point °C	294	109
Butylparaben		
Physical Form	Crystals or powder	114
Color	White	114
Odor	Odorless	114
Molecular Weight g/mol	194.23	114
Vapor pressure mmHg @ 25°C	$1.86x10^{-4}$	114
Melting Point °C	68-69	6
	68-72	6
Boiling Point °C	309.2±15.0	a
Water Solubility g/L @ 20°C	$0.0027x10^{2}$	114
	Insoluble	6
Other Solubility g/L		
Alcohol	Soluble	6
Ether	Soluble	6
Glycerin	Slightly soluble	6
Disassociation constants (pKa, pKb)		
pK_a	8.37	6
	8.47	114
Isobutylparaben		
Physical Form	Solid, powder	12
Color	White	12
Molecular Weight g/mol	194.25	6
Density g/cm^3 @ 20°C	1.105±0.06	a
Vapor pressure mmHg @ 25°C	0.000381	12
Melting Point °C	72.95 est.[d]	12
Boiling Point °C	302.3±15.0	a
Water Solubility g/L @ 25°C	2.24	12
log P_{ow}	3.04[e]	12

Table 3. Chemical and physical properties of parabens.

Property	Value	Reference
Benzylparaben		
Physical Form	Solid, crystalline	13
Color	White	13
Odor	Odorless	13
Molecular Weight g/mol	228.25	6
Molecular Volume m^3/kmol		
Density g/cm^3 @ 20°C	1.224±0.06 est.	a
Vapor Density mmHg	0 est.	13
Melting Point °C	110-112	6
Boiling Point °C	389.8±17.0 est.	a
Water Solubility g/L @ 25°C	1.08 10	13 6
Other Solubility g/L		
Propylene glycol	130	6
log P_{ow}	3.97	13
Disassociation constants (pKa, pKb)		
pK_a	8.18±0.15 est.	a

Decomp=decomposes on melting

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2017 ACD/Labs)

[b] US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Jan 9, 2007: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm

[c] SPARC; pKa/property server. Ver 3. Jan, 2006. Available from, as of Feb 13, 2007: http://ibmlc2.chem.uga.edu/sparc/

[d] Danish (Q)SAR Database. Database developed by National Food Institute, Technical University of Denmark, with support from the Danish Environmental Protection Agency, the Nordic Council of Ministers and the European Chemicals Agency.

[e] SRC Physprop database. 2016. SRC (Syracuse Research Corporation) PhysProp Database.

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	7
Ethylparaben	50±4.3	307.5±21.9	770.6	8
Sodium Ethylparaben	6.5±0.3	49.5±6.4	147.1±28.3	10
Sodium Propylparaben	6.7±0.3	37.8±4.9	164.5±36.7	11

Table 5. Current and historical frequency and concentration of use of parabens according to duration and exposure.[6,25,26]

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

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

Table 5. Current and historical frequency and concentration of use of parabens according to duration and exposure.[6,25,26]

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2017	**2006**	**2016**	**2003**	**2017**	**2006**	**2016**	**2003**
	Butylparaben				**Isobutylparaben**			
Totals*	**4685**	**3001**	**0.00000006-0.5**	**0.00002-0.54**	**2291**	**642**	**0.00000006-0.3**	**0.000007-0.5**
Duration of Use								
Leave-On	*3754*	*2409*	*0.0000000-0.5*	*0.00002-0.4*	*1729*	*435*	*0.00000006-0.3*	*0.000007-0.5*
Rinse-Off	*890*	*551*	*0.0000004-0.33*	*0.00004-0.54*	*528*	*178*	*0.0000004-0.23*	*0.0001-0.4*
Diluted for (Bath) Use	*41*	*41*	*0.00002-0.1*	*0.00004-0.07*	*34*	*29*	*0.000012-0.005*	*0.00002-0.2*
Exposure Type								
Eye Area	999	812	0.000002-0.5	0.00002-0.3	263	59	0.00000006-0.14	0.000007-0.5
Incidental Ingestion	297	219	0.0000026-0.2	0.0008-0.1	73	11	0.000004-0.09	0.0001-0.4
Incidental Inhalation-Spray	36; 773[a]; 733[c]	27; 453[a]; 320[c]	0.00000011-0.1; 0.00059-0.22[a]	0.0004-0.2; 0.03-0.4[a]; 0.0004-0.4[c]	29; 447[a]; 499[c]	7; 109[a]; 129[c]	0.00004-0.023; 0.00002-0.18[a]	0.01-0.2; 0.0002-0.3[a]; 0.02-0.4[c]
Incidental Inhalation-Powder	172; 7[b]; 733[c]	88; 21[b]; 320[c]	0.0057-0.3; 0.0001-0.24[b]	0.07-0.14; 0.05[b]; 0.0004-0.4[c]	28; 2[b]; 499[c]	8; 5[b]; 129[c]	0.0029-0.0086; 0.0000007-0.24[b]	0.00001-0.04; 0.02-0.4[c]
Dermal Contact	3840	2406	0.0000004-0.4	0.00004-0.54	1883	525	0.0000006-0.3	0.00001-0.5
Deodorant (underarm)	10[a]	10[a]	0.000025[d]	0.002[a]	7[a]	3[a]	NR	0.002[a]
Hair - Non-Coloring	323	246	0.00000011-0.22	0.0004-0.25	166	83	0.0000004-0.17	0.01-0.3
Hair-Coloring	48	28	0.0000005-0.05	0.03	42	1	0.000036-0.00008	NR
Nail	48	21	0.00000006-0.07	0.003-0.2	41	3	NR	0.006
Mucous Membrane	601	312	0.0000026-0.2	0.00004-0.11	297	63	0.000004-0.09	0.00002-0.4
Baby Products	12	28	NR	0.05	5	7	NR	NR

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

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

Table 6. Frequency of use according to duration and exposure of parabens.[25,26]

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

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

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

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

NR=Not Reported

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

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

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

Table 7. Parabens with no current reported uses in 2017 or historic according to the VCRP and the Council survey.[6,25,26]

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

Table 8. SCCP opinions on parabens.

Year	Conclusion	Reference
2005	It is the opinion of the SCCP that, viewing the current knowledge, there is no evidence of demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics.	16
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.	17
2006	The conclusion of opinion SCCP/0873/05 remains unchanged.	18
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.	19
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.	20
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.	21
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.	22

Table 9. Dermal penetration and penetration enhancement studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
Dermal Penetration In Vitro							
Methylparaben	Pig	Skin from the upper half of the ears of 6-month-old pigs	0.1% in aqueous, or hydrogel or emulsion oil-in-water formulations with and without a penetration enhancer (urea, Transcutol or propylene glycol), 0.1%, pH=5.5	Porcine skin used fresh or after storage at 4°C for 18 h or frozen, clamped between donor and receptor chambers of Franz-type diffusion cells	Receptor fluid and skin samples (~3.3 cm^2 discs, intact or tape-stripped 20 times; diffusion area 2 cm^2) maintained at 32°C; 20 µL aqueous solution was added to the donor chamber or ~20 mg of hydrogel or emulsion was applied to the skin sample at t=0; 50 µL samples removed from the receptor chamber at intervals for up to 4 h or 24 h (depending on the experiment) for analysis by HPLC and replaced by fresh receptor medium	For freshly excised intact skin and previously frozen intact skin, concentrations of unmetabolized Methylparaben in receptor fluid <LOD-2.3% and 2.3%-3.3% of applied dose, respectively, after 4-h exposure; for previously frozen intact and tape-stripped skin, concentrations of unmetabolized Methylparaben in receptor fluid were 2.0%-5.8% and 2.9%-7.6% respectively, after 24-h exposure; absorption rate was higher from emulsions vs. hydrogels, enhancer-containing formulations vs. enhancer-free formulations, and when skin was tape stripped; Freezing skin samples reduces hydrolysis of Methylparaben slightly	37
Methylparaben Ethylparaben Propylparaben Butylparaben	Pig	Ears (~1 mm thick) collected from young animals	0.1% in 20%(v/v) or 50% (v/v) ethanol/PBS	Full-thickness porcine skin, stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (20% or 50% ethanol/PBS) and skin samples (diffusion area 1.77 cm^2); system maintained at 37°C; 2 mL solution added to the donor chamber at t=0; 400 µL samples removed from the receptor chamber at intervals for up to 6 h or 7.5 h (depending on the experiment) for analysis by capillary electrophoresis (CE) and replaced by fresh receptor medium	Permeability coefficients (cm/h x 10^{-4}), in descending order: Methylparaben, 214.8 ± 40, Ethylparaben, 197.5 ± 10; Propylparaben, 101.9 ± 15; Butylparaben 31.3 ± 1.6; skin penetration was inversely proportional to lipophilicity; Increasing ethanol concentration and exposure duration increased parabens retention in dermis compared epidermis; Binary combinations of the parabens reduced their permeation rates, attributed by the authors to high retention in the epidermis and dermis	38
Methylparaben Ethylparaben Propylparaben	Rabbit (mixed breed)	Skin excised from ears of 6-month-old animals	3 commercial facial moisturizing creams containing 0.23%-0.32% (w/w) Methylparaben, 0%-0.1% Ethylparaben, and 0.04%-0.19% Propylparaben.	Full-thickness skin, stored 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	39

Table 9. Dermal penetration and penetration enhancement studies of parabens

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Exposure Route	Procedure	Results	Reference
Methylparaben Propylparaben Butylparaben	Human Mouse (hairless)	Human cadaver epidermis (commercially available) Skin from 8-week-old male mice	0.1%, 0.4%, and 2% in a general oil-in-water cream formulation	Human epidermis (~0.03 mm thick) and mouse skin (~0.25 mm thick), stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (1:1 ethanol/water, v/v) and skin samples (diffusion area 0.785 cm^2) maintained at 32°C; 10 mg cream applied to the skin surface at t=0; 1 mL samples removed from the receptor chamber at intervals for up to 24 h for analysis by LC-MS/MS and replaced by fresh receptor medium	Permeability coefficients (K_ps; cm/h x 10^{-4}) were similar regardless of concentration tested; K_ps were directly related to paraben concentration K_ps for human skin ranged from 0.74 ± 0.19 to 0.91 ± 0.44 for Methylparaben, 0.54 ± 0.14 to 0.91 ± 0.22 for Propylparaben, and 0.37 ± 0.15 to 0.56 ± 0.32 for Butylparaben K_ps for mouse skin ranged from 1.41 ± 0.12 to 1.66 ± 0.21 for Methylparaben, 1.52 ± 0.13 to 1.76 ± 0.39 for Propylparaben, and 1.17 ± 0.15 to 1.27 ± 0.20 for Butylparaben Residual quantities of parabens remaining in skin increased with increasing concentration tested, with greater amounts in human epidermis than in mouse skin; Residual quantities in human epidermis (µg/ml x 10^{-4}): Methylparaben, 235 ± 132 to 7198 ± 4662; Propylparaben, 375 ± 212 to 4120 ± 2344; Butyl paraben, 436 ± 226 to 5480 ± 2593; Residual quantities in mouse skin: Methylparaben, 14 ± 5 to 286 ± 104; Propylparaben, 21 ± 9 to 410 ± 112; Butyl paraben, 15 ± 2 to 358 ± 118 Authors state results show that parabens may be classified as moderate penetrants	[40]
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	Abdominal skin samples collected during surgery from 8 women	Commercial body lotion containing 0.1% (w/w) Methylparaben, 0.08% Ethylparaben, 0.2% Propylparaben, and 0.15% Butylparaben.	Human skin samples, stored frozen, thawed and mounted on Franz diffusion cells	Receptor fluid (3% bovine serum albumin in isotonic saline solution) and skin samples (diffusion area 3.14 cm^2) maintained at 32°C; single 100 µL (45 mg) lotion applied to skin surface at t=0, which was repeated for some skin samples at t=12 h and t=24 h; fluid was removed from the receptor chamber at intervals for up to 36 h for analysis by HPLC and replaced by fresh receptor medium	Penetration was inversely proportional to lipophilicity of parabens tested, and increased with repeated applications; penetration 36 h after single application (percentage of applied dose): Methylparaben, 0.057% ± 0.03; Ethylparaben, 0.045% ± 0.01; Propylparaben, 0.028% ± 0.01; Butylparaben, 0.007% ± 0.003; Penetration 12 h after last of 3 repeated applications: Methylparaben, 0.6 ± ± 0.1%; Ethylparaben, 0.3% ± 0.1; Propylparaben, 0.2% ± 0.05; Butylparaben, 0.04% ± 0.01	[41]

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

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
IN VITRO						
Methylparaben Ethylparaben Propylparaben Benzylparaben	Rat (strain not specified)	AFP in rat amniotic fluid	Five to 6 concentrations between 10^{-9} M and 10^{-4} M	Competitive binding to AFP in rat amniotic fluid assayed against 2,4,5,7-[^{3}H]-estrone, with assay tubes containing no "cold" radio-inert test competitor provided the 100% binding level, and 1.5 x 10^{-6} M "cold" competitor maximally competed with 10^{-6} M 2,4,5,7-[^{3}H]-estrone; radioactivity remaining above this standard was considered nonspecific and was subtracted from assay measurements to estimate specific binding	The concentration of Benzylparaben inhibiting the binding of 2,4,5,7-[^{3}H]-estrone to AFP by 50% (IC_{50}) was 0.012 µM; AFP did not exhibit binding affinity for Methylparaben, Ethylparaben, and Propylparaben	44
Butylparaben	Rat (Wistar)	S9 fraction of 5-week old males (n not specified)	Twelve concentrations between about 5 µM and 90 µM	Reactions performed in PBS, pH 7.4, at 37°C in shaking water bath and stopped by adding ice-cold methanol; supernatant was separated by HPLC and formation of *p*-hydroxybenzoic acid metabolite was monitored using UV detector at 254 nm; Michaelis-Menten parameters were estimated by Lineweaver-Burk plot (no further details provided)	Butylparaben was biotransformed to *p*-hydroxybenzoic acid in the reaction mix with the maximum rate achieved by the system, at saturating substrate concentration (V_{max})=8.8 nmol/min/mg protein and the substrate concentration at which the reaction rate is half of V_{max} (Km)=28.6 mM	45
Butylparaben	Human Rat (Harlan Sprague-Dawley)	Hepatocytes from human subjects (59-year-old woman an 45-year-old man, both non-smokers) and 8 to 12 week old male and female rats	1 µM radiolabeled Butylparaben (phenyl ring-^{14}C(U) – 53.1 mCi/mmol); 10 µM radiolabeled Butylparaben in metabolism studies	The plates were then pre-incubated for 5 min at 37°C and Butylparaben added in acetonitrile (<0.5% final concentration) at t=0; 50 µL aliquots were collected at t=300 min for metabolism studies and at intervals up to t= 300 min for clearance studies for LC-MS/MS analysis	Butylparaben was rapidly cleared in hepatocytes from rats, with little or no sex difference ($t_{1/2}$=3.8 ± 0.3 min and 3.3 ± 0.1 min for hepatocytes from males and females, respectively, corresponding to Cl_{int}=811 ± 53 and 903 ± 28 mL/min/kg); Butylparaben was cleared more slowly in hepatocytes from humans but, again, there was no sex difference ($t_{1/2}$=23.9 ± 1.3 min and 29.6 ± 5.2 min for hepatocytes from males and females, respectively, corresponding to Cl_{int}=92 ± 5 and 111 ± 22 mL/min/kg); Butylparaben was extensively hydrolyzed to *p*-hydroxybenzoic acid as the major metabolite for both sexes and species (92% to 100% in rat, 78% to 84% in human) after 5 h of incubation. The other metabolite observed in human hepatocytes was 4-hydroxyhippuric acid (16% to 22%)	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
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (Sprague-Dawley) Monkey (African green)	Pooled human liver and small intestine microsomes available commercially Rat liver, skin, kidney, pancreas, and small intestine microsomes and blood plasma S9 from COS cells (Monkey-kidney derived, fibroblast like)	100 nmol paraben and tissue microsomes or plasma in final volume of 1 mL 0.1 M K, Na-phosphate buffer (pH 7.4)	Incubation was for 7 min at 37°C, then 10 mg 2,4-dihydroxybenzophenone (internal standard) and 1 mL acetonitrile added; aliquot of the supernatant was collected for analysis of paraben hydrolase activity by HPLC Carboxylesterase activity was determined by measuring deacetylase activities toward 4-nitrophenol acetate and 4-methylumbelliferyl acetate: 4-nitrophenol acetate deacetylase activity measured by spectrophotometry at 405 nm; 4-methylumbelliferyl acetate deacetylase activity measured by fluorophotometry at 329 nm (excitation) and 448 nm (emission)	Rat liver microsomes showed the highest hydrolytic activity towards Butylparaben, with activity decreasing with decreasing side-chain length – carboxylase 1 exhibited a similar activity pattern; Rat small-intestinal microsomes exhibited higher activity toward longer-side-chain parabens – carboxylase 2 showed a similar activity pattern; In contrast, human liver microsomes showed the highest hydrolytic activity toward Methylparaben, with activity decreasing with increasing side-chain length; human small-intestinal microsomes showed a specificity pattern similar to that of rat small-intestinal microsomes	47
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	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
Methylparaben Ethylparaben Propylparaben Butylparaben	Human Rat (strain not specified)	HLM, HSM, HLC, and HSC RLM, RSM, RLC, and RSC	100 µM in 50 mM potassium phosphate, pH 7.4	Reactions were initiated with the addition of 100 µM paraben; mixture incubated for 30 min at 37°C; p-hydroxybenzoic acid formation measured by HPLC-analysis of supernatants	Hydrolysis of parabens by HLM was about 10-fold more rapid than by HLC; Metabolism rates were inversely proportional to chain length (the longer the alcohol moiety, the slower the hydrolysis); this trend was also observed for HSM and HSC, but at much lower rates of hydrolysis; Paraben metabolism in HLM was 300- to 500-fold faster than in HSM, depending on the ester compared; Paraben hydrolysis rates in rat liver and skin were greater than in human liver and skin; RLM and RSM metabolized parabens 7-fold and 5-fold faster than RLC and RSC, respectively; In all rat tissue fractions tested, hydrolysis rates for the parabens increased as the ester chain length increased, in contrast to human tissue fractions; Methylparaben and Propylparaben was the preferred substrate for human tissue fractions and rat tissue fractions, respectively; Rat skin displayed 3 to 4 orders of magnitude faster hydrolysis rates than human skin	[48]

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
ANIMAL						
Dermal						
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	n=9/sex/group for the toxicokinetics study and n=3/sex/group for the mass balance study	Single 100 mg/kg bw dosage of radiolabeled (ring-U-^{14}C) paraben, in 60% aqueous ethanol vehicle, applied to the skin	Isotopic mixtures were applied to the interscapular/back region (on an area equivalent to approximately 10% of the total body surface) over a 6-h period; hair at the administration site was clipped before application; animals wore an Elizabethan collar during the 6-h exposure period Blood samples were taken from the retro-orbital sinus of the toxicokinetic animals pre-dose and then at 0.5, 1, 2, 4, 8, 12, 22, and 24 h after oral dosing; 3 rats/sex/group were sampled each time; Animals were killed after the last sampling; Blood, excreta were collected from all mass balance animals pre-dose and then after the periods 0–6, 6–24, 24–48, 48, 72–96, 96–120, 120–144 and 144–168 h after oral dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection After sacrifice, the brain, heart, kidneys, liver, lungs, spleen, stomach and forestomach, small and large intestine, pancreas, and skeletal muscle sample were collected and weighed, and analyzed for radioactivity	For all 3 parabens, C_{max} (≥693 and ≥614 ng eq/g in males and female, respectively) occurred within 8 h post-gavage, and blood concentrations decreased until the last quantifiable concentration within 24 h; Most of the dosage (≥46.4%) as unabsorbed and recovered in the swabs used for cleaning of the application site at the end of the exposure period; ≤25.8% of the applied radioactivity was found in the urine; urinary excretion was the main route of elimination; radioactivity was eliminated rapidly in the urine with averages ≥11.9% recovered in the first 48 h; ≤0.16 % of the radioactive dose of Methylparaben was found in the skin strips and biopsies from the treated sites after necropsy; for all of the parabens tested, a large part of the radioactivity (≥20.7%) was retained in the carcasses; Metabolic profiling of pooled plasma collected 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of *p*-hydroxybenzoic acid	[50]

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben	Rat (Harlan Sprague-Dawley)	8 to 10 week old males, n=4	Single 10 or 100 mg/kg dosage of radiolabeled Butylparaben (phenyl ring-^{14}C(U) – 53.1 mCi/mmol; 50 µCi dose/animal) in 95% ethanol, applied to the skin	Single dermal dosages (0.5 mL/kg bw) were applied onto a 4 cm^2 (2 cm × 2 cm) area of shaved skin on the backs of the rats; a protective foam appliance was glued onto the skin using medical adhesive, the doses were administered evenly to the dose area, and a non-occlusive cloth cover was attached over the appliance Urine and feces of rats were collected separately for up to 72 h post-exposure; the animals were then killed, blood was collected via cardiac, and the following tissues were excised and weighed: liver, kidney, brain, muscle (hind leg), abdominal skin, adipose (perirenal), spleen, heart, lung, ovaries, uterus and testes; protective appliance was removes, dose-site skin was excised and washed with a series of water-wetted gauzes and appliance, dose-site skin, rinsate, and gauzes were stored frozen before analysis	Absorption of 10 mg/kg and 100 mg/kg Butylparaben 72 h following application was about 52% and 8%, respectively; total absorbed dosage was comparable (5.2 mg and 8 mg for 10 and 100 mg/kg, respectively); authors stated that nonlinearity with increasing dosage indicates saturation of the capacity for dermal absorption; About 21% of the 10 mg/kg dosage remained unabsorbed; about 16% was recovered in the dose-site skin; About 3% and 8% of the 100 mg/kg dosage was absorbed at 24 h and 72 h, respectively; the amount recovered in the dose-site skin increased from 19% at 24 h to 43% at 72 h; Urine was the primary route of elimination, with about 46% of 10 mg/kg recovered in urine and in cage rinse at 72 h; fecal elimination of radioactivity accounted for 1.7%; Tissues contained about 4.3% of the 10 mg/kg dosage; highest concentrations of radiolabel were in bladder, liver and kidney, which contained about twice the concentration of residues found in liver	[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
ANIMAL						
Oral						
Methylparaben Propylparaben Butylparaben	Rat (Sprague-Dawley)	n=9/sex/group for the toxicokinetics study and n=3/sex/group for the mass balance study	Single 100 mg/kg bw dosage of radiolabeled (ring-U-^{14}C) paraben, in 60% aqueous ethanol vehicle, administered by gavage	Blood samples were taken from the retro-orbital sinus of the toxicokinetic animals pre-dose and then at 0.5, 1, 2, 4, 8, 12, 22, and 24 h after oral dosing; 3 rats/sex/group were sampled each time; Rats were killed after the last sampling; Blood, excreta were collected from all mass balance rats pre-dose and then after the periods 0–6, 6–24, 24–48, 48, 72–96, 96–120, 120–144, and 144–168 h after oral dosing, and samples were analyzed for radioactivity; all animals were sacrificed after the last excreta collection After sacrifice, the brain, heart, kidneys, liver, lungs, spleen, stomach and forestomach, small and large intestine, pancreas, and skeletal muscle sample were collected and weighed, and analyzed for radioactivity	For all 3 parabens, C_{max} (≥11432 and ≥21040 ng eq/g in males and female, respectively) occurred within 1 h post-gavage, and blood concentrations decreased until the last quantifiable concentration at 12 h; Mean total cumulative excretion (urine, feces and cage wash) of the administered radioactive dose over a 168-h collection period was complete and amounted to ≥89%; most of the administered dose (≥71%) was eliminated in urine, while ≤3.3% was eliminated in the feces; radioactivity was eliminated rapidly with averages ≥69.6% recovered in the urine during the first 24 h; A small amount of radioactivity (<0.1%) was observed in the collected tissues, and the levels of radioactivity were below the LOQ in the carcasses of most animals; Metabolic profiling of pooled plasma collected at 0.5, 1, 2, 4, and 8 h post-dose detected a single radioactive peak, which corresponded to the retention time of *p*-hydroxybenzoic acid	[50]

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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	[49]
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	[51]

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Butylparaben Isobutylparaben	Human	Healthy 31-year old volunteers, n=3 (1 woman and 2 men)	10 mg deuterated (D4-ring-labeled) paraben/dose, dissolved in ethanol and added to a cup of breakfast coffee or tea	Each subject ingested a dose of each paraben, a different paraben each time, with at least 2 weeks between exposures; the first urine samples were collected before exposure and then at 4 13-h intervals for 48 h after exposure for HPLC analysis; ring-deuterated standards included ethyl 4-hydroxybenzoate-2,3,5,6-d4, iso-butyl 4-hydroxybenzoate-2,3,5,6-d4, n-butyl 4-hydroxybenzoate-2,3,5,6-d4, and 4-hydroxybenzoic-2,3,5,6-d4 acid	Free and conjugated parabens and their known, non-specific metabolites, *p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid, were detected in the urine samples; new oxidized metabolites with hydroxy groups on the alkyl side chain (3OH-n-butylparaben and 2OH-iso-butylparaben) and species with oxidative modifications on the aromatic ring were discovered; 17.4 %, 6.8 %, 5.6% of the doses of Methylparaben, Isobutylparaben and Butylparaben, respectively, were excreted in the urine; about 16% and 6% of Isobutylparaben and Butylparaben were excreted as 2OH-iso-butylparaben and 3OH-n-butylparaben, respectively; less than 1% was excreted as ring-hydroxylated metabolites; For all parabens tested, *p*-hydroxybenzoic acid was the major metabolite (57.2% - 63.8%) and urinary *p*-hydroxyhippuric acid ranged from 3.0% - 7.2% of the doses; 80.5% - 85.3% of the doses were excreted as the metabolites detected in this study within 24 h after exposure	[52]
			HUMAN			
			Aggregate			
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Human	Female breast cancer patients undergoing radical mastectomy, n=40	Aggregate exposures (undefined sources)	Human breast tissue was collected from 40 mastectomies for primary breast cancer in England between 2005 and 2008; concentrations of parabens were measured (HPLC-MS/MS) in breast tissue samples excised from four serial locations (quadrants) across the breast, from axilla to sternum	One or more paraben ester was detected 99% of the tissue samples and all 5 esters were detected in 60% of the samples; Median concentrations in the 160 tissue samples were highest for Propylparaben (16.8 ng/g tissue) and Methylparaben (16.6 ng/g tissue), lower for Butylparaben (5.8 ng/g tissue) and Ethylparaben (3.4 ng/g tissue, and least for Isobutylparaben (2.1 ng/g tissue); Maximum concentrations ranged from 95.4 ng Butylparaben/g tissue to 5103 ng Methylparaben/g tissue; Propylparaben concentrations were statistically significantly higher in samples excised from the axilla, compared with those from the mid or medial regions of the breasts	[53]

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

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
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	[54]

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

Table 11. Short-Term Toxicity Studies.

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
Short-Term							
Animal							
Dermal							
Isopropylparaben Isobutylparaben	Rat (Sprague-Dawley)	5-week old males and females, n=10/sex/ group, 13 groups	50, 100, 300, or 600 mg/kg bw/day Isopropylparaben, Isobutylparaben, or 100, 200, 600 and 1200 mg/kg bw/day of a 1:1 mixture of Isopropylparaben and Isobutylparaben, in 99% ethanol	28 days	Protocol followed current OECD TG 410 for short-term repeated dermal exposure studies; test material was topically applied to shaved dorsal skin and covered with a porous gauze dressing and non-irritating tape, 5 days/week; 8 hematological parameters were evaluated; brains, hearts, kidneys, the large lobe of livers, and sectioned dorsal skin were harvested for histological evaluation; hormone concentrations were measured by ELISA, including concentrations of T3, FSH, estradiol, insulin, T, and TSH	There were no significant changes in body and organ weights in any group; macroscopic and microscopic histopathological examinations revealed mild-to-moderate skin damage in female rats; NOAELs for Isobutylparaben and Isopropylparaben were 600 mg/kg bw/day, and 50 mg/kg bw/day, respectively; a LOAEL for hyperkeratosis of 50 mg/kg bw/day was estimated for the mixture; Analysis of serum concentrations showed that FSH was dose-dependently decreased in animals treated with ≥200 mg/kg bw/day of the mixture (i.e. ≥100 mg/kg bw/day each of Isopropylparaben and Isobutylparaben combined)	[55]

Table 11. Short-Term Toxicity Studies.

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
Short-Term							
Animal							
Oral							
Propylparaben	Rat (Wistar)	Adult males, n=8/group, 3 groups	100 or 300 mg/kg bw/day, suspended in a few drops of Tween-80 (stock solution) and diluted in distilled water (vehicle)	4 weeks	At the end of the treatment period, blood was collected from the abdominal aorta, liver, kidneys, heart and testes were excised, organ to total body weight ratio was calculated, right lobe of the liver and the left testis were fixed for histological examination and homogenates of the remaining liver and testis were prepared ALT, AST, ALP, and LDH activities were analyzed using ELISA; TP, Alb and creatinine concentrations were measured using commercial assay kits; reduced GSH, lipid peroxides (as MDA) and total NO were determined in liver and testis homogenates by the colorimetric methods and CAT and SOD activities were determined; Serum free T and E2 concentrations were measured by ELISA	Statistically-significant effects included dose-dependent increase in relative liver weights, increases in serum ALT, AST, ALP and LDH activities, and reduced total serum protein and albumin (at both dosage rates) and serum globulin (at 300 mg/kg bw/day) concentrations; Serum urea concentrations and urea/creatinine ratios were statistically-significantly increased (at both dosage rates), as was the serum creatinine concentration (at 300 mg/kg bw/day); Statistically-significant decrease in GSH, CAT and SOD activities, and increase of lipid peroxidation and NO generation (at both dosage rates); Statistically-significant dose-dependent reduction in serum testosterone concentration and T/E2 ratio, and elevation in serum E2; Livers exhibited presence of dilated congested central and portal veins, focal areas of dilated sinusoids, highly proliferated bile ducts with fibrotic reactions around them, expanded portal areas with edema, multifocal areas of necrotic hepatocytes with inflammatory cells infiltration and severe cytoplasmic vacuolization of hepatocytes (at both dosage rates); Testes exhibited evidence of severe spermatogenic arrest, seminiferous tubules occupied with ill-defined eosinophilic mass structure and giant cells in the lumen, detached spermatogenic lineage, edematous eosinophilic interstitial space with congested blood vessels and a mild loss of Leydig cells population	[56]

Table 11. Short-Term Toxicity Studies.

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Exposure Duration	Procedure	Results	Reference
Methylparaben	Rats (Wistar)	Females (146 ± 10 g bw), n=10/group	250 mg/kg bw/day, administered in the diet	10 days	Blood samples were collected from the retro-orbital sinuses of the animals on the 10th day of the experiment; plasma was analyzed for total MDA concentrations by HPLC and for 2,3-DHBA by LC-MS/MS	Serum MDA (lipid-peroxidase end-product) and 2,3-DHBA (marker of in vivo hydroxyl radical production) concentrations were statistically-significantly elevated compared with controls ($p<0.01$)	[57]
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	[58]

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	[59]
Butylparaben	Rat (Wistar)	Pregnant females, n=7 or 8/group, 5 groups	0, 64, 160, 400, and 1000 mg/kg bw/day in corn oil, by gavage	Dams were dosed daily from GD7 to PND21	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	[60]
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	[61]

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	[62]
Methylparaben Ethylparaben Propylparaben Isopropylparaben Butylparaben Isobutylparaben	Rat (Sprague-Dawley)	Prepubertal (8-week-old) females, N=200, n=10/group, 20 groups	0, 62.5, 250 or 1000 mg/kg bw/day in corn oil (vehicle), by gavage	Prepubertal females were dosed daily with a paraben in corn oil from PND21 to PND40; EE was used as a positive control (1 mg/kg bw/day)	Treatment with Methylparaben (1000 mg/kg bw/day) or Isopropylparaben (250 or 1000 mg/kg bw/day) resulted in a statistically-significant delay in vaginal opening in prepubertal females ($p < 0.05$); in contrast, the positive control (EE) significantly accelerated the date of vaginal opening; In the 1000 mg/kg bw/day groups, there were statistically-significant ($p<0.05$) decreases in ovary weights (Methylparaben or Isopropylparaben) and kidney weights (Ethylparaben, or Isopropylparaben) and increases in adrenal gland weights (Methylparaben, Ethylparaben, or Propylparaben) and thyroid gland weights (Methylparaben); Liver weights increased at all dosage rates of Butylparaben ($p < 0.05$); Histological analysis of the ovaries indicated decrease in the number of corpora lutea, increase in the number of cystic follicles, and thinning of the follicular epithelium; Morphological studies of the uterus revealed myometrial hypertrophy after exposure to 1000 mg/kg bw/day Propylparaben or Isopropylparaben and in animals of all dose groups of Butylparaben and Isobutylparaben; In the 1000 mg/kg bw/day groups, serum estradiol concentrations were statistically-significantly reduced (Ethylparaben or Isopropylparaben) and prolactin concentrations were increased (Methylparaben); Serum concentrations of T4 were statistically-significant reduced after treatment with 1000 mg/kg bw/day Methylparaben or 250 mg/kg bw/day Propylparaben or Isopropylparaben, or 62.5 mg/kg bw/ Isobutylparaben, propyl- and Isopropylparaben; The parabens exhibited affinities for ERα and ERβ (IC_{50}s ranging from 2.07×10^{-6} to 5.55×10^{-5}) in the following order: Isobutylparaben>Butylparaben>Isopropylparaben=Propylparaben>Ethylparaben; IC_{50} for 17β-estradiol was approximately 3×10^{-9}, by comparison	[63]

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

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben	Rat (Wistar)	Young adult, pregnant females, n=8/group	0, 100 mg/kg bw/day (vehicle not specified), by gavage	Pregnant females were dosed daily from GD7 to GD21; fetuses were removed on PND21, blood from the fetuses of each litter were pooled (males and females separately) for measurement of plasma insulin, leptin, MCP1, IL-1B, PAI-1 active, IL6, and TNFα concentrations Livers, adrenals and testes were collected from GD21 males for histopathology examination, gene expression analysis, or hormone measurements (estradiol and testosterone)	Butylparaben reduced plasma leptin concentrations in male and female offspring ($p<0.01$)	[64]
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	[65]
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	[66]

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

Test Substance(s)	Species/ Strain	Test Population- Sex	Dosage (Vehicle)	Procedure	Results	Reference
Butylparaben	Rat (Sprague-Dawley)	7-week-old males, n=5/group, 4 groups	0, 10, 100 and 1000 mg kg in corn oil (vehicle), by gavage	Performed in accordance with OECD TG 407 for repeated 28-day oral toxicity studies; 24 h after the last dose, testes, tails and epididymal spermatozoa samples were collected, DNA was extracted, and the DNA samples from each group were pooled, digested (methylation-specific restricted restriction digestion), and analyzed by differential display random amplification of polymorphic DNA (RAPD)	Among 57 RAPD amplicons, six were methylation specific. Densitometric analysis of stained agarose gels revealed that five of these amplicons were elevated 1.4- to 3.8-fold in epididymal sperm DNA in treated vs. control animals, indicating an epigenetic effect on spermatogenic germ cells in adult rats	[115]
Methylparaben Butylparaben	Rat (Wistar – Crl:WI [BR])	Males, 22 days of age, n=16/group, 4 groups	0, 100, 1000 or 10,000 ppm in the diet	Rats were 22 days of age at the start of exposure, which was continued for 8 weeks; parameters evaluated included organ weights, histopathology of reproductive tissues, sperm production, motility, and morphology; reproductive hormone concentrations (LH, FSH, and T) were measured in blood samples from Butylparaben-treated rats and corresponding controls	Methylparaben exposure resulted in a statistically-significantly higher incidence of abnormal sperm in the 1000-ppm ($p \leq 0.01$) and 10,000-ppm ($p \leq 0.05$) exposure groups, mostly sperm with no head in 4% to 5% of sperm, vs. 2.3% in 100-ppm and control groups; 100-ppm Methylparaben in the diet corresponds to 11.2 ± 0.5 mg/kg bw/day; Hormone concentrations were comparable across groups and were not altered from controls, with the following exceptions: Testosterone concentration was statistically-significantly reduced in the 1000-ppm and 10,000-ppm Butylparaben-treated groups after 3 weeks of exposure – removing two rats with aberrantly high testosterone measurement from the control group resulted in a mean control values that were comparable to those of the other groups; T and FSH concentrations were statistically-significantly higher (by 72% and 53%, respectively) in the 10,000-ppm Butylparaben-treated group, compared with the control group; LH concentrations were statistically-significantly lower ($p \leq 0.01$) in the 1000-ppm (by 35%) and 10,000-ppm (by 30%) exposure groups, compared with controls, but only at the 5-week exposure point The authors concluded that none of the parameters evaluated for either paraben showed compound- or dosage-dependent adverse effects, and the NOAEC was the highest concentration tested (10,000 ppm), corresponding to a NOAEL of 1141.1 ± 58.9 and 1087.6 ± 67.8 mg/kg/day for Methylparaben and Butylparaben, respectively	[45]

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	[71]
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Chinese hamster	CHO cells, AR-transfected	0, 12 concentrations within the range of 0.025 - 50 µM	Cells were transfected with the expression vector pSVAR0 and the MMTVLUC reporter plasmid; test compounds were added to the cells with or without 0.01 nM of the AR agonist R1881; The principle of concentration addition was applied to predict the effects caused by an equimolar (1:1:1:1:1) of the parabens; concentration-response relationship for the mixture was calculated using data fitted from the concentration-response curves of the individual compounds	Only Isobutylparaben antagonized the AR; the effect was statistically significant at ≥ 25 µM; Butylparaben and Propylparaben inhibited the R1881-induced response, but only at cytotoxic concentrations; The mixture was predicted to antagonize the AR at concentrations ≥ 2 µM	[72]
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	[116]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, 10 µM, ethanol vehicle (0.1% final concentration)	BT-474 cells are HER2 negative and ERα-positive; MCF-7 cells are ERα-positive and HER2-negative; SKBR3 cells are HER2-positive and ERα-negative; All cells were grown in phenol red-free culture medium and incubated for 2 h (for RT-PCR and Western blot analysis) or from 1 to 3 h (for chromatin immunoprecipitation analysis), with and without Butylparaben, with and without the HER2 HRG at 27°C	Propylparaben and Butylparaben statistically-significantly, synergistically, elevated c-Myc mRNA expression in BT-474 cells in the presence of HRG; Butylparaben was selected for further study because it was most effective; In BT-474 cells, no increase in c-Myc protein concentrations was observed with Butylparaben or HRG alone; in the presence of HRG with 1 µM and 10 µM Butylparaben, the increase in c-Myc protein concentrations was similar to that induced by 0.01 µM E2 plus HRG; the increase was blocked by ER antagonists ICI 182,780, raloxifene, and tamoxifen; MCF-7 cells treated Butylparaben exhibited a similar enhancement of HRG-induced c-Myc protein expression; no synergistic increase in c-Myc protein concentrations was observed in SKBR3 cells Butylparaben increased the number of BT-474 cells entering S-phase (EC_{50}=0.551 µM); the effect was enhanced in the presence of HRG (EC_{50}=0.024 µM After 1-h treatment with HRG and Butylparaben together, maximal 8-fold enhancement of ERα binding to c-Myc enhancer sequence was observed in BT-474 cells; Butylparaben enhanced binding about 4-fold and HRG <2-fold, by comparison	[73]
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	[74]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	Human	MDA-kb2 human breast carcinoma cells	0, and 25 µM in DMSO (vehicle)	MDA-kb2 cells are stably transformed with the MMTV luciferase neo reporter gene construct, and express high levels of functional endogenous AR and GR, which can both act through the MMTV promoter; cells were cultured and then incubated for 24 h, in the presence or absence of paraben, with and without the AR antagonist flutamide (5 µM), in Leibovitz's L-15 medium supplemented with 10% FBS, with 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C	Butylparaben statistically-significantly enhanced the hydrocortisone-induced GR signal by 85%; Methylparaben, Ethylparaben, and Propylparaben did not; Without hydrocortisone but with flutamide, Ethylparaben, Propylparaben, and Butylparaben increased GR activity by more than 50%, and Methylparaben by more than 20%	[75]
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	[76]
Methylparaben Ethylparaben Propylparaben Butylparaben Isobutylparaben	Human	MCF-7 human breast adenocarcinoma cells	Range of concentrations tested was not specified, ethanol vehicle	Cells prepared as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 5% (v/v) FCS, 10 mg/mL insulin, and 10-8 M E2 at 37°C; incubated with or without paraben or E2 for 7 or 14 days; cellular proliferation was measured using a Coulter counter EC_{100}, EC_{50}, LOEC, and lowest concentration which gave an increase in cell number statistically different ($P<0.05$) from the LOEC were reported	After 14 days of exposure, the EC_{50}s for cellular proliferation ranged from 0.4 - 40 µM, LOECs from 0.1 - 20 µM, and NOECs from 0.05 - 8 µM for the parabens; the parabens, in descending order of these values, were Isobutylparaben>Butylparaben> Propylparaben>Ethylparaben> Methylparaben; In comparison, corresponding values for E2 were $EC_{50}=2 \times 10^{-6}$ µM, LOEC=10^{-6} µM, and 1 x10^{-7} µM; A mixture of all 5 parabens, each at its 7-day NOEC, increased the number of cell doublings above that with any of the parabens tested individually, but lower than with E2	[77]
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	[78]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben	Human Mouse (FVB)	MCF-7 and MDA-MB-231 human breast adenocarcinoma cells HCI-7-Luc2 ER+ PDX human breast tumor cells; Normal cells from murine mammary glands of 8-week-old FVB mice	10 nM in ethanol (vehicle control, 0.1%)	Cells were grown in accordance with standard protocols; mammospheres were established, treated with 0.1% ethanol, 10 nM E2, 10 nM Methylparaben, 1 µM tamoxifen or 100 nM fulvestrant on days 4 and 7, and imaged on day 10	10 nM E2 exposure stimulated the proliferation of MCF-7 cells 7-fold after 1 week of exposure; 10 nM Methylparaben did not have this effect, and also failed to increase expression (mRNA) of p52 (TFF1) or progesterone receptor (canonical estrogen-responsive genes) MCF-7 mammospheres treated with Methylparaben exhibited increased expression of ALDH1 (marker of human mammary stem cells) and were larger than control and E2-treated mammospheres; HCI-7-Luc2 and normal murine mammospheres treated with 10 nM Methylparaben were also larger than controls; Methylparaben statistically-significantly increased NANOG, OCT4, and ALDH1 (all of which are stem cell markers) mRNA expression in both MCF-7 and HCI-7-Luc2 mammospheres; Methylparaben also upregulated NANOG protein expression in MCF-7 mammospheres; none of these effects were seen in MDA-MB-231 mammospheres; Neither tamoxifen nor fulvestrant inhibited effects of Methylparaben on MCF-7 mammospheres	[79]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	Mouse (strain not specified) Human	Murine 3T3-L1 fibroblasts Differentiated hADSCs	0, 1, 10, 100 µM in DMSO (vehicle)	Murine 3T3-L1 cells were grown in DMEM containing 10% calf serum at 37°C until they reached confluence; hADSCs were grown and differentiated according to the supplier's instructions; For the detection of early target genes, Butylparaben or DMSO was added to the media with or without dexamethasone or the differentiation cocktails (cortisone, methylisobutyxanthine, and insulin) For the studies of the antagonists of GR or PPARγ, cells were pretreated with the antagonists of PPARγ (GW9662 and BADGE) or GR (RU-486) or DMSO for 1 h before the cells were treated with Butylparaben or DMSO in the presence of the antagonist	Butylparaben in the presence of differentiation cocktail enhanced 3T3-L1 cell differentiation, as revealed by ORO-stained lipid accumulation, adipocyte morphologies and ORO absorbance; Parabens enhanced differentiation with potencies that increased with the length of the linear alkyl chain (Methylparaben < Ethylparaben < Propylparaben < Butylparaben), and the extension of the linear alkyl chain with an aromatic ring in Benzylparaben further augmented adipogenicity; 4-hydroxybenzoic acid or benzoic acid did not have these effects; In 3T3-L1 cells, the parabens also induced mRNA expression of adipocyte marker genes as well as adiponectin and leptin mRNA, in a concentration-related manner, and activated GR and/or PPARγ; no direct binding to, or modulation of, the ligand binding domain of GR was detected in competitor assays; 50 µM Butylparaben or Benzylparaben, in the presence of differentiation media promoted lipid accumulation in hADSCs as early as day 3 and throughout the differentiation process; on day 14, Benzylparaben showed the most potent adipogenic effects (upregulation of mRNA expression of adipocyte marker gene and lipid-filled adipocyte morphology); 1 µM Butylparaben had the strongest adipogenic effects of the parabens tested, whereas Ethylparaben, Propylparaben, and Benzylparaben had no effect at 1 or 10 µM)	[80]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
ANIMAL						
Oral						
Benzylparaben	Rat (Sprague-Dawley and Wistar)	Immature females, n=13 - 14/group	0, 0.0064, 0.032, 0.16, 0.8, 4, and 20 mg/kg bw/day by gavage, in peanut oil (vehicle)	Rats were exposed to Benzylparaben for 3 days, beginning on PND 21; on PND 24, the rats were weighed and killed, and uteri dissected and weighed	Relative uterine weights (ratios of uterine weights to final body weights) of Sprague-Dawley rats increased after treatment with ≥5 µg/kg bw/day E2, but Wistar rats given up to 100 µg/kg bw/day E2 showed no obvious effect; 400 µg/kg bw/day E2 increased relative uterine weight in Sprague-Dawley rats by 281% and in Wistar rats by 83%; Relative uterine weights were elevated in Sprague-Dawley rats after treatment with ≥0.16 mg/kg bw/day ($p<0.05$) in a dose-dependent manner; relative uterine weights increased by 3%, 7%, 19%, 24%, 27%, 31%, and 36% in the 0.0064, 0.032, 0.16, 0.8, 4, 20 and mg/kg bw/day groups, respectively The Wistar rats were not tested for sensitivity to Benzylparaben in this study	[81]
Methylparaben Ethylparaben	Rat (Sprague-Dawley)	Immature females (PND 20); n=6 - 9/ group (n=17 in one of the control groups)	0, 0.8, 4, and 20 mg/kg bw/day (20 mg/kg bw/day when tested with 10 mg/kg bw/day fulvestrant) in peanut oil, by gavage	Rats were exposed to a paraben for 3 days, beginning on PND 21; rats were then weighed and sacrificed, and uteri dissected and weighed, and relative uterine weights calculated, except for 1 group that was transferred on PND 23 to individual metabolic cages in which only pure water was available, ad libitum, and from which urine was collected for 24 h and analyzed for Methylparaben and Ethylparaben concentrations; Relative expressions of estrogen-responsive genes in the uteri were evaluated by quantitative real-time RT-PCR	LOELs for increased relative uterine weight after treatment with Methylparaben and Ethylparaben were 20 and 4 mg/kg bw/day, respectively; NOELs for Methylparaben and Ethylparaben were 4 and 0.8 mg/kg bw/day, respectively; The uterotrophic effects of 25 µg/kg bw/day E2 or 20 mg/kg be/day Methylparaben or Ethylparaben were antagonized by 10 mg/kg bw/day fulvestrant; Expression of icabp, itmap1, CaBP-9k, and/or Pgr biomarker genes were elevated in a concentration-dependent manner after treatment with 4 or 20 mg/kg bw/day Methylparaben or Ethylparaben; Mean urinary concentrations of the Methylparaben and Ethylparaben increased in a dose-dependent manner, from 491 to 17,635 ng/mL for Methylparaben and 376 to 11,906 ng/mL for Ethylparaben in rats that received 0.8 to 20 mg/kg/day Methylparaben or Ethylparaben	[82]

Table 13. Endocrine Activity

Test Substance(s)	Species/ Strain	Sample Type/Test Population-Sex	Concentration/ Dosage (Vehicle)	Procedure	Results	Reference
Ethylparaben Propylparaben	Mouse (C57BL/6J)	Ovariectomized females, 8 weeks of age, n=6/group, 11 groups	0, 1000 mg/kg bw/day in corn oil, by gavage	Study was performed in compliance with OECD TG 440 (Uterotrophic Bioassay in Rodents); mice were dosed daily for 7 consecutive days; 6 µg/kg bw/day E2 was given orally as the positive control in the test for agonism, and subcutaneously 15 min after administration of the test compound in the test for antagonism; 24 h after the last treatment, the animals were killed, and uteri were excised and weighed	Ethylparaben and Propylparaben were negative for estrogen agonism and antagonism	[83]
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.	[84]

Table 13. Endocrine Activity

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

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

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Prospective Studies						
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	185 pregnant women (18 to 45 years of age) recruited from Brooklyn's Prenatal Clinic and their singleton infants	Subjects recruited from 10/2007 to 12/2009	- Random "spot" urine specimens were provided once per participant during last 4 months of pregnancy - Convenience subset of the subjects were followed to delivery, when umbilical cord blood was collected - Maternal urinary concentrations were measured - Random subset of umbilical-blood plasma samples were analyzed for free and total parabens - Questionnaire was used to gather demographic - Neonate outcome data were from patient charts - Urinary biomarker concentrations were corrected for creatinine levels and were log-transformed - Non-detect values were treated as the MDL divided by the square root of 2 - Covariates were selected if they achieved $p < 0.05$ in Spearman correlations or Chi-square tests in relation to biomarker concentrations or birth outcomes - Measures of birth outcomes (body length, gestational age at birth, birth weight, and head circumference) were analyzed using linear models - Multiple linear regression analysis was used to evaluate concentration-outcome associations adjusted for maternal age, nativity, neonate gender, and alcohol and tobacco use; additional adjustments were made for confounders independently associated with outcomes or which changed the magnitude of effects by ≥ 5% -Relationships between concentrations and dichotomous outcomes were analyzed by logistic regression <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 <u>Low Birth Weight and Maternal Urine Concentrations</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <u>Low Birth Weight and Cord Blood Concentrations</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <u>Preterm Birth and Maternal Urine Concentrations</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben <u>Preterm Birth and Cord Blood Concentrations</u> Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	<u>**OR**</u> 0.83 (0.37-1.87) 1.18 (0.74-1.89) 0.92 (0.44-1.94) 1.45 (0.88-2.39) NA NA 1.89 (0.62-5.81) 1.52 (0.66-3.45) 10.27 (0.68-156.07 0.18 (0.01-2.63) 0.78 (0.40-1.54 1.15 (0.78-1.69) 1.27 (0.67-2.43) 1.42 (0.93-2.16) NA NA 2.65 (0.83-8.48) 1.86 (0.84-4.08) **60.77 (2.60-1417.93)** **0.03 (0.01-0.44)**	[88]

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Propylparaben Butylparaben	245 women who completed ≥1 IVF cycle and provided ≥1 urine sample/IVF cycle between November 2004 and April 2012 at the Massachusetts General Hospital (MGH) Fertility Center	Subjects recruited from 11/2004 to 4/2012	- Subjects provided up to two spot urine samples per IVF cycle; first collected between Day 3 and Day 9 of the gonadotrophin phase, second collected on day of oocyte retrieval - Urinary concentrations of total parabens were measured by HPLC-MS/MS - Clinical information was abstracted from the patient electronic medical records - Serum concentrations of FSH and E2 were measured - Each subject was assigned an infertility diagnosis by a physician - Subjects underwent one of three controlled ovarian stimulation IVF treatment protocols, after completing a cycle of oral contraceptives - Embryologists determined the total number of oocytes retrieved per cycle and classified them - Oocytes underwent either conventional IVF or ICSI, and embryologists determined fertilization rate 17-20 h after insemination - Embryo quality was classified based on morphology and number of blastomeres, ranging from 1 (best) to 5 (worst) on day 2 and 3 - In women who underwent an embryo transfer, implantation was assessed and pregnancy was confirmed by ultrasound at 6 weeks - Live birth was defined as birth of a neonate on or after 24 weeks gestation - Exposures were categorized into quartiles of urinary concentrations; the lowest quartile used as the reference group - Associations between urinary concentrations and demographics and baseline reproductive characteristics were evaluated using Kruskal-Wallis and Chi-squared tests - Multivariable generalized linear mixed models were used to evaluate associations between concentrations and IVF outcomes - Poisson distributions and log link functions were specified for oocyte counts, and a binomial distributions and logit link functions for embryo quality, fertilization rates, and clinical outcomes (implantation, clinical pregnancy and live birth) - Potential confounders considered include factors previously related to IVF outcomes in this or other studies and factors associated with paraben exposure and IVF outcomes in this study - Final models were adjusted for age, BMI, race (white vs nonwhite), smoking status (never vs ever), and infertility diagnosis (male factor, female factor, unexplained) <u>Limitations</u> - Study design may not allow extrapolation of the findings to the general population - Misclassification of paraben exposure based on concentrations from spot urine samples is possible	Urinary paraben concentrations were not associated with IVF outcomes; Geometric means of urinary concentrations of Methylparaben, Propylparaben, and Butylparaben were 133, 24 and 1.5 μg/L, respectively; The urinary concentrations were not associated with total or mature oocyte counts, proportion of high embryo quality, fertilization rates, implantation rats, clinical pregnancy, or live births	None of the ORs calculated for total oocyte yield, metaphase II oocyte yield, >1 best embryo quality, and fertilization rate in the 2^{nd}, 3^{rd}, and 4^{th} quartiles of Methylparaben, Propylparaben, and Butylparaben urinary concentrations were statistically-significantly different from those of the 1^{st} quartile, adjusted or unadjusted	89

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
Methylparaben Ethylparaben Propylparaben Butylparaben	520 mother-son pairs with complete data on prenatal (3 ultrasound measurement), neonatal (biometry), and postnatal growth up to 3 years of age (≥4 weight/height measurements and clinical exam), recruited before the end of gestation week 28 from Poitiers and Nancy University hospitals (France)	Subjects recruited from 4/2003 to 3/2006	- Biparietal diameter was measured by ultrasound during gestation weeks 12.6, 22, and 32.6 (on average) - Fetal head circumference, abdominal circumference, and femur length were assessed during the last 2 ultrasound examinations - Fetal weights were estimated from measures of abdominal circumferences, femur lengths, head circumferences, and biparietal diameter - Weight and length at birth were extracted from hospital records - Infants were weighed and measured at 1 and 3 years of age - Mothers were mailed questionnaires at 4, 8, 12, 24, and 36 months about the boys' weight and height measures - Jenss nonlinear model was used to evaluate growth and predict weight and height at 6, 12, 24, and 36 months - Head circumference was assessed within 4 days after birth and at 3 years - Abdominal circumference was measured at 3 years - Urine samples were collected between gestation weeks 22 and 29 - Total paraben concentration was calculated by summing molar concentrations of the 4 parabens - Non-detects were replaced by the lowest instrumental reading value divided by the square root of 2 - Concentrations were standardized for collection conditions, including creatinine concentrations - Cross-sectional analyses and linear regression models with a random effect variable corresponding to the mother-son pair were used to study associations between concentrations and growth parameters - Models for prenatal and postnatal growth were adjusted for maternal and paternal height, pre-pregnancy weight, maternal active and passive smoking during pregnancy, maternal education, recruitment center, and parity - Model for head circumference was also adjusted for number of days between birth and assessment of head circumference - Analyses of postnatal growth were additionally adjusted for breastfeeding duration - Effect estimates were reported for an increase by 1 IQR of ln-transformed standardized concentrations <u>Limitations:</u> - Use of only 1 urine sample to assess paraben concentrations increases the chances of exposure misclassification - Use of estimates of caloric intake (rather than specific food usually eaten) increases the chance of confounding by differences in eating behavior.	No statistically-significant associations were found between maternal urinary paraben concentrations during pregnancy and prenatal or postnatal growth of male newborns. However, maternal urinary concentrations during pregnancy appeared to be positively associated with body weights: <u>Body Weight at Birth</u> **<u>β Coefficient</u>** Methylparaben 36.0 (-12.4-84.4) Ethylparaben 49.9 (-2.21-102) Propylparaben 48.0 (-3.64-99.6) Butylparaben 50.1 (-5.69-106) <u>Body Weight at 6 Months</u> Methylparaben 85.3 (-16.5-187) Ethylparaben 17.8 (-92.9-129) Propylparaben 80.1 (-27.4-188) Butylparaben 55.8 (-62.0-174) <u>Body Weight at 12 Months</u> Methylparaben 81.2 (-45.4-208) Ethylparaben 2.60 (-135-140) Propylparaben 79.1 (-54.9-213) Butylparaben 54.5 (-91.1-200) <u>Body Weight at 24 Months</u> Methylparaben 128 (-31.88-287) Ethylparaben 45.3 (-128-219) Propylparaben 116 (-53.3-285) Butylparaben 111 (-71.2-294) <u>Body Weight at 36 Months</u> Methylparaben 193 (-3.88-389) Ethylparaben 113 (-101-327) Propylparaben 159 (-49.4-368) Butylparaben 179 (-45.3-404)		90

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
				β coefficients calculated for Ethylparaben and Butylparaben, body weights estimated at the 3rd ultrasound examination, were 13.00 (-13.1-39.1) and 23.5 (-3.96-50.9), respectively; coefficients for all other parameters were < 7.5 with CIs spanning across negative and positive values		
				REROSPECTIVE STUDIES		
Methylparaben Ethylparaben Propylparaben Butylparaben	28 boys diagnosed with cryptorchidism and/or hypospadias at San Cecilio University Hospital of Granada: 19 cryptorchidism cases (n=9 unilateral, 6 bilateral), 12 hypospadias cases, 1 case with both disorders; 51 matched controls	Subjects recruited from 10/2000 to 7/2002	- This was a case-control study nested within a prospective birth cohort study of risk factors for male urogenital malformations - All boys in the cohort were examined at birth and those diagnosed with cryptorchidism and/or hypospadias were re-examined at 1month of age -Information on potential confounding variables related to parents, pregnancy/delivery and activities were gathered from structured interviews with the mother within 48 h after delivery - There was a larger proportion of mothers reporting historical (pre-pregnancy) use of oral contraceptives in the selected versus non-selected cases (21% vs. 53%, p=0.034), although not in the selected versus non-selected controls (37% vs.42%, p=0.686) - Placentas were collected immediately after delivery and analyzed by UPLC–MS/MS - Crude and adjusted ORs and corresponding 95% CIs were calculated by conditional logistic regression - Concentrations of parabens were used as independent variables and analyzed both as continuous variables and in tertiles, with the first tertile as the reference group - Concentrations below the LOQ were assigned a value of half of the LOQ - Potential confounding variables were selected if they were statistically-significantly associated with outcomes in bivariate analyses or changed the β coefficient by >20% in the multivariable analysis - Only maternal age and newborn birthweight had a substantial effect on results - In the bivariate analyses, differences between groups were tested with Pearson's chi-square test or Fisher's exact test, when appropriate Limitations - Relatively small sample size prevented adjustment for some potential confounders, such as the type of delivery, fetal presentation, weeks of gestation, child length, head size, presence of other malformations and season of birth - Exposure assessment made in term placentas may have resulted in exposure misclassification - Cryptorchidism and hypospadias grouped together for statistical analysis discounts the fact that these conditions are related to inset	Methylparaben <0.4 ng/g 0.44-1.91 ng/g 1.96-11.69 ng/g Concentration as continuous variable Ethylparaben <LOD 0.07-0.89 ng/g 0.91-5.49 ng/g Concentration as continuous variable Propylparaben <LOD 0.06-1.15 ng/g 1.16-5.52 ng/g Concentration as continuous variable Butylparaben <0.08 ng/g 0.16-0.74 ng/g 0.79-1.60 ng/g Concentration as continuous variable Methylparaben <0.4 ng/g 0.44-1.91 ng/g 1.96-11.69 ng/g Concentration as continuous variable Ethylparaben <LOD 0.07-0.89 ng/g	**OR (unadjusted)** 1.00 1.00 (0.32-3.09) **3.18 (0.88-11.48)** 1.17 (0.94-1.46) 1.00 0.29 (0.08=1.06) 1.51 (0.44-5.15) 1.07 (0.74-1.55) 1.00 1.23 (0.30-5.04) **4.72 (1.08-20.65)** **1.90 (1.12-3.22)** 1.00 2.29 (0.65-8.05) 2.31 (0.72-7.46) 2.27 (0.8-6.42) **OR (adjusted)** 1.00 1.04 (0.33-3.26) 3.24 (0.83-12.69) 1.17 (0.93-1.48) 1.00 0.26 (0.07-1.00)	91

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			mechanisms occurring at different critical stages in gestation	0.91-5.49 ng/g	1.25 (0.34-4.60)	
				Concentration as continuous variable	1.00 (0.68-1.47)	
				Propylparaben		
				<LOD	1.00	
				0.06-1.15 ng/g	1.39 (0.33-5.91)	
				1.16-5.52 ng/g	6.42 (1.16-35.47)	
				Concentration as continuous variable	2.16 (1.16-4.01)	
				Butylparaben		
				<0.08 ng/g	1.00	
				0.16-0.74 ng/g	2.26 (0.62-8.21)	
				0.79-1.60 ng/g	2.11 (0.62-7.16)	
				Concentration as continuous variable	2.07 (0.71-6.06)	
Methylparaben Ethylparaben Propylparaben Butylparaben Benzylparaben	436 3-year old children recruited from Sheyang Maternal and Child Health Care Centre (China)	Subjects recruited between 7/2012 and 4/2013	- Questionnaire survey was administered to each child's caregiver by trained interviewers, covering sociodemographics, living environment and lifestyles - Pregnancy and maternal health information was obtained from medical records and questionnaires - Spot urine sample was collected from each child, and urinary paraben concentrations were measured by LVI-GC-MS/MS - EDI_{urine} of parabens was calculated based on urinary concentrations and a steady-state toxicokinetic model - Anthropometry measurements were compared with sex-specific WHO child growth standards, and age- and sex-standardized z scores were calculated - Generalized linear models were used to examine associations between SG-adjusted concentrations and body growth outcomes - Individual paraben concentrations and the $P_{parabens}$ were adjusted for SG - Analyses of quartiles of $P_{parabens}$ were conducted separately - Urinary concentrations were log transformed for univariate and multivariate analyses - Associations between concentrations and sociodemographic	Weight z Score (Boys)	**β Coefficient**	92
				Methylparaben	0.08 (-0.06-0.23)	
				Ethylparaben	**0.16 (0.03-0.28)**	
				Propylparaben	0.00 (-0.16-0.17)	
				Butylparaben	0.12 (-0.09-0.32)	
				Benzylparaben	-0.04 (-0.18-0.10)	
				∑Parabens	0.17 (-0.04-0.39)	
				Height z Score (Boys)		
				Methylparaben	0.11 (-0.02-0.26)	
				Ethylparaben	**0.15 (0.03-0.27)**	
				Propylparaben	0.05 (-0.11-0.21)	
				Butylparaben	0.14 (-0.06-0.34)	
				Benzylparaben	0.08 (-0.06-0.21	
				∑Parabens	**0.23 (0.03-0.43)**	

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			characteristics were examined using a Wilcoxon rank-sum or Kruskal-Wallis rank sum test - Log-transformed concentrations were assessed using Pearson correlation coefficients - Concentrations below LOD were substituted with LOD divided by the square root of two - Covariates considered included: maternal and paternal BMI, child's sex, maternal education, family income, habitation in town, suburb or countryside, feeding pattern, smoking status, time spent outdoors, sampling season, and birth outcome - Potential confounders that were separately include: urinary bisphenol A, triclosan, and benzophenone-3 concentrations Limitations: - Spot urine samples may cause exposure misclassification - Specific diet information was not sufficiently obtained and evaluated	All β coefficients calculated for girls and all other β coefficients for boys were not statistically significant		
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 subsample of U.S. NHANES participants n=185 adolescent males (ages 12 to 19) males, 171 adolescent females, 785 adult (ages ≥20) males, and 708 adult females	2007-2008	- Stratified multistage probability sample of civilian U.S. population was surveyed via household interviews, physical exams, and collection of medical histories and biologic specimens. - Urinary parabens concentrations were measured - Spot urine samples were analyzed by HPLC-MS/MS - LOD values were estimated as 3 x standard deviation as concentrations approached zero - Serum thyroid measures included free and total T3 and T4, thyroglobulin, and TSH (or thyrotropin) - Potential confounders considered: age, sex, BMI, urinary creatinine levels, race/ethnicity, poverty income ratio , education, serum cotinine levels and alcohol intake - Variables used as the basis for creation of sample weights, including race/ethnicity, PIR, and education, were not included in final models to avoid over-adjustment - Following ln-transformation of the remaining variables with log-normal distributions, Pearson correlations, one-way ANOVA, and t-tests were used to evaluate potential confounders - Covariates were adjusted for in the final models if there were statistically-significantly associated with one exposure or outcome variable based on a priori evidence or the analysis, and if they altered parameter estimates of the main effects by more than 10% - Final regression models included age, sex, BMI, and urinary creatinine - Concentrations of urinary parabens below the LOD were replaced with values equal to the LOD divided by the square root of two. - Parabens were analyzed on a creatinine-adjusted basis for univariate and bivariate analyses; unadjusted urinary concentrations were used in regression models with urinary	Adults, Total T4 (µg/dL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, ln-Free T3 (pg/mL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, ln-Free T4 (ng/mL) Methylparaben Ethylparaben Propylparaben Butylparaben Adult Females, T4 (µg/dL) Methylparaben Ethylparaben Propylparaben Butylparaben	β Coefficient -0.04 (-0.12-0.03) ***-0.5 (-0.10 - -0.002)*** -0.19 (-0.46-0.07) ***-0.20 (-0.36 - -0.03)*** 0.005 (-0.01-0.000) ***-0.006 (-0.001- -0.0001)*** ***-0.02 (-0.04- -0.002)*** ***-0.02 (-0.03- -0.002)*** ***-0.01 (-003- -0.000)*** ***-0.01 (-0.02- -0.003)*** -0.02 (-0.05-0.01) ***-0.04 (-0.07- -0.004)*** -0.09 (-0.26-0.08) -0.08 (-0.20-0.05) -0.30 (-0.65-0.06) ***-0.36 (-0.57- -0.16)***	[93]

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			creatinine included as a covariate - Final multivariate linear regression models included serum thyroid concentrations (continuous variable) as the dependent variable and an individual urinary Methylparaben and Propylparaben concentration (continuous) as a predictor, along with age (continuous), sex (dichotomous), BMI (continuous), and ln-transformed urinary creatinine (continuous) Limitations: - Causality cannot be established because NHANES is an observational, cross-sectional study - Exposures were evaluated based on spot urine measurements; - Spot urine samples served as the basis for estimating exposures, so time of sample collection could be a source of intra-individual variability and the concentrations may not accurately represent average body burdens	All other β coefficients calculated were not statistically significant		
Methylparaben Propylparaben Butylparaben	Female participants of a prospective fertility study at the MGH Fertility Center, undergoing infertility evaluation, n=109 to 142, depending parameter measured	2004-2010	- Subjects had at least one hormonal or ultrasonographic marker of ovarian reserve measured and contributed at least one urine sample - Clinical information was abstracted from medical records - Intravenous blood sample was drawn on the 3rd day of the menstrual cycle, and the serum was analyzed for FSH - AFC and OV were measured for both ovaries using transvaginal ultrasound - Each patient was given an infertility exam and diagnosis by a physician at the MGH Fertility Center - Demographic data were collected using a nurse-administered questionnaire at entry into the study - Convenience spot urine sample was collected at recruitment and at subsequent visits during infertility treatment cycles - Paraben concentrations were measured by HPLC-MS/MS - Distribution of exposures was summarized using the median, IQR, and range of urinary paraben concentrations - Urinary concentrations below LOD were assigned a value equal to the LOD divided by the square root of two - Concentrations were corrected for SG - Spearman's rank correlation coefficients (r_S) were calculated for markers of ovarian reserve, age, and BMI - Multivariable linear regression was used to estimate associations between within-person paraben concentrations (divided into tertiles) and day-3 FSH and OV; OV was ln-transformed before all	Methylparaben Tertile 1 (5.13-132 µg/L) Tertile 2 (145-377 µg/L) Tertile 3 (381-2,428 µg/L) p_{trend} =0.31 Propylparaben Tertile 1 (<LOD-25.2 µg/L) Tertile 2 (26.3-81.8 µg/L) Tertile 3 (87.8-727 µg/L) p_{trend} =0.07 Butylparaben Tertile 1 (<LOD-0.73 µg/L) Tertile 2 (0.75-5.12 µg/L) Tertile 3 (5.44-177 µg/L) p_{trend} =0.86 All MPCs and p_{trens} calculated for AFC and OV were not statistically significant	**MPC in AFC** 0 (Reference) -6.8 (-23.5-13.7) -10.6 (-28.2-11.2) 0 (Reference) -5.0(-23.7-18.4) -16.3 (-30.8-1.3) 0 (Reference) -4.8 (-22.5-16.8) -2.0 (-21.0-21.6)	94

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			regression analyses - Poisson regression was used to estimate associations between within-person paraben concentrations (tertiles) and AFC - Covariates considered included age at time of outcome and BMI determinations at study entry into the study - MPC in outcome from the lowest tertile of paraben concentrations was calculated for both OV and AFC - Secondary analysis combined concentrations of parabens using two methods: an EEQ factor approach, and summation of concentrations - Multivariable linear regression was used to evaluate association between EEQ (parabens) and Σ(parabens) with day-3 FSH and OV Limitations: - Time period of collection of the urine samples was up to 3 years before the outcome measure - Relatively small sample size - Not all subjects had all three of the outcome measures - Inclusion of high proportion of Caucasian and older women and sole inclusion of women from a fertility clinic undergoing in vitro fertilization or intrauterine insemination (all with varied SART diagnoses) may limit generalizability of findings			
Methylparaben Ethylparaben Propylparaben Butylparaben	Randomly selected 1/3 sub-sample of the U.S. NHANES participants ≥6 years of age, n=860 (450 males, 410 females)	2005-2006	- Sociodemographic data, urinary paraben levels, total and specific IgE levels, respiratory disease and medical condition questionnaire data were included in the dataset - Urinary parabens levels were collected - Subject answered the following questions: Has a doctor or other health professional ever told you that you have asthma? In the past 12 months, have you had wheezing or whistling in your chest? - Atopic asthma was defined as having doctor-diagnosed asthma in addition to at least 1 positive aeroallergen-specific IgE level - Nonatopic asthma was defined as having doctor-diagnosed asthma with negative specific IgE test results - Atopic wheeze was defined as having a history of wheezing in the past 12 months in addition to at least 1 positive aeroallergen-specific IgE level - Nonatopic wheeze was defined as having a history of wheezing in the past 12 months with negative specific IgE test results - Parabens were measured in urine samples by HPLC-MS/MS - Serum total IgE levels and aeroallergen-specific IgE levels were measured, including IgE specific for cat, dog, mouse, rat, Dermatophagoides, cockroach, ragweed, thistle, rye, Bermuda, oak, birch, *Alternaria* species, and *Aspergillus* species - Food-specific IgE levels measured were for milk, egg, peanut, and shrimp - Subjects were considered to have aeroallergen or food	Aeroallergen and Food Sensitization (males and females) Methylparaben Tertile 1 Tertile 2 Tertile 3 P_{trend}=0.4 Propylparaben Tertile 1 Tertile 2 Tertile 3 **P_{trend}=0.04** Propylparaben Tertile 1 Tertile 2 Tertile 3 P_{trend}=0.2 Butylparaben	 **OR (unadjusted)** 1.0 (Reference) 1.11 (0.82-1.47) **1.74 (1.02-3.11)** 1 (Reference) 1.35 (1.00-1.82) 1.74 (0.98-3.08) **OR (adjusted)** 1.0 (Reference) **1.51 (1.15-1.99)** **2.04 (1.12-3.74)**	95

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			sensitization if the specific IgE level was ≥0.35 kU/L - Urinary paraben concentrations were divided into tertiles or dichotomized when 50% or fewer of the subjects had detectable levels (as was the case for Butylparaben) - Linear regression was used to determine whether mean urinary concentrations varied by race/ethnicity. - Logistic and linear regression were used to determine associations between paraben concentrations and food and aeroallergen sensitization, atopic and nonatopic asthma and wheeze, and total IgE levels - Test for trend was performed by using the variable for tertiles of the paraben concentrations - Multivariate models were adjusted for age, sex, race/ethnicity, urinary creatinine level, and PIR Limitations: -Data are drawn from a cross-sectional study, which introduces the possibility of reverse causation (i.e., subjects with allergy might use more products containing parabens} - Use of allergen sensitization as an outcome was limited by lack of clinical correlation of allergic disease - Urinary paraben levels were used as biomarkers of exposure, which might not reflect actual exposure	Tertile 1 Tertile 2 p_{trend}=0.9 Nonatopic Asthma (males and females) Methylparaben Tertile 1 Tertile 2 Tertile 3 **p_{trend}=0.04** Nonatopic Wheeze (males and females) Methylparaben Tertile 1 Tertile 2 Tertile 3 p_{trend}=0.47 In addition, the OR and p_{trend} calculated for Propylparaben concentrations and aeroallergen and food sensitization in males were statistically significant The ORs and p_{trend}s calculated for all other comparisons were not statistically significant	1 (Reference) **1.55 (1.02-2.33)** **OR (adjusted)** 1.0 (Reference) 0.43 (0.47-3.73) **0.25 (0.07-0.90)** 1 0.51 (0.18-1.46) **0.23 (0.05-0.99)**	
Methylparaben Propylparaben Butylparaben	194 male partners (18 to 55 years old; mean = 36.7 years of age) of subfertile couples seeking treatment from the Vincent Memorial Obstetrics and Gynecology Service, Andrology Laboratory, Massachusetts General Hospital (MGH)	2000-2004	- A single spot urine sample was collected on day of each subject's clinic visit; 2nd and 3rd samples were collected from a subset of men at subsequent visits - Concentrations of total (free + conjugated) parabens were measured in urine samples by HPLC-MS/MS, - One nonfasting blood sample was drawn on the same day and time as the first urine sample - Serum testosterone, E2, sex-hormone-binding globulin, inhibin B, FSH, LH, prolactin, free thyroxine (T4), total triiodothyronine	Comet Tail % Butylparaben <0.2 µg/L 0.2-0.6 µ/L >0.6 µg/L **P_{trend}=0.03**	**β Coefficient (adjusted)** 0 6.81 (-1.80-15.4) 8.23 (-0.41-16.9)	96

Table 14. Epidemiological studies of parabens.

Ingredient(s)	Population/ Geographical Area	Study/ Diagnosis Years	Methods and Limitations	Findings	OR, β, or MPC (95% C.I.)*	Reference
			(T3), and TSH were measured - Free androgen index (FAI), testosterone:LH ratio, FSH:inhibin B and E2:testosterone ratios were calculated - Semen quality parameters and motion characteristics were measured: sperm concentration, motility, and motion parameters - Total sperm count was calculated and sperm morphology was assessed - Sperm damage was assessed by comet assay: comet extent, tail distributed moment (TDM), and percent DNA located in the tail (Tail%) were determined - Multivariable linear regression was used to explore relationships between urinary paraben concentrations and hormone levels, semen quality parameters, and sperm DNA damage measures - Distribution of sperm count, sperm concentration, FSH, LH, SHBG, prolactin, TSH, all calculated hormone ratios, and paraben concentrations were ln-transformed for statistical analyses - Paraben concentrations < LOD were assigned values of LOD/2 - Inclusion of covariates in the multivariable models was based on statistical and biologic considerations - Age and BMI were modeled as continuous variables; abstinence period was treated as an ordinal categorical variable - Race, smoking status, and timing of the clinic visit by season and time of day were considered for inclusion as dichotomous variables - Covariates with $p < 0.2$ in their relationship with one or more paraben or ≥ 1 outcome measure in preliminary bivariate analyses were included in a "full" model - Covariates with $p > 0.15$ in full models for all measures within the three sets of outcomes (hormone levels, semen quality, sperm DNA damage) were removed from the final models <u>Limitations</u>: - Urine samples were collected weeks or months after, rather than before, serum and semen samples were collected - Only a single blood or semen sample was available for assessment of hormone levels, semen quality, and sperm DNA damage - Cross-sectional design restricts the ability to draw conclusions about causal relationships - Relatively small sample size provided low statistical power	No other comparisons were statistically significant in this study		

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

AFC=Anthral follicle count; ANOVA=Analysis of variance procedures; BMI=Body mass index; CI=Confidence interval; E2=Estradiol; EDI=Estimated daily intake; EEQ=Estrogen equivalency; FSH=Follicle stimulating hormone; HPLC-MS/MS=High-performance liquid chromatography-mass spectrometry/mass spectrometry; ICSI=Intracytoplasmic sperm injection; IQR=Interquartile range; IVF=In vitro fertilization; LOD=Limit of detection; LOQ=Limit of quantification; LVI-GC-MS/MS=Large volume-injection gas chromatography with tandem mass spectrometry; MDL=Method detection limit; MGH=Massachusetts General Hospital; MPC=Mean percent change; NA=Not applicable; NHANES=National Health and Nutrition Examination Survey; OR=Odds ratio; OV=Ovarian volume; $P_{parabens}$=Sum molar concentrations of the parabens; PIR=Poverty income ratio; PTB=Preterm birth; SART= Society for Assisted Reproductive Technology; SG=Specific gravity; UPLC-MS/MS=Ultra-high-performance liquid chromatography-tandem mass spectrometry; WHO=World Health Organization

Table 15. Margins of safety for parabens in cosmetics as a function of exposed population and single versus multiple paraben usage.[6]

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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3

Final Report on the Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben

The Parabens are esters of *p*-hydroxybenzoic acid (PHBA) and are the most commonly used as preservatives in cosmetic formulations. Data obtained from chronic administration studies indicate that Parabens are rapidly absorbed, metabolized, and excreted.

Acute chronic and subchronic toxicity studies in animals indicate that Parabens are practically nontoxic by various routes of administration. Methylparaben and Ethylparaben at 100 percent concentration were slightly irritating when instilled into the eyes of rabbits.

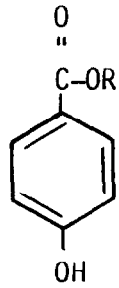
Numerous in vitro mutagenicity studies indicate that the Parabens are nonmutagenic. Methylparaben was noncarcinogenic when injected in rodents or when administered intravaginally in rats. Cocarcinogenesis studies on Propyl- and Methylparaben were negative. Teratogenic studies on Methyl- and Ethylparaben were also negative.

Parabens are practically nonirritating and nonsensitizing in the human population with normal skin. Paraben sensitization has been reported when Paraben-containing medicaments have been applied to damaged or broken skin. Photo-contact sensitization and phototoxicity tests on product formations of Methyl-, Propyl-, and/or Butylparaben gave no evidence of significant photoreactivity.

It is concluded that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben are safe as cosmetic ingredients in the present practices of use.

INTRODUCTION

This report on the Parabens summarizes much of the available data published between 1920 and 1982 and all of the unpublished data submitted to the Cosmetic Ingredient Review. The following references are review articles that contain supplemental information on these ingredients (especially regarding very early studies).[1-8]



CHEMISTRY

Structure and Preparation

The Parabens are esters of *p*-hydroxybenzoic acid (PHBA) and conform to the following structure[9]:

where R = methyl (CH_3), ethyl (C_2H_5),
propyl (C_3H_7), butyl (C_4H_9).

The following nonspecific trade names apply to these ingredients[9]:

Betacides (Beta)
Aseptoforms (Greeff)
Parasepts (Tenneco)
Nipagins (Nipa)
Protabens (Protameen)
Tegosepts (Inolex)

The Parabens are prepared by esterifying PHBA with the corresponding alcohol in the presence of an acid catalyst, such as sulfuric acid, and an excess of the specific alcohol. The acid is then neutralized with caustic soda, and the product is crystallized by cooling, centrifuged, washed, dried under vacuum, milled, and blended.[4]

Properties

The Parabens form small colorless crystals or white crystalline powders with practically no odor or taste. Parabens are soluble in alcohol, ether, glycerine, 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.[4,10-16] Table 1 summarizes other physicochemical properties of the Parabens.

Analytical Methods

The literature contains many references pertaining to the determination of Paraben preservatives in foods, cosmetics, and pharmaceuticals. Chromatography, especially high-pressure liquid chromatography, is used presently for many of these determinations. The Parabens may be determined directly, or they may

TABLE 1. Physicochemical Properties of the Parabens.

Property	*Methyl*	*Ethyl*	*Propyl*	*Butyl*	*Reference*
Molecular weight	152.16	166.18	180.21	194.23	11,22
Melting point (°C)	131	116–18	96.2–98	68–69	11,23
	125–128	115–118	95–98	68–72	13–16
Boiling point (°C)	270–280	297–298	—	—	11
Density	—	—	1.0630	—	11
Refractive index	1.5250	1.5050	1.5050	—	11,24
$\lambda max^{(*)}$ in H_2O	—	256 (1.5×10^{-2})	256 (1.5×10^{-2})	256 (1.55×10^{-2})	25
pKa	8.17	8.22	8.35	8.37	26
Inorganic impurities*					
As	1 ppm	—	1 ppm	1 ppm	27
Pb	10 ppm	—	10 ppm	10 ppm	27
Ash	0.1%	0.1%	0.1%	0.1%	13–16
Residue on ignition*					
(%)	0.05	0.05	0.05	0.05	13–16
Loss on drying* (%)	0.5	0.5	0.5	0.5	13–16
Acidity* (mEq/750 mg)	0.02	0.02	0.02	0.02	13–16
Solubility†					
Alcohol	vs	vs	s	s	11,23
Water	sl	sl	i	i	11,23
Ether	vs	vs	s	s	11,23,28
Acetone	vs	s	s	s	11,23,28
Benzene	sl	—	—	—	27
Carbon tetrachloride	sl	—	—	—	27
Glycerin	sl	sl	—	sl	29,30

*Maximum recommended; no information available on organic impurities.
†vs = very soluble; s = soluble; sl = slightly soluble; i = insoluble.

be chemically modified and the derivative subsequently identified. Table 2 lists reported analytical methods for Paraben determination.

Reactivity/Stability

The Parabens are stable in air and are resistant to hydrolysis in hot and cold water, as well as in acidic solutions. Resistance to hydrolysis increases as the size of alkyl sidechain increases. Above pH 7, appreciable hydrolysis occurs, producing PHBA and the corresponding alcohol. In strongly alkaline solutions, Parabens hydrolyze to the corresponding carboxylic acid, which then becomes ionized. The rate of hydrolysis is pH-dependent. Parabens are resistant to hydrolysis under usual conditons of sterilization (autoclaving) and also resist saponification.[2,3,8,17,18]

Ishizaki et al.[19] reported a reaction of 1 percent Butylparaben with potassium nitrate or sodium nitrite. The reactants were mixed constantly and irradiated with UV light for 5 days under a high voltage mercury lamp. Butyl 3-nitro-4-hydroxybenzoate was isolated as a reaction product.

Potential Interaction with Other Cosmetic Ingredients

Parabens interact with a number of cosmetic ingredients, including gelatin, sodium lauryl sulfate, polysorbates, polyethylene glycols (PEGs), cellulose esters, and polyvinylpyrrolidone (PVP). Bolle and Mirimanoff[20] reported that 2 percent

TABLE 2. Analytical Methods for Paraben Determination.

Method	*Reference*	*Method*	*Reference*
Thin-layer chromatography (TLC)/ ultraviolet spectroscopy (UV spec)	25,31–34	High-pressure liquid chromatography	43–62
Gas chromatography (GC) w/flame ionization	35,36	Reversed phase TLC/UV spec	63
Densitometry/TLC/UV spec	37,38	Saponification/bromometric titration	64,65
UV SPEC	39	Microrefractive index determination	24
Gel electrophoresis	40	Isotachophoresis	66
Etherification	41	Saponification	67
Saponification/TLC	42	Partition chromatography/UV spec	68
Ion exchange chromatography	43,44	Partition chromatography/GC	69
Fluorescence	45	Nuclear magnetic resonance (NMR) spectrometry	70
TLC	46–52	Fractional sublimation/ polarimetry	94
Microbiological Assay (*Candida albicans*)	71	Sublimation/UV spec	95
Colorimetric test	72–74	Microdetermination of refractive index	96
Column chromatography/GLC	75,76	Mass spectroscopy	97
Column chromatography/UV spec	77	TLC/paper chromatography	98
Trimethyl silyl ether conversion/GC	78	Spectrophotometric assay	99
High-speed gel permeation chromatography	79	Polyamide TLC	100–102
Extraction/TLC/colorimetric test	80,81		
Paper chromatography/UV spec	82–84		
Paper electrophoresis	85		
GC	86–91		
Liquid chromatography	92–93		

Tween 81, Tween 60, and Arlacel 83 interfered with the preservative properties of 0.1 percent Methylparaben. De Navarre[21] observed that 1 percent Tween (2, 4, 6, or 8) improved the preservative effect of 0.1 percent Methylparaben, whereas 2 percent Tween inhibited the effect of 0.2 percent Methylparaben. At 2 percent, an oleyl alcohol ethylene oxide adduct (Emulphor OW-870) also interfered with 0.2 percent Paraben. Ishizaki et al.[19] reported that 0.7 percent Tween 80 inactivated Butylparaben.

Most nonionic surfactants that are based on the addition of ethylene or propylene oxide to fatty acids, alcohols, esters, or polyglycols interfere with the preservative properties of the Parabens. The interference appears to be due to the formation of complexes through hydrogen bonding. The addition of anionics or quaternary compounds to products may prevent Paraben inactivation by nonionics.[103]

The interaction of fatty acid esters of sucrose and Parabens was studied by Valdez et al.[104] The authors suggested that the Paraben molecules may become incorporated within surfactant micelles and associate, through a combination of hydrogen and hydrophobic bonding, to form a stable Paraben–sucrose ester complex. The formation of such a complex would result in a loss of Paraben preservative activity. Hydrophobic bonding was indicated when it was observed that Methylparaben complexed to a greater degree than Propylparaben. According to Rosen and Berke,[105] if a 5 percent nonionic surfactant is added to

Paraben-containing water–oil emulsion, as much as 75 percent of the total preservative will migrate to the nonionic surfactant micelle, leaving only 25 percent of the concentration to distribute between the oil and water phases of the emulsion.

Goto and Endo[106] studied the hydrogen bonding of the Parabens to sodium lauryl sulfate (SLS) micelles. They suggested that the sulfuric group of SLS hydrogen bonds with the hydroxyl group of the Paraben resulting in short penetration of the Paraben molecule into the palisade layer of the micelle.

Parabens are bound by various macromolecules (such as methylcellulose and gelatin), nonionic emulsifiers (especially those which contain PEG groups), and proteins.[105]

USE

Cosmetic

The Parabens are the most commonly used preservatives in cosmetics. Found in all types of formulations, they have a total use in over 13,200 formulations.[107] The Parabens formulate well because they have no perceptible odor or taste, they are practically neutral, they do not have discoloration, and they do not cause hardening or muddying.[1]

As the carbon number of the alkyl chain increases, antimicrobial activity increases, but water solubility decreases and oil solubility increases. Since microbial replication generally occurs in the water phase of oil/water bases, the amount of Paraben dissolved in the water phase generally determines the preservative efficiency.[108] Various concentrations of Methylparaben and Propylparaben can be added to the base's water and oil phases, respectively, taking advantage of each Paraben's solubility characteristics.[109]

Propylparaben is a stable, nonvolatile preservative that is active at low concentrations and used to prevent decay of gum binders in cosmetic creams, lotions, and powders. Mixtures of Parabens may be used in dentifrices, since they apparently are absorbed by the oral mucosa and have a prolonged antiseptic effect. Parabens are also used to preserve proteins in nail creams, stabilize hydrogen peroxide in bleaches, prevent discoloration and deterioration in soaps, and prevent rancidity of fat and vegetable oils.[1,109]

According to the industry's voluntary submissions to the FDA in 1981 (Table 3), the number of product formulations and maximum use concentrations for the individual Parabens are as follows: Methylparaben (6606 uses), 25 percent; Propylparaben (5868 uses), 25 percent; Butylparaben (693 uses), 5 percent; and Ethylparaben (115 uses), 1 percent.[107] Commonly, formulations contain Parabens in concentrations up to 1 percent. Similar data from 1976 and 1979 indicate that the concentrations of use have remained the same, and the number of uses has steadily increased.[110,111]

The cosmetic product formulation computer printout that is made available by the FDA is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[112] Ingredients are listed in prescribed concentration ranges under specific product type categories. Certain cosmetic ingredients are supplied by the manufacturer at less than 100 percent concentration. The value reported by the cosmetic formulator in such a case

TABLE 3. Product Formulation Data.[107]

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)					
			Unreported Concentration	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Methylparaben								
Baby shampoos	35	12	—	—	—	—	8	4
Baby lotions, oils, powders, and creams	56	13	—	—	—	—	12	1
Other baby products	15	4	—	—	—	—	3	1
Bath oils, tablets, and salts	237	36	—	—	—	—	25	11
Bubble baths	475	142	—	—	—	—	125	17
Bath capsules	3	3	—	—	—	—	2	1
Other bath preparations	132	73	—	—	—	1	57	15
Eyebrow pencil	145	14	—	—	—	—	14	—
Eyeliner	396	114	—	—	—	—	95	19
Eye shadow	2582	883	—	—	—	—	730	153
Eye lotion	13	9	—	—	—	—	8	1
Eye makeup remover	81	33	—	—	—	1	22	10
Mascara	397	227	—	—	—	1	209	17
Other eye makeup preparations	230	73	—	—	—	1	53	19
Colognes and toilet waters	1120	44	—	—	—	—	9	35
Perfumes	657	28	—	—	—	—	12	16
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	152	—	—	—	1	87	64
Sachets	119	77	—	—	—	—	59	18
Other fragrance preparations	191	53	—	—	—	—	33	20
Hair conditioners	478	163	—	—	—	1	114	48
Hair sprays (aerosol fixatives)	265	6	—	—	—	—	3	3
Hair straighteners	64	6	—	—	—	—	3	3
Permanent waves	474	28	—	—	—	1	18	9
Hair rinses (noncoloring)	158	39	—	—	—	—	28	11
Hair shampoos (noncoloring)	909	364	—	—	—	—	284	80
Tonics, dressings, and other hair grooming aids	290	56	—	—	—	—	33	23

Wave sets	180	52	—	—	—	1	20	31
Other hair preparations (noncoloring)	177	20	—	—	—	—	15	5
Hair dyes and colors (all types requiring caution statement and patch test)	811	7	—	—	—	—	1	6
Hair shampoos (coloring)	16	4	—	—	—	—	4	—
Hair bleaches	111	2	—	—	—	—	—	2
Other hair coloring preparations	49	5	—	—	—	—	2	3
Blushers (all types)	819	274	—	1	—	2	230	41
Face powders	555	186	—	—	—	1	125	60
Makeup foundations	740	301	—	—	—	1	279	21
Lipstick	3319	144	—	—	—	1	128	15
Makeup bases	831	419	—	—	—	—	362	57
Rouges	211	34	—	—	—	—	19	15
Makeup fixatives	22	6	—	—	—	—	3	3
Other makeup preparations (not eye)	530	61	—	—	—	1	51	9
Nail basecoats and undercoats	44	1	—	—	—	—	1	—
Cuticle softeners	32	15	—	—	—	—	12	3
Nail creams and lotions	25	10	—	—	—	—	10	—
Nail polish and enamel remover	41	1	—	—	—	—	—	1
Other manicuring preparations	50	9	—	—	—	—	7	2
Dentifrices (aerosol, liquid, pastes, and powders)	42	17	—	—	—	—	8	9
Other oral hygiene products	3	1	—	—	—	—	1	—
Bath soaps and detergents	148	34	—	—	—	—	31	3
Deodorants (underarm)	239	28	—	—	—	1	21	6
Douches	26	4	—	—	—	—	1	3
Feminine hygiene deodorants	21	2	—	—	—	—	—	2
Other personal cleanliness products	227	41	—	—	—	—	27	14
Aftershave lotions	282	38	—	—	—	—	17	21
Beard softeners	4	1	—	—	—	—	1	—
Men's talcum	13	3	—	—	—	—	1	2
Preshave lotions (all types)	29	3	—	—	—	—	1	2
Shaving cream (aerosol, brushless, and lather)	114	46	—	—	—	—	27	19

TABLE 3. (Continued.)

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)					
			Unreported Concentration	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Methylparaben (cont)								
Other shaving preparation products	29	13	—	—	—	—	11	2
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	421	—	—	—	—	328	93
Depilatories	32	3	—	—	—	—	2	1
Face, body, and hand skin care preparations (excluding shaving preparations)	832	556	—	—	—	6	455	95
Foot powders and sprays	17	2	—	—	—	—	—	2
Hormone skin care preparations	10	8	—	—	—	—	6	2
Moisturizing skin care preparations	747	532	—	—	—	4	433	95
Night skin care preparations	219	135	—	—	—	—	114	21
Paste masks (mud packs)	171	123	—	—	—	—	92	31
Skin lighteners	44	22	—	—	—	—	18	4
Skin fresheners	260	117	—	—	—	1	56	60
Wrinkle smoothers (removers)	38	20	—	—	—	—	14	6
Other skin care preparations	349	143	—	—	—	1	97	45
Suntan gels, creams, and liquids	164	68	—	—	—	—	53	15
Indoor tanning preparations	15	10	—	—	—	—	8	2
Other suntan preparations	28	12	—	—	—	—	10	2
1981 TOTALS		6606	—	1	—	27	5148	1430
Propylparaben								
Baby shampoos	35	8	—	—	—	—	2	6
Baby lotions, oils, powders, and creams	56	10	—	—	—	—	3	7
Other baby products	15	4	—	—	—	—	—	4
Bath oils, tablets, and salts	237	25	—	—	—	—	7	18
Bubble baths	475	95	—	—	—	—	13	82

Bath capsules	3	3	—	—	—	—	—	3
Other bath preparations	132	42	—	—	—	1	20	21
Eyebrow pencil	145	17	—	—	—	—	6	11
Eyeliner	396	106	—	—	—	—	59	47
Eye shadow	2582	857	—	—	—	—	440	417
Eye lotion	13	5	—	—	—	—	2	3
Eye makeup remover	81	36	—	—	—	1	8	27
Mascara	397	191	—	—	—	1	87	103
Other eye makeup preparations	230	100	—	—	—	—	35	65
Colognes and toilet waters	1120	22	—	—	—	—	6	16
Perfumes	657	14	—	—	—	—	4	10
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	105	—	—	—	—	14	91
Sachets	119	48	—	—	—	—	27	21
Other fragrance preparations	191	37	—	—	—	—	14	23
Hair conditioners	478	100	—	—	—	1	21	78
Hair sprays (aerosol fixatives)	265	3	—	—	—	—	1	2
Hair straighteners	64	6	—	—	—	—	—	6
Permanent waves	474	23	—	—	—	—	—	23
Hair rinses (noncoloring)	158	28	—	—	—	—	3	25
Hair shampoos (noncoloring)	909	190	—	—	—	—	31	159
Tonics, dressings, and other hair grooming aids	29048	—	—	—	—	—	17	31
Wave sets	180	14	—	—	—	—	5	9
Other hair preparations (noncoloring)	177	13	—	—	—	—	2	11
Hair dyes and colors (all types requiring caution statement and patch test)	811	1	—	—	—	1	—	—
Hair shampoos (coloring)	16	3	—	—	—	—	—	3
Other hair coloring preparations	49	3	—	—	—	—	—	3
Blushers (all types)	819	284	—	—	—	—	125	159
Face powders	555	179	—	—	—	1	54	124
Makeup foundations	740	316	—	—	—	1	130	185
Lipstick	3319	357	—	—	—	—	167	190
Makeup bases	831	429	—	—	—	—	88	341
Rouges	211	68	—	—	—	—	22	46
Makeup fixatives	22	5	—	—	—	—	2	3

TABLE 3. (Continued.)

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)					
			Unreported Concentration	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Propylparaben (cont)								
Other makeup preparations (not eye)	530	130	—	—	—	—	44	86
Nail basecoats and undercoats	44	2	—	—	—	—	—	2
Cuticle softeners	32	13	—	—	—	—	7	6
Nail creams and lotions	25	12	—	—	—	1	5	6
Nail polish and enamel	767	1	—	—	—	—	—	1
Other manicuring preparations	50	8	—	—	—	—	3	5
Dentifrices (aerosol, liquid, pastes, and powders)	42	11	—	—	—	—	—	11
Bath soaps and detergents	148	26	—	—	—	—	4	22
Deodorants (underarm)	239	17	—	—	—	—	9	8
Douches	26	2	—	—	—	—	—	2
Other personal cleanliness products	227	39	—	—	—	—	5	34
Aftershave lotions	282	21	—	—	—	—	5	16
Beard softeners	4	1	—	—	—	—	—	1
Men's talcum	13	2	—	—	—	—	—	2
Preshave lotions (all types)	29	2	—	—	—	—	—	2
Shaving cream (aerosol, brushless, and lather)	114	34	—	—	—	—	6	28
Other shaving preparation products	29	8	—	—	—	—	3	5
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	350	—	—	—	1	101	248
Depilatories	32	3	—	—	—	—	3	—
Face, body, and hand skin care preparations (excluding shaving preparations)	832	467	—	1	—	—	184	282
Foot powders and sprays	17	1	—	—	—	—	—	1
Hormone skin care preparations	10	5	—	—	—	—	4	1

Moisturizing skin care preparations	747	481	—	—	—	2	170	309
Night skin care preparations	219	111	—	—	—	—	52	59
Paste masks (mud packs)	171	64	—	—	—	—	22	42
Skin lighteners	44	15	—	—	—	—	6	9
Skin fresheners	260	32	—	—	—	—	2	30
Wrinkle smoothers (removers)	38	16	—	—	—	—	5	11
Other skin care preparations	349	104	—	—	—	—	24	80
Suntan gels, creams, and liquids	164	77	—	—	—	—	35	42
Indoor tanning preparations	15	7	—	—	—	—	2	5
Other suntan preparations	28	11	—	—	—	—	4	7
1981 TOTALS		5868	—	1	—	11	2120	3736
Butylparaben								
Baby lotions, oils, powders, and creams	56	1	—	—	—	—	—	1
Bath oils, tablets, and salts	237	8	—	—	—	—	—	8
Bubble baths	475	10	—	—	—	—	2	8
Other bath preparations	132	4	—	—	—	—	—	4
Eyebrow pencil	145	11	—	—	—	—	—	11
Eyeliner	396	8	—	—	—	—	2	6
Eye shadow	2582	42	—	—	—	—	7	35
Eye makeup remover	81	18	—	—	—	—	3	15
Mascara	397	14	—	—	—	—	4	10
Other eye makeup preparations	230	18	—	—	—	—	4	14
Colognes and toilet waters	1120	4	—	—	—	—	—	4
Perfumes	657	11	—	—	—	—	—	11
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	14	—	—	—	—	—	14
Sachets	119	16	—	—	—	—	2	14
Other fragrance preparations	191	4	—	—	—	—	1	3
Hair conditioners	478	7	—	—	—	—	1	6
Hair rinses (noncoloring)	158	1	—	—	—	—	—	1
Hair shampoos (noncoloring)	909	6	—	—	—	—	—	6
Tonics, dressings, and other hair grooming aids	290	9	—	—	—	—	2	7
Wave sets	180	6	—	—	—	—	1	5

TABLE 3. (Continued.)

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)					
			Unreported Concentration	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Butylparaben (cont)								
Other hair coloring preparations	49	1	—	—	—	—	—	1
Blushers (all types)	819	4	—	—	—	—	2	2
Makeup foundations	740	46	—	—	—	—	12	34
Lipstick	3319	44	—	—	—	—	20	24
Makeup bases	831	10	—	—	—	—	3	7
Rouges	211	1	—	—	—	—	1	—
Makeup fixatives	22	3	—	—	—	—	—	3
Other makeup preparations (not eye)	530	20	—	—	—	—	8	12
Cuticle softeners	32	1	—	—	—	—	—	1
Nail creams and lotions	25	2	—	—	—	—	—	2
Other manicuring preparations	50	2	—	—	—	—	1	1
Deodorants (underarm)	239	2	—	—	—	—	1	1
Other personal cleanliness products	227	3	—	—	—	—	—	3
Aftershave lotions	282	1	—	—	—	—	—	1
Men's talcum	13	1	—	—	—	—	—	1
Shaving cream (aerosol, brushless, and lather)	114	1	—	—	—	—	—	1
Other shaving preparation products	29	2	—	—	—	—	—	2
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	58	—	—	—	1	11	46
Face, body, and hand skin care preparations (excluding shaving preparations)	832	104	—	—	—	1	17	86
Hormone skin care preparations	10	1	—	—	—	—	1	—

Moisturizing skin care preparations	747	91	—	—	—	—	17	74
Night skin care preparations	219	33	—	—	—	—	5	28
Paste masks (mud packs)	171	11	—	—	—	—	1	10
Skin lighteners	44	2	—	—	—	—	—	2
Skin fresheners	260	3	—	—	—	—	1	2
Wrinkle smoothers (removers)	38	4	—	—	—	—	—	4
Other skin care preparations	349	11	—	—	—	—	4	7
Suntan gels, creams, and liquids	164	15	—	—	—	—	5	10
Other suntan preparations	28	4	—	—	—	—	—	4
1981 TOTALS		693	—	—	—	2	139	552
Ethylparaben								
Bubble baths	475	5	—	—	—	—	—	5
Eye shadow	2582	4	—	—	—	—	1	3
Mascara	397	1	—	—	—	—	—	1
Other eye makeup preparations	230	1	—	—	—	—	1	—
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	4	—	—	—	—	—	4
Wave sets	180	5	—	—	—	—	3	2
Blushers (all types)	819	1	—	—	—	—	—	1
Face powders	555	2	—	—	—	—	—	2
Makeup foundations	740	8	—	—	—	—	—	8
Lipstick	3319	2	—	—	—	—	—	2
Makeup bases	831	2	—	—	—	—	2	—
Other makeup preparations (not eye)	530	1	—	—	—	—	1	—
Nail creams and lotions	25	1	—	—	—	—	1	—
Other personal cleanliness products	227	1	—	—	—	—	—	1
Aftershave lotions	282	1	—	—	—	—	—	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	13	—	—	—	—	10	3

TABLE 3. (Continued.)

Product Category	*Total No. of Formulations in Category*	*Total No. Containing Ingredient*	*No. of Product Formulations Within Each Concentration Range (%)*					
			Unreported Concentration	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Ethylparaben (cont)								
Face, body, and hand skin care preparations (excluding shaving preparations)	832	31	—	—	—	—	20	11
Moisturizing skin care preparations	747	9	—	—	—	—	2	7
Night skin care preparations	219	7	—	—	—	—	3	4
Paste masks (mud packs)	171	13	—	—	—	—	6	7
Skin fresheners	260	1	—	—	—	—	—	1
Other skin care preparations	349	1	—	—	—	—	1	—
Suntan gels, creams, and liquids	164	1	—	—	—	—	1	—
1981 TOTALS		115	—	—	—	—	52	63

may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for a two- to tenfold overestimation of the actual concentration of an ingredient in a particular product.

The Parabens are used in all 13 product formulation categories. Products containing these ingredients may contact the skin, hair and scalp, lips, mucosae (oral, ocular, vaginal), axillae, and nails. Products containing Parabens are used daily or occasionally; their use may extend over a period of years. Frequency and duration of application could be continuous.

Food

The Parabens have been used in foods for more than 50 years because of their low toxicity to humans and their effective antimicrobial activity, especially against molds and yeasts. Under FDA regulation, Methylparaben and Propylparaben are generally recognized as safe (GRAS) when used as chemical preservatives in foods, with use limits of 0.1 percent for each (21 CFR 121.101). They are used in processed vegetables, baked goods, fats and oils, seasonings, sugar substitutes, and frozen dairy products in concentrations of 0.00001 to 0.1 percent (0.1 to 1000 ppm). The possible average daily intake, based on the types and quantities of food consumed, is approximately 1 to 16 mg/kg for infants and 4 to 6 mg/kg for persons aged two or older. These estimates are considered to be maximum possible intakes.[5]

Butylparaben, Methylparaben, and Propylparaben are permitted as direct food additives for use as synthetic flavoring substances and adjuvants in the minimum quantities required to produce their intended effect (21 CFR 172.515). Both Methylparaben and Propylparaben preseratives are included among optional ingredients permitted in artificially sweetened fruit jellies and jams. They may be used alone or in combination with sorbates, propionates, and benzoates, with total preservative concentrations not to exceed 0.1 percent (21 CFR 29.4). As indirect food additives, Methylparaben and Propylparaben are permitted by prior sanction as antimycotics in food-packaging materials with no limits or restrictions (21 CFR 181.23); Ethylparaben is similarly allowed when used for packaging, transporting, or holding food (21 CFR 175.105).

The Parabens are officially approved food additives in 12 countries. In Italy, Ethylparaben is permitted as a direct food additive; in Japan, Butylparaben is used as a food additive.[3]

Pharmaceutical

Parabens were first used in drug products in 1924.[113] Since then, they have been incorporated as preservatives in a wide variety of drug formulations. They are colorless, odorless, inert, nontoxic, nonvolatile, and effective at low concentrations against a wide range of microorganisms in acid, neutral, and slightly alkaline conditions. Combinations of Parabens are more active than individual esters.[114] As preservatives, Parabens are or have been used in suppositories, anesthetics, eyewashes, pills, syrups, weight-gaining solutions, injectable solutions, and contraceptives. Use concentrations vary from product to product, but maximum levels seldom exceed 1 percent.[1,114-117]

Ritzau and Swangsilpa[118] studied the prophylactic effect of Propylparaben

on alveolitis sicca dolorosa (ASD). They had previously noted that this compound to some degree disturbs and inhibits bone healing in experimental cavities in the iliac crests of rabbits.[119] Each of 45 patients received three tablets containing 33 mg Propylparaben or a placebo in the socket immediately after removal of a mandibular third molar. None of the patients receiving Propylparaben developed ASD, whereas 24 percent of the placebo group did. The prophylatic effect of Propylparaben was highly significant, and no side effects to treatment were reported.

Methylparaben and Propylparaben are used in a number of over-the-counter (OTC) drugs as preservatives. The Ophthalmic Drug Panel of the Food and Drug Administration's Bureau of Drugs has determined that these two ingredients, if used alone, are unsuitable as preservatives in OTC ophthalmic products because they are irritating to the eyes if used at concentrations effective against microorganisms. However, eye irritation by Parabens could not be confirmed by the references cited in the OTC ophthalmic report. The OTC Panel reported that Parabens are good antifungals with limited antibacterial action but that a combination of Methylparaben and Propylparaben in concentrations up to 0.25 and 0.04 percent, respectively, may be useful as an antibacterial preparation in ophthalmic products. It was suggested that further formulation studies and safety testing be done on these two ingredients.[120] Other OTC panels have concluded that Methylparaben is a safe and effective preservative in concentrations of 0.1 to 0.2 percent in products for anorectal application and other antimicrobial uses.[121,122] Methylparaben and Propylparaben have been classified as inactive ingredients in dentifrices, contraceptives, and topical analgesics.[123-125]

Other

Parabens are used in textiles as antifungal agents, in gelatins and photographic emulsions, in bone glues, and as antifermentation agents in malt.[1] Methylparaben may be used as an arachnacide. Bronswijk and Koekkoek[126] tested its activity against *Dermatophagoides pteronyssinus* (house dust mite). Methylparaben at 0, 1, 5, or 7 percent was added to cultures, which were then incubated for 28 days. Growth of mites was suppressed by 1 percent Methylparaben; at 5 and 7 percent, mite growth was completely inhibited.

GENERAL BIOLOGY

Absorption, Metabolism, and Excretion

Jones et al.[127] studied the pharmacology of the Parabens. Intravenous (IV) injections at 50 mg/kg Methylparaben, Ethylparaben, Propylparaben, or Butylparaben were administered to groups of three or more fasted dogs. Blood and urine were analyzed at predetermined intervals. Similarly, these compounds were administered orally at a dose of 1.0 g/kg. Immediately following IV injection, very little ester remained in the blood. Metabolites were detectable in the blood up to 6 hours postinjection and 24 hours postingestion. Recovery of all esters but Butylparaben ranged from 58 to 94 percent of the administered dose. Absorption was essentially complete. Recoveries of Butylparaben after oral and IV administration were only 40 and 48 percent, respectively. This was considered

a result of less effective hydrolysis of this ingredient by the body. A fasted man was given orally 70 mg/kg Methylparaben. No ester was detected in his blood or urine. After 12 hours half of the dose was excreted in the urine as metabolites (11 percent as *p*-hydroxybenzoic acid). No accumulation of Parabens was observed in the tissues of dogs given orally 1 g/kg/day Methylparaben or Propylparaben for 1 year. Rate of urinary excretion of esters and metabolites increased over the course of the study until 96 percent of the dose was excreted per day. When 10 percent Methylparaben or Propylparaben in hydrophilic ointment was applied to the skin of rabbits for 48 hours, esters and metabolites were not detected in the kidneys. Dogs were given intravenously 100 mg/kg of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben and were then killed for the determination of organ distribution of esters and metabolites. Pure ester was recovered only in the brain, spleen, and pancreas. High concentrations of metabolites were detected in the liver and kidneys. With in vitro assays, it was found that esterases in the liver and kidneys were extremely efficient in hydrolyzing Parabens (100 percent hydrolysis after 3 minutes for all Parabens except Butylparaben, which took 30 to 60 minutes).

Mouse liver perfused with Ethylparaben rapidly hydrolyzed it to the free acid within 60 minutes. No Ethylparaben was detected in the blood of six humans 4 hours following oral administration of 10 to 20 mg/kg. When given orally to dogs at 25 to 500 mg/kg, no Ethylparaben was detected in their blood until a dose of 500 mg/kg was reached. High serum concentrations of *p*-hydroxybenzoic acid were reported. The results indicated that Ethylparaben, ingested in food by man, was probably completely hydrolyzed within 3 minutes after absorption.[128]

Tsukamoto and Terada[129-131] studied the metabolic fate of Methylparaben in rabbits. The compound was given by gastric intubation, and urine was analyzed by paper chromatography. Three major metabolies, *p*-hydroxybenzoic acid, *p*-hydroxyhippuric acid, and *p*-carboxyphenyl glucuronide, as well as two minor metabolites, *p*-hydroxybenzoyl glucuronide and *p*-carboxyphenyl sulfate, were identified. Rabbits given orally 0.4 or 0.8 g/kg Methylparaben, Ethylparaben, Propylparaben, or Butylparaben excreted only 0.2 to 0.9 percent of the uncharged ester by 24 hours. Urinary excretion of *p*-hydroxybenzoic acid was slower with increasing carbon chain length of the Paraben alkyl group. Excretion of the conjugated acid was approximately that of the free acid. Twenty-four hours following Paraben administration, 25 to 39 percent was recovered as *p*-hydroxybenzoic acid, 15 to 29 percent as the glycine conjugate, 5 to 8 percent as the ester glucuronide, 10 to 18 percent as the ether glucuronide, and 7 to 12 percent as the sulfate.

The pharmacology of Methylparaben, Ethylparaben, and Propylparaben was studied in rats by Derache and Gourdon.[132] Animals were given orally 100 mg of ester. Blood and urine were collected regularly and analyzed by paper chromatography. Paraben metabolites were identified in the urine 30 minutes after dosing. No unchanged Paraben was detected. Ninety minutes after dosing, excretion of metabolites was maximum; thereafter, excretion decreased. *p*-Hydroxyhippuric acid appeared in the urine after 30 minutes; its concentration then increased evenly during the next 4 hours. The glucuronide and ethereal sulfate metabolites appeared only between 30 and 75 minutes postingestion. After 90 minutes, 67 to 75 percent of the total Paraben dose was excreted as *p*-hydroxybenzoic acid, 10 to 12.5 percent as *p*-hydroxyhippuric acid, and 8 to 10 percent as glucuronyl derivatives. The concentration of free *p*-hydroxybenzoic

acid in the blood remained extremely low. A continuous rise occurred within the first hour, but the concentration thereafter decreased and leveled off 1 to 2 hours after ingestion. The authors concluded that there were two stages of Paraben detoxification: massive absorption of Paraben and excretion in urine of *p*-hydroxybenzoic acid, and metabolic detoxification by glucuronic-, sulfo-, and glycinoconjugation.

A metabolic study was conducted on ^{14}C ring-labeled Ethylparaben and Propylparaben. Compounds were administered orally to groups of four male cats at doses of 156 and 158 mg/kg, respectively. Urine was collected at 24, 48, and 72 hours; feces were collected at 72 hours. At 72 hours, total recovery was 96 percent for Ethylparaben and 95.6 percent for Propylparaben. Approximately 90 percent of the label was recovered in the urine at 24 hours, whereas 6 and 3 percent, respectively, were recovered in the feces. Analysis of urine by thin-layer chromatography revealed only two major metabolites: *p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid. The authors concluded that both Parabens were rapidly and totally excreted in the urine within 72 hours following oral administration.[133]

Radiolabeled (ring) Ethylparaben was injected into the femoral vein or the duodenum of rats at a dose of 2 mg/kg. Excretion of it and its metabolites in the urine and bile was determined at fixed intervals by scintillation counting. Excretion was complete within 5 hours. Little unmetabolized Ethylparaben was detected in samples of urine (0.03 percent) and bile (0 percent). Radiolabeled metabolites recovered in the urine were 83.5 percent of the dose injected into the duodenum and 91.3 percent of that injected intravenously. Those recovered in the bile were 12.8 and 5.97 percent, respectively. The results suggested that hydrolysis of Ethylparaben to *p*-hydroxybenzoic acid and metabolism of the latter was rapid and complete.[134]

Frogs were immersed in solutions of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben for 2 hours to study the percutaneous absorption properties of these ingredients. Uptake increased as the length of the ester carbon chain length increased. Absorption was fastest during the first 20 minutes of immersion. The authors suggested that the greater the lipid solubility of the Paraben, the greater the rate of absorption.[135]

Parabens (15 percent in Vaseline) were applied to the skin of each of three healthy humans. Presence of residual Parabens on the skin was determined at 1 and 8 hours. One hour after application, Parabens were identified; at 8 hours, they were not detected.[136]

Komatsu and Suzuki[137] studied the percutaneous absorption of Butylparaben (0.015 to 0.1 percent aqueous) through guinea pig skin in vitro. The authors had previously shown that Butylparaben was absorbed percutaneously from several ointments through mouse skin. The presence of a solubilizer (such as polysorbate 80, propylene glycol, or PEG 400) increased antimicrobial activity and reduced percutaneous absorption of Butylparaben. The amount of Butylparaben that passed through the skin was dependent on the partition coefficient of the system. Total penetration of Butylparaben from an aqueous vehicle was a combination of the penetration through the epidermis and the penetration through the adnexal structures. Over time, transient penetration through the latter became less important than the steady-state penetration through unbroken skin.

Antimicrobial Effects

In antimicrobial studies, the Parabens were effective in low concentrations against fungi and bacteria. These compounds are more active against fungi than bacteria and are more active against gram-positive bacteria than gram-negative bacteria. Table 4 summarizes the antifungal and antibacterial properties of the alkyl Parabens. These compounds have a more static than lethal effect on microorganisms. Combinations of individual esters are additive in effect. The Parabens are effective in acid, neutral, or slightly alkaline solutions. Beyond pH 8, hydrolysis can occur and reduce preservative efficiency.[2,105] Activity of the Parabens increases as the length of the alkyl chain increases.[138] Inhibition of microbial growth results from the Parabens' action on germinative and vegetative phases of development, although spore germination is much more inhibited by Parabens than vegetative growth in both fungi and bacteria.[139,140] According to Shiralkar et al.,[141] growth inhibition is present only after a minimum concentration of Paraben is reached; once this value is exceeded, inhibition is rapid. Nonionic surfactants at low concentrations may have a synergistic effect with Parabens, whereas higher concentrations of the surfactant inhibit preservative activity.[142]

The specific action of Parabens as antimicrobial agents has been studied extensively. Lang and Rye[143] observed that regardless of molecular size, all Paraben esters were equally effective in inhibiting bacterial growth at the site of action. The higher activity of the long-chain esters over the shorter-chain esters resulted from greater uptake of the former by bacterial cells. The authors suggested that since the Parabens are lipophilic, the action site was probably the cell membrane. In 1973, Lang and Rye[144] reported that, at equilibrium in treated cell suspensions, Paraben concentration within the cell was greater than that in the external medium. They speculated that the intracellular Paraben was largely present in the lipid-containing region of the cell (i.e., the cell membrane), and that Parabens acted by affecting membrane permeability to disrupt growth. They stated that uptake of Paraben by the cell proceeded by general dissolution. No specific sites existed for uptake at the cell surface. They noted that as the chain length of the ester increased, so did its tendency to be concentrated within the cell.

Furr and Russell[145] observed that Propylparaben and Butylparaben induced leakage of intracellular material through the cell wall of *Serratia marcescens* (a bacterium). These Parabens, however, were not lysing agents. No gross cellular damage was observed. They concluded that Parabens act by causing damage to the cytoplasmic membrane. The immediate loss of selective permeability to small molecules reflected a structural disorganization of the cell membrane. Furr and Russell[146] noted that Methylparaben and Ethylparaben were not taken up by whole cells and isolated cell walls of *S. marcescens,* whereas Propylparaben and Butylparaben were. The lack of preservative activity of Methylparaben and Ethylparaben was due to their lack of uptake by the cell. Less of the ester was able to reach the cytoplasmic membrane due to decreased partition into oil phases. Parabens were absorbed from aqueous solution and diffused through the cell wall to the membrane, and the cell wall acted as a permeability barrier. The high proportion of lipid in the lipoprotein membrane allowed high concentrations of Propylparaben and Butylparaben to pass readily from the cell wall to the cell

TABLE 4. Antimicrobial Effectiveness of Parabens.

Microorganism	*Species*	*Effective Concentration (% by Weight)*				
		Methylparaben	*Ethylparaben*	*Propylparaben*	*Butylparaben*	*Reference*
Fungi	*Rhizopus nigricans*	0.05	0.025–0.05	0.0125	0.0063	22,147
	Trichoderma lignorum	0.025	0.0125	0.0125	0.0063	22
	Chactonium globosum	0.05	0.025	0.0063	0.0031	22
	Candida albicans	0.1	0.1	0.0125–0.1	0.0125–0.1	22,148
	Saccharomyces cerevisiae	0.1–0.23	0.05–0.1	0.01–0.0125	0.0063	22,141,150,156
	S. pastorianus	0.1	0.05	0.0125	0.0063	22
	Aspergillus flavus	0.04–0.125	0.03	0.08	0.02	1,149
	A. niger	0.08–0.27	0.04–0.06	0.02–0.07	0.02	22,141,149,150
	Penicillium digitatum	0.05	0.025	0.0063	0.0031	22
	P. crysoqenum	0.01	—	—	—	149
	P. glaucum	0.04–0.1	0.03–0.15	0.15	0.02–0.15	1
	P. expansum	—	—	—	0.02	1
	Mucor mucedo	0.04–0.15	0.03–0.04	0.05–0.1	0.02	1
	Torula sp.	0.125–0.15	0.025–0.1	0.05–0.1	—	1,147
	Epidermophyton floccosum	0.025–0.1	—	0.01	0.01	148,151
	Microsporum audovini *M. canis* *M. gypseum* *Trichophyton ferrugineum* *T. tonsuransivio* *T. mentagrophytes* *T. rubrum*	0.01–0.1 0.025–0.1 >0.008	— — 0.008	0.01 0.01 0.004	0.01 0.01 0.002	148,151 148,151 22
	Hormodendrum compactum *H. pedrosoi*	0.025–0.1	—	0.01	0.01	148,151
	Phialophora verrucosa	0.025–0.1	—	0.1	0.1	148,151
	Geotrichum sp.	0.05	—	—	—	151
	Monosporum apiospermum	0.1	—	0.1	0.01	148,151
	Sporotrichum schenckii	0.05–0.1	—	0.01	0.01	148,151
	Blastomyces dermatitidis *B. brasiliensis*	0.01–0.1	—	0.01–0.1	0.01	148,151
	Cryptococcus neoformans	0.05–0.1	—	0.01	0.01	148,151

	Haplosporangium parvum	0.025	—	—	—	151
	Histoplasma capsulatum	0.1–0.025	—	0.01	0.01	148,151
	Trichosporon beigelii	0.1	—	0.01	0.01	148
	Piedraia hortai	0.1	—	0.01	0.01	148
	Other fungi	—	0.1–0.025	—	—	147
Bacteria	*Bacillus subtilis*	0.12–0.25	0.1–0.2	0.025–0.2	0.0125	22,150,152
	B. cereus	0.2	0.1	0.125	0.0063	22
	B. coli	0.125–0.15	—	0.05–0.1	0.02	1
	B. coagulans	0.15–0.35	—	0.05–0.07	—	141
						150
	B. megaterium	0.14	0.06	0.03	0.01	153
	Staphylococcus aureus (*Micrococcus pyogenes aureus*)	0.16–0.4	0.065–0.15	0.04–0.15	0.0125–0.02	1,22,148,152,153
	S. pyogenes	0.063	0.063	0.05	—	152
	Sarcina lutea	0.25–0.4	0.25–0.1	0.25–0.05	0.0125	22,152
	Klebsiella pneumoniae	0.1	0.05	0.025	0.0125	22
	Escherichia coli	0.125–0.4	0.1–0.125	0.05–0.1	0.4	1,22,152
	Salmonella typhosa	0.2	0.1	0.1	0.1	22
	S. schottmulleri	0.2	0.1	0.05	0.1	22
	S. typhimurium	—	—	0.020–0.025	—	154
	Proteus vulgaris	0.2	0.1–0.15	0.05–0.15	0.05	1,22
	Aerobacter aerogenes	0.125–0.24	0.1	0.05–0.1	0.4	1,22,141
	Pseudomonas aeruginosa	0.1–0.4	0.2–0.4	0.2–0.8	0.8	22,152,155
	P. fluorescens	0.15–0.4	0.2	0.05–0.2	0.4	1,22
	Streptococcus hemolyticus	0.01	—	0.1	0.1	148
	S. faecalis	—	0.130	0.04	0.012	156
	Serratia marcescens	0.08	0.049	0.04	0.019	153
	Achromobacter sp.	0.23–0.24	—	0.05–0.07	—	141
	Arthrobacter simplex	0.36–0.38	—	0.07–0.09	—	141
	Clostridium botulinum	0.1–0.12	0.04	0.04	0.02	26,157
	Corynebacterium acnes (5 strains)	—	—	1.0	—	158
	Nocardia asteroides	0.025–0.1	—	0.1	0.01	148,151

membrane where they acted. In an in vitro experiment, more Propylparaben and Butlyparaben was taken up by fattened *Bacillus subtilis* cells (grown on medium containing 3 percent gylcerol) than by normal cells, but higher concentrations were needed to inhibit growth of fattened cells. Cell leakage was also reduced in fattened cells. Extra lipid in the cell walls of fattened bacteria increased the permeability barrier to the Parabens; less ester was able to reach the cytoplasmic membrane to cause damage. Furr and Russell[153] also studied the effect of Parabens on spheroplasts (cells with defective cell walls) and protoplasts (cells with no cell wall) of *S. marcescens*. The Parabens (especially the Propyl and Butyl esters) did not induce significant lysis or gross disruption of the cytoplasmic membrane but did induce leakage of cytoplasmic contents. According to Freese et al.,[159] the Parabens inhibit cellular oxidation by inhibiting compounds which donate electrons to the electron-transport mechanism of the cell. The deficiency of these donating compounds resulted from Paraben-induced transport inhibition of substrates into the cell. In membrane vesicles of *B. subtilis,* uptake of L-serine, L-leucine, and L-malate was inhibited by Parabens. Lipophilic acids, such as the Parabens, are known to uncouple substrate transport and oxidative phosphorylation of the electron transport system of the cell.

Shiralkar et al.[10,160] reported that Propylparaben was taken up by cells of *Aerobacter* sp.; 90 to 95 percent of the ester was taken up within 2 minutes after introduction into cultures. These results indicated that the uptake was a physical phenomenon rather than a result of active biological transport. Propylparaben was primarily absorbed by the cell, but its inhibitory effect was due to its being on the cytoplasmic particulates. Experiments indicated that Parabens have no effect on nutrient transfer into the cell or on hydrolytic enzymes. Parabens have a significant inhibitory effect on oxygen consumption (respiration) and most oxidative enzymes.

Eklund[161] studied the effect of Parabens on the uptake processes of three bacteria. Parabens had a dose-dependent inhibitory action on growth and amino acid uptake. Growth inhibition was a consequence of transport inhibition. The author suggested that Parabens increase membrane permeability such that both chemical and electrical components of the proton-motive force are neutralized and also inhibit NADH oxidation and cellular oxygen consumption.

Murata and Shiroura[162] reported that Parabens are lysing agents for phage-infected *Lactobacillus casei*. Premature lysis of infected cells was induced when the Parabens were added during the bacterial latent period. Upon lysis, the infecting phage was lost, and no new phage was produced. The lytic reaction was determined to be due to a Paraben-induced increase in the permeability of the bacterial cytoplasmic membrane.

Concerning the structural relationship to Paraben preservative activity, both the ester chain and the *p*-hydroxy group of the molecule have been implicated. Gottfried[163] stated that location of the phenolic hydroxy group on the benzene ring can increase or decrease the antimicrobial activity of the Parabens. The ester chain was also necessary for activity; any branching reduced the effectiveness of the Paraben.[164] Shiralkar et al.[10] stated that if a microorangism possessed an esterase that could hydrolyze the ester linkage of the Paraben, they would survive in the presence of these preservatives. Such an organism was identified by Close and Neilson.[165] A Propylparaben-resistant strain of *Pseudomonas cepacia* was cultured, and the bacteria were able to use Propylparaben as a carbon source once it was hydrolyzed. This organism was also able to hydrolyze Methylparaben but was unable to use it as a carbon source.

The in vitro effectiveness of Paraben preservatives has been studied in rabbits and man. In a study involving 186 patients, oral, vaginal, and rectal administration of Methylparaben and Propylparaben effectively inhibited development of candidiasis (from *Candida albicans*) during aureomycin treatment. In three patients with candidal vaginitis, intravaginal insertion of 200 mg Paraben daily ameliorated symptoms. No toxic effects of Parabens were observed.[166]

Three times daily for 3 days, each of 17 patients was given 90 mg Methylparaben plus 22.5 mg Propylparaben along with aureomycin. Stool samples were analyzed daily for yeast. Results indicated that the Parabens exerted antiyeast activity when compared to control patients receiving aureomycin only. The authors concluded that Parabens may be useful in controlling intestinal yeast overgrowth during antibiotic treatment.[167]

Biochemical Effects

In an early study of the effect of Methylparaben and Ethylparaben on various enzymes, and amylolytic activity was not observed. Peptic proteolysis and lipolysis were inhibited, and Ethylparaben was a more potent inhibitor than Methylparaben. Trypsin, dehydrogenase, and peroxidase were all activated by addition of Parabens. Methylparaben was a better activator than Ethylparaben.[168]

Tzortzatou and Hayhoe[169] reported that Methylparaben and Propylparaben increase the activity of dihydrofolate reductase and methotrexate-inhibition of this enzyme. The authors suggested that the action of the Parabens is due to induced conformational changes in the enzyme, which increase its affinity for dihydrofolate.

All four alkyl Parabens bind to bovine serum albumin (BSA). Binding increased with increasing ester chain length. The binding process is endothermic and hydrophic in nature. Additionally, protein-bound Paraben is devoid of its antifungal activity.[170] A fluorescent probe was used in determining that the Paraben sidechain is the primary binding site to BSA.[171] Brodersen[172] and Echeverria et al.[173] observed that Methylparaben and Propylparaben are competitive inhibitors of bilirubin binding to serum albumin at concentrations of 400 μg/ml. Rasmussen et al.[174] observed that, while Methylparaben and Propylparaben bind to serum albumin, only Methylparaben displaces bilirubin from albumin. Methylparaben is a weak primary site competitor and a strong secondary site competitor. They reported that at plasma concentrations of 340 μmol/L or greater, Methylparaben competes with bilirubin only when the high-affinity binding sites on serum albumin approach saturation. Loria et al.[175] observed that Methylparaben interacts with components of icteric newborn sera, increasing the availability of free, unconjugated bilirubin. Otagiri and Perrin[176] reported that the serum albumin-binding constant increases significantly from Propylparaben to Butylparaben.

Cytotoxicity

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were studied for their effects on human and rabbit erythrocytes in vitro. Butylparaben, at 0.02 percent, induced hemolysis in 12 percent of the rabbit and 6 percent of the human erythrocytes. Concentrations of 0.25 percent Methylparaben, 0.17 percent Ethylparaben, and 0.05 percent Propylparaben induced no hemolysis.[177]

When tested in cultures of embryonic mouse fibroblasts, Methylparaben, Ethylparaben, and Propylparaben significantly reduced biosynthesis of RNA and

DNA. The incorporation of ^{32}P into RNA and DNA of whole cells was inhibited by 0.2 g/L Ethylparaben only. None of the Parabens affected the protein content of the cell cultures.[178]

Shev et al.[179] determined that the IC_{50}s (dose for 50 percent cell inhibition) of Methylparaben, Ethylparaben, and Propylparaben in HeLa cells were 1.3, 0.6, and 0.22 mM, respectively. These were similar to IC_{50} values in *B. subtilis* and *Escherichia coli.* In HeLa cells, Parabens induced jagged cell shapes; cell processes were shortened, branched, rough-edged, and curved. Many perinuclear and cytoplasmic granules were also observed. Growth inhibition of bacteria by Parabens was due to inhibition of cellular uptake of amino acids and other compounds needed for substrate and energy supply.

Contact lenses treated with 0.02 percent Propylparaben were cytotoxic to the L929 strain of mouse fibroblasts and S_3 HeLa cells.[180]

Tissue Effects

Methylparaben was studied for toxicity to tissue cultures of embryonic chicken spleen and adult human skin. In splenic tissue, doses of 520 to 1040 μg/ml inhibited growth, whereas doses of 30 to 60 μg/ml induced detectable injury. In cultures of skin, doses required for least growth inhibition and detectable injury were 175 to 350 μg/ml and 140 to 175 μg/ml, respectively.[181]

The effects of Methylparaben and Propylparaben on cultured embryonic chicken femoral bones were studied in vitro. At doses of 0.25 and 2.5 μg/ml Methylparaben, bone weight was significantly increased. Significant growth also occurred at 0.025 to 2.5 μg/ml Propylparaben concentration. When mixtures of the two were tested, growth inhibition occurred, even at the lowest dose tested (0.025 μg/ml of each). The authors suggested that the Parabens' effect may be due to their ability to stabilize lysosomes.[182]

The effects of 0.1 and 0.2 percent Methylparaben on vagus and sympathetic nerves, as well as spinal roots, were studied in vivo in cats. When applied directly, Methylparaben blocked nerve impluse conduction in myelinated and unmyelinated nerves. Conduction block was reversible and anestheticlike. The authors suggested that injection of Methylparaben may cause degeneration in a number of the surrounding nerves.[183]

Kitamura[184] studied the anesthetic effect of perfused Parabens on the isolated peripheral nerve and isolated spinal cord of the frog. Methylparaben, Ethylparaben, and Propylparaben blocked nerve conduction. The action of Propylparaben was higher than that of Methylparaben. Total nerve block occurred at concentrations of 1 mM for the former and 5 mM for the latter. The lowest concentration of Methylparaben required for conduction block was higher than that of all local anesthetics tested, whereas effective concentrations of Propylparaben were comparable to the anesthetics. The author concluded that, as preservatives in anesthetic solutions, Methylparaben and Propylparaben may intensify the action of the anesthetic.

The effect of Methylparaben on the sensitivity of the isolated frog rectus abdominus muscle to acetylcholine (ACh) was studied. Methylparaben application instantaneously potentiated the sensitivity of the muscle to ACh. Activity increased gradually with higher Methylparaben concentrations. The authors suggested that the action of Methylparaben may be a result of its ability to increase permeability and facilitate the penetration of ACh into the motor endplates.[185]

The effect of Methylparaben and Propylparaben on smooth muscle of isolated guinea pig trachea was studied by Geddes and Lefcoe.[186] Both compounds induced dose-dependent, rapid, reversible relaxation of tracheal smooth muscle. In addition, these ingredients potentiated isoproterenol and dibutyryl cyclic AMP at doses of 10 μg/ml Methylparaben and 1.5 μg/ml Propylparaben. The authors suggested that the bronchodilation effect of Parabens may be due to their inhibition of phosphodiesterase.

Jones et al.[187] studied the effect of Methylparaben on the isolated trachea of guinea pigs, isolated jejunum of rabbits, and mammalian atrial preparations. Methylparaben induced weak, dose-dependent relaxation of smooth muscle; it did not, however, affect atrial preparations. Subthreshold concentrations significantly enhanced the tracheal response to three catecholamines and two noncatechol sympathomimetics, but did not enhance the response to a xanthine derivative. These results suggest that Methylparaben has a nonspecific spasmolytic action, possibly related to its anesthetic effects. Enhancement of catecholamine response suggested that Methylparaben inhibits extraneural removal of catecholamine. Other data support the lack of interaction with β-receptors by Methylparaben. The authors noted that the direct action of Methylparaben could have clinical implications, since injection of drugs containing as little as 1.5 mg/ml Methylparaben would result in a dose of this compound much greater than that required to augment the catecholamine response.

The effects of Methylparaben and Propylparaben on the ciliary activity of epithelial cells in cultures of ferret tracheal rings were studied. Propylparaben, at 0.06 mg/ml and greater, paralyzed cilia; at 0.5 mg/ml and greater, paralysis was irreversible. Methylparaben was a potent inhibitor of ciliary activity. The authors suggested that topical respiratory anesthesia with Paraben-containing solutions may result in prolonged ciliary paralysis.[188]

Physiological Effects

Bubnoff et al.[189] studied the anticonvulsive and vasodilating effects of Parabens. They reported that Methylparaben and Ethylparaben had anticonvulsive effects in rats with cocaine-induced cramps. Intravenous administration was four times more effective than oral administration in controlling cramps. Methylparaben, Ethylparaben, Propylparaben, and Butylparaben had vascular-widening properties in cat brain blood vessels upon intra-arterial injection. Only slight effects were observed upon intravenous injection. The authors concluded that a relationship may exist between the Parabens' effects as vasodilators and anticonvulsants.

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were tested for surface analgesia in rats, infiltration analgesia in guinea pigs, and conduction anesthesia in frogs. Surface analgesia was studied by applying the Parabens (0.01 percent) to rabbit skin and measuring the response time to stimulation. All Parabens tested had no anesthetic effect. Infiltration analgesia was tested by injecting intradermally 0.25 ml of a 1 percent Paraben solution into the dorsal skin of guinea pigs. Analgesic effect was measured as the time following injection until the animal reacted to three of five pin pricks at the injection site. All Parabens had no siginificant effects. In the conduction anesthesia study, isolated frog muscle-nerve preparations were treated with 1 percent Parabens and then electrically stimulated. Conduction was measured by the electric poten-

tial required to stimulate muscle contraction. Only Butylparaben and Propylparaben significantly (but slightly) inhibited contraction when compared to controls.[190]

Methylparaben was identified by gas chromatography and mass spectroscopy as a component of vaginal secretions of female dogs in estrus. Analysis of secretions at other points of their estrous cycle revealed no presence of Methylparaben. Male and female dogs (not in estrus) were introduced for 5 to 7 minutes, during which time no sexual behavior was exhibited by the males. A small amount of Methylparaben was then applied to the vulva of each female; animals were again paired. In 18 of 21 individual trials, males attempted intercourse following intense anogenital investigation of the females. The authors suggest that Methylparaben is a sex pheromone of the dog.[191]

Animal Toxicology

Acute Toxicity

Oral

Schuebel[192] reported that the acute toxic/lethal oral doses for individual Parabens in dogs and rabbits were as follows: Methylparaben, 2 and 3 g/kg, respectively; Ethylparaben, 4 and 5 g/kg; and Propylparaben, 3 to 4 g/kg and 6 g/kg. Toxicity decreased as the alkyl chain length increased.

A 60:40 mixture of the sodium salts of Propylparaben and Ethylparaben, respectively, was administered orally to groups of 5 to 10 guinea pigs at doses of 4.75 to 6.0 g/kg to determine the minimum lethal dose (the smallest dose required to induce 60 to 80 percent mortality). Animals were observed for 10 days posttreatment. Doses of 5.0 and 6.0 g/kg induced 60 percent mortality, although surviving animals became progressively worse with increasing doses. The minimum lethal dose was determined to be 5.0 g/kg.[193]

The acute oral toxicity of Parabens and their sodium salts was determined in an unspecified number of mice. Test compounds were suspended in 3 percent starch, propylene glycol, or olive oil. Animals were observed for 1 week posttreatment. The acute oral LD_{50}s. were: Methylparaben, >8000 mg/kg; Methylparaben (Na salt), 2000 mg/kg; Ethylparaben (Na salt), 2500 mg/kg; Propylparaben, >8000 mg/kg; Propylparaben (Na salt), 3700 mg/kg; and Butylparaben (Na salt), 950 mg/kg. The authors concluded that as the Parabens' alkyl chain length increased, toxicity increased due to longer hydrolysis times.[194]

Sado[195] studied the acute oral toxicity of Ethylparaben, Propylparaben, Butylparaben, and Paraben combinations in dd-strain mice. The acute oral LD_{50}s for Ethyl-, Propyl-, and Butylparabens were 6.008, 6.332, and 13.200 g/kg, respectively. Additional tests revealed that the toxicity of mixtures did not exceed theoretical values, indicating that these compounds do not exhibit synergistic toxicity.

The acute oral toxicity of Methylparaben was determined in rats. Methylparaben in 0.85 percent saline was administered orally to groups of 5 to 10 rats at doses of 100 to 5000 mg/kg. Animals were observed for 10 days and then killed. All 10 animals receiving 5000 mg/kg died within 24 hours. Necropsy findings included reddened gastric mucosa and congested lungs. No animals died at 100 and 500 mg/kg. The acute oral LD_{50} was 2100 mg/kg. Methylparaben as a 21.8 percent saline suspension was given orally to each of 10 rats at a dose of 5000

mg/kg. Animals were observed for 7 days and then killed. No toxicity, abnormal behavior, or gross lesions were observed; the acute oral LD_{50} was >5000 mg/kg. As a 37 to 79 percent saline suspension, Methylparaben was administered orally to groups of six male rats at doses of 2600 to 5600 mg/kg. Animals were observed for 7 days and then killed. No toxicity, abnormal behavior, or gross lesions were observed; the acute oral LD_{50} was >5600 mg/kg.[196]

Ethylparaben was administered by gastric intubation to groups of four female rats at doses of 2, 20, and 200 mg/kg. Rats were observed for 1 week and then killed. No animals died as a result of treatment, and body weight increased normally. No macroscopic abnormalities were found at necropsy.[197]

Methylparaben was administered by gastric intubation to five female rats at a dose of 15 g/kg. All animals appeared normal throughout the study, and there were no gross lesions at necropsy on the seventh day.[198]

Ethylparaben was tested for acute oral toxicity as a 20 percent dilution in propylene glycol. Doses of 4.64 g/kg or 2.15 g/kg were administered by gastric intubation to groups of five female rats. Three deaths resulted from administration of the higher dose and none from the lower dose. There were no gross lesions at necropsy on the seventh day. The acute oral LD_{50} was 4.30 g/kg.[199]

Product formulations containing various concentrations of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben have been tested for acute oral toxicity in rats. Products containing 0.2 percent or 0.8 percent Methylparaben administered by gastric intubation at doses up to 15 g/kg caused no deaths.[200-204] Products containing 0.2 percent or 0.3 percent Propylparaben caused no deaths when administered at doses of 15 g/kg.[205,206] Products containing both Methylparaben at 0.2 percent and Propylparaben at 0.1 percent had LD_{50} values in excess of 5 g/kg in one study[207] and 98.9 g/kg in another.[208] Products containing 0.2 percent or 0.3 percent Butylparaben produced no deaths when administered orally to rats at doses of 5 g/kg and 25 g/kg, respectively.[209,210] A product containing both 0.2 percent Propylparaben and 0.1 percent Butylparaben produced no deaths when administered at 5 ml/kg to 10 rats.[211] Products containing 0.2 percent Ethylparaben produced no deaths when administered to groups of five rats at a dose of 15 g/kg.[212,213]

Dermal

A hairdressing product containing 0.2 percent Methylparaben was tested for acute dermal toxicity in three male and three female albino rabbits. Doses of 2.0 ml/kg were applied to intact and abraded skin and occluded for 24 hours. No toxic effects were observed for 14 days posttreatment.[214]

The acute dermal toxicity of eye makeup formulations containing 0.2 percent Butylparaben or a mixture of 0.2 percent Methylparaben and 0.1 percent Propylparaben was studied. The LD_{50} values were greater than 2 g/kg.[207,210]

Subcutaneous

Methylparaben was administered subcutaneously to mice in doses up to 333 mg/kg. Doses greater than 165 mg/kg temporarily induced exhaustion, ataxia, and respiratory distress. Because of solubility limitations, higher doses could not be tested. The acute lethal subcutaneous dose was greater than 333 mg/kg, since no animals died from this dose.[215]

The sodium salts of Methylparaben, Ethylparaben, Propylparaben, and Butyl-

paraben were administered subcutaneously to groups of five mice. The resultant acute LD_{50}s were 1.20, 1.65, 1.65, and 2.5 g/kg, respectively.[190]

Groups of eight C57BL/6 mice were given single subcutaneous injections of 125 mg/kg Methylparaben (in tricaprylin). This was the maximum tolerated dose for repeated injection. Injection sites in the majority of animals developed small, ill-defined soft cysts and small ulcerations that later healed.[216]

Methylparaben was administered subcutaneously to five groups of 20 Fischer rats at doses up to 500 mg/kg (10M/10F per group). No animals died and the acute LD_{50} was >500 mg/kg.[217]

Intravenous

Methylparaben was administered to three rabbits at doses of 0.289, 0.69, and 0.92 g/kg. The lowest dose induced a temporary, small drop in arterial blood pressure. The animal receiving 0.69 g/kg had transitory hypotension and reduced respiration. The rabbit that received 0.92 g/kg died.[218]

Methylparaben and Propylparaben were administered intravenously in dogs in increasing doses (1 to 1400 mg/kg), and the effects on the cardiovascular and autonomic nervous system were monitored. The only effect was a sharp but brief fall in blood pressure and a corresponding rise in the jugular venous pressure. Death was associated with the hypotensive action. The rate of injection and the cardiovascular effect were correlated. The Parabens had no effect on the nervous system.[194]

The acute intravenous LD_{50}s in mice of the sodium salts of Methylparaben and Propylparaben were 170 and 180 mg/kg, respectively.[194]

Six A/Jax mice were each given 2.5 mg Methylparaben. Gasping respiration and shock were observed immediately. Animals returned to normal within 90 minutes.[216]

Intraperitoneal

The acute intraperitoneal LD_{50}s in mice for various Parabens and their salts are as follows: Methylparabens, 960 mg/kg; Methylparabens (Na salt), 760 mg/kg; Ethylparaben (Na salt), 520 mg/kg; Propylparaben, 640 mg/kg; Propylparaben (Na salt), 490 mg/kg; and Butylparaben (Na salt), 230 mg/kg. Test animals had fluid in the peritoneal cavity caused by local irritation.[194]

Subarachnoid

Adams et al.[219] studied the effect of 0.1, 0.3, and 1 percent Methylparaben (in saline) on the spinal cords and spinal nerve roots of rabbits following subarachnoid injection. Vehicle and negative controls were also used. Injections were administered to groups of four albino male rabbits; 3 days later, the animals were killed and the spinal cords dissected and examined grossly as well as microscopically. No animal exhibited any overt toxic effects to the Paraben treatment. Although mechanical trauma caused by the injection procedure resulted in morphologic changes in the spinal cords, no abnormalities could be attributed to Methylparaben. The authors concluded that this material produces no neurotoxic effects, even when administered at 10 times the concentration commonly used in parenteral preparations.

Subchronic Toxicity

Oral

Bijlsma[215] administered 18 mg/kg/day Methylparaben to a dog for 28 days and 53 mg/kg/day to another dog for 4 days. The animals were killed at the end of the study. No toxicity was reported, and no gross lesions were noted upon necropsy.

Ethylparaben was administered orally to groups of 10 rats (5M/5F per group) at concentrations of 2.0, 1.0, and 0.2 percent in the diet for 25 weeks. During the test, no significant differences in general appearance, behavior, food consumption, mortality, or survival times were observed between experimental and control groups. Significant increases in mean body weight occurred in males at the 0.2 percent level from Weeks 22 to 25. Significant decreases were observed in males at the 1.0 and 2.0 percent levels. Values for erythrocyte numbers, hemoglobin, hematocrit and white blood cell counts were normal in all animals throughout the study. No macroscopic or microscopic abnormalities were observed[195]

Ethylparaben was administered by gastric intubation to three groups of four female rats at doses of 2, 20, and 200 mg/kg for 6 consecutive days. After this time, animals were killed for necropsy. In this study, no animals died, body weight increased, and no abnormalities were observed upon necropsy.[197]

A product formulation containing 0.2 percent Methylparaben and 0.2 percent Propylparaben was administered orally to groups of 10 male and 10 female rats at doses of 0, 40, or 200 mg/kg/day for 1 month. The test material was prepared as a 2 percent and 10 percent dispersion in corn oil and administered daily in dose volumes of 2 ml/kg. An equal volume of corn oil was given to control rats. All but one rat survived, and there were no signs of toxicity in the survivors. The one high-dose male rat that died had pneumonia, presumably caused by test material accidentally placed in the trachea. Body weight gain and food consumption were unaffected by treatment. Slight changes in hematologic and blood chemistry values and organ weights were not biologically significant. Microscopic examination of the tissues revealed no treatment-related changes.[220]

A product formulation containing 0.2 percent Propylparaben and 0.1 percent Butylparaben was tested in a 1-month oral toxicity assay identical to the one described above. All animals survived, and there were no signs of toxicity. Body weight gain, food consumption, and hematologic values were similar for treated and control animals. Slight changes in blood chemistry and organ weights were considered toxicologically insignificant. Microscopic examination of the tissues revealed no treatment-related changes.[221]

Dermal

A 3-month dermal toxicity study was conducted to test the effects of daily dermal exposure to a product formulation containing 0.2 percent Methylparaben. A treatment group of five male and five female albino rabbits received daily topical doses of 5.5 mg/cm^2/8.4 percent body surface area; an untreated group of seven males and seven females served as a control. The product caused persistent well-defined to moderate erythema, slight edema, and intermittent slight desquamation. Three test animals died during the study of conditions unrelated to treatment. Body weight gain, food consumption, hematologic, and blood

chemistry values were unaffected by treatment. The presence of glucose and blood in the urine of some untreated and treated rabbits was considered clinically unimportant. Histopathologic examination of tissues of all animals was negative for treatment-related changes other than mild inflammation at the application site.[222]

A 3-month dermal toxicity study similar to that described above was conducted on another product formulation containing 0.2 percent Methylparaben. The formulation was administered to groups of five male and five female rabbits at doses of 6.6 mg/cm²/8.4 percent body surface area and 11 mg/cm²/8.4 percent body surface area. The product caused persistent well-defined to moderate erythema, slight edema, and intermittent slight desquamation. Two untreated control animals died during the study; all treated animals survived. Body weight gain, food consumption, hematologic, blood chemistries and urinalysis values, and organ weights were negative for toxicologically significant changes. No treatment-related changes other than mild inflammation at the application site were found.[223]

A 3-month dermal toxicity study similar to those described above was conducted on a product formulation containing 0.2 percent Methylparaben and 0.2 percent Propylparaben. Rabbits were assigned to two untreated control groups and three treatment groups. Each group contained six or eight animals, with an equal distribution of males and females. The formulation was administered at doses of 2 mg/cm²/10 percent body surface area and 6 mg/cm²/10 percent body surface area. After dosing, rabbits in one control group and one group treated with 6 mg/cm² of the product were exposed daily to one-half the minimal erythema dose of ultraviolet light (4 minutes at 6 inches from Westinghouse FS-20 lamps producing a continuous spectrum from 2800 to 4000 A). The product caused persistent moderate erythema, slight edema, and mild desquamation. Epidermal fissures with bleeding and papuloerythema were observed occasionally. The high dose was slighly more irritating than the low dose. Ultraviolet light exposure had no apparent effect on the severity of the irritation. Two test animals died during the study of conditions unrelated to treatment. Body weight gain, food consumption and hematologic, blood chemistries, and urinalysis values were negative for toxicologically significant findings. Mild to severe dermal inflammation and hyperkeratosis with acanthosis were found at microscopic examination of the skin. There were no significant effects produced by ultraviolet light exposure.[224]

A 13-week of dermal toxicity study in rats was conducted on one product formulation containing 0.7 percent Methylparaben and another containing 0.3 percent Propylparaben. Groups of 10 rats received daily topical doses of 4.12 g/kg; a control group consisted of 10 untreated animals. All applications were made to the anterior dorsal shaved skin, which represented 10 to 15 percent of the total body surface area. All animals survived the full term of the study. Significant depression in body weight gain was noted for males of both test groups. Slight changes in hematologic and blood chemistry parameters and organ weights were considered toxicologically insignificant. Significant gross and histopathologic changes were limited to the treated skin site. The investigators concluded that there were no cumulative systemic toxic effects from these products.[225]

Chronic Toxicity

Oral

A 60:40 mixture of the sodium salts of Propylparaben and Ethylparaben, respectively, was fed to rats for 18 months. Forty rats were given 0.014 g/kg/day. At 2 and 4 months, 10 rats each were killed for necropsy and collection of tissues for histopathologic examination. At 18 months, the remaining animals were killed. Two groups of 20 rats each received 0.14 or 1.4 g/kg/day for 18 months and then were killed for necropsy. The mixture, even when fed at 1.4 g/kg/day did not induce significant pathologic changes when compared to control groups. At the highest dose tested, a significant decrease in body weight gain was observd from months 4 to 8. Some evidence of growth stimulation was observed at the lower doses.[226]

In a chronic oral toxicity study, Methylparaben and Propylparaben were incorporated into the diets at 2 or 8 percent and the diets fed to groups of 24 rats for 96 weeks. Ethylparaben and Butylparaben were fed to the same numbers of rats at concentrations of 2 or 8 percent in the diet for 12 weeks. Negative controls were included in the study. Rats, especially the males, fed the 8 percent Methylparaben or Propylparaben diets had decreased weight gain in the early part of the study. At 8 percent dietary concentration, Ethylparaben reduced growth rate, decreased motor activity, and, in some cases, caused death within the first week. All males fed 8 percent Butylparaben died before the twelfth week. Females fed this diet exhibited signs of toxicity. At 2 percent of the diet, Parabens exerted no toxic effect. Rats killed at the conclusion of the feeding test had no treatment related abnormalities.[194]

Weanling dogs were dosed as follows: six dogs, 1 g/kg/day Methylparaben or Propylparaben for 378 to 422 days; and three dogs, 0.5 g/kg/day Methylparaben or Propylparaben for 318 to 394 days. Two untreated dogs served as a control group. All dogs were killed for necropsy upon completion of the feeding. No toxicity to the Parabens was observed. All animals were in excellent condition throughout the experiment. All tissues were normal.[194]

Subcutaneous

Methylparaben at doses of 3.5, 2.0, 1.1, and 0.6 mg/kg was administered to groups of 80, 60, 40, and 20 Fischer rats, respectively, twice weekly for 52 weeks. At this time, some animals were killed; others were observed for an additional 6 months and then killed for necropsy. Toxicity was determined by survival time, weight changes, and drug-related organ changes. When compared to controls, Paraben-treated rats had no significant differences in mortality, weight gain or lesions.[217]

Primary Irritation

Skin

Pastes containing hydrophilic ointment and either 10 percent Methylparaben or Propylparaben were applied to the shaved backs of albino rabbits for 48 hours. No irritation was observed. Animals were then killed and their kidneys removed for analysis of Paraben metabolites. Methylparaben, Propylparaben and their degradation products were not detected.[22]

Undiluted Methylparaben was tested with the Draize skin irritation technique using nine rabbits. A 0.1 ml sample of the ingredient was applied to the shaved skin and occluded for 24 hours. The resultant Primary Irritation Index (PII) was 0.67 (maximum score 4.0), a value indicative of mild skin irritation.[227]

The Draize skin irritation technique was used to test Ethylparaben at 100 percent and at 10 percent in water on groups of nine rabbits. The undiluted and diluted ingredient produced no signs of irritation; the PII was 0.0.[228] Several Draize rabbit skin irritation tests have been conducted on product formulations containing the Parabens. Product formulations containing 0.2 to 0.8 percent Methylparabens produced PIIs of 0.0 to 1.0 (out of 4.0), values indicative of no to mild irritation. There was no relation between the concentration of Methylparaben and degree of irritation.[229-233] A product containing 0.2 percent Propylparaben produced minimal irritation with a PII of 0.5.[234] A product containing both 0.2 percent Methylparaben and 0.1 percent Propylparaben was minimally irritating with a PII of 0.5.[207] A product containing 0.2 percent Butylparaben was reported to be nonirritating, but the PII of 2.75 indicates moderate irritation.[210] There were no signs of irritation with a product formulation containing 0.2 percent Propylparaben and 0.1 percent Butylparaben.[211] Products containing 0.2 percent Ethylparaben produced minimal to mild irritation with PIIs of 0.17 to 0.56.[235,236]

A product formulation containing 0.3 percent Propylparaben was applied daily to the shaved skin of nine albino rabbits for 4 consecutive days. The product produced minimal irritation with a maximum PII of 0.5 (maximum score 4.0).[237] A product containing 0.3 percent Butylparaben was similary tested on the backs of six rabbits for 3 consecutive days. Almost all rabbits showed mild irritation with grade ¼ erythema and/or edema.[238]

Eye

Methylparaben, at concentrations up to 0.20 percent, was instilled in the eyes of rabbits. At the highest concentration tested, Methylparaben induced slight, transient conjunctival hyperemia.[218] In an investigation concerning the irritancy of various ophthalmic drug ingredients, 0.1 to 0.2 percent Methylparaben in isotonic solution did not induce ocular irritation when instilled in the eyes of rabbits and guinea pigs.[239]

Methylparaben at 100 percent concentration was instilled into the eyes of six albino rabbits. The ingredient produced slight transient irritation with an eye irritation score of 1/110 on Day 1.[240]

Ethylparaben at 100 percent and 10 percent in water was instilled into the eyes of two groups of six albino rabbits. The undiluted ingredient was slightly irritating, with a maximum eye irritation score of 2/110 on Day 1. The diluted ingredient produced no signs of irritation.[241]

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 percent. Most products produced no signs of eye irritation.,[207,210,242-248] Other products produced slight or minimal eye irritation, with scores of 1.0 to 3.3/110.[211,249-252]

Mucous Membrane

A product formulation containing 0.2 percent Propylparaben and 0.1 percent Butylparaben was applied to the genital mucosa of six albino rabbits. The

single 0.1 ml application of the undiluted product produced no evidence of mucosal irritation during the 7-day observation period.[211]

Subchronic Irritation

A hairdressing product formulation containing 0.2 percent Methylparaben was tested in a 21-day dermal irritation study. A volume of 0.5 ml of the undiluted product was applied topically to the intact and abraded skin of six albino rabbits once a day for 21 days. Twenty-four hours after each application and prior to the next application, the skin sites were examined and scored for erythema and edema according to the Draize scale. The abraded sites were reabraded once a week, and the hair was clipped as needed. The test material initially produced slight irritation, which increased to mild to moderate by the end of the first week and remained moderate throughout the remainder of the study. This degree of irritation was considered typical for this type of product.[253]

Sensitization

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben (0.1 percent in saline) were injected intracutaneously into an unspecified number of guinea pigs, three times weekly for 3 weeks (10 injections). No reaction was observed 24 hours after the first injection. Two weeks following the last induction injection, a challenge injection was administered into an adjacent site and observed for 48 hours. No allergic response was induced by any of the Parabens.[22]

The same four Parabens (at 0.1 percent) were each injected intracutaneously into the shaved dorsal skin of 10 guinea pigs per ingredient according to the Draize method. Injections were made three times weekly for 3 weeks (10 injections). Two weeks after the final induction injection, a challenge injection was administered into an adjacent site and observed 24 hours later. There were no reactions in the animals to any of the Parabens. It was observed that these ingredients are nonsensitizing.[194]

In a procedure described by Marzulli et al.,[254] dinitrochlorobenzene (DNCB)-hypersensitive guinea pigs were given intradermal injections or occlusive topical patches of Methylparaben or Propylparaben solutions every other day for 3 weeks (10 applications). Two weeks after the last induction application, a challenge was administered; reactions to challenge and induction phases were compared. DNCB (0.5 ml) was then injected intradermally into each animal. Two weeks later, 0.5 and 1.0 percent DNCB were applied to two sites per animal. Only the results of those guinea pigs showing a hypersensitivity to DNCB were used to evaluate Paraben hypersensitivity. None of the 23 DNCB-sensitive animals was sensitized to 3 percent Propylparaben by the intradermal route at induction and both intradermal and topical routes at challenge. None of the 21 DNCB-sensitive animals was sensitized to Methylparaben 5 percent intradermally at induction, and 1 percent intradermally or 10 percent topically at challenge.

Methylparaben (0.1 percent) was injected intradermally into the shaved dorsal skin of four guinea pigs 5 days per week for 8 weeks. Sites were scored 24 hours after each injection. Results indicated that the frequency as well as the intensity of positive skin reactions decreased slightly with repeated exposures, suggesting a desensitizing effect.[255]

Twenty albino guinea pigs were given intradermal injections of Freund's

complete adjuvant on Days 0 and 9; 5 percent Butylparaben was applied under 48-hour occlusive patches to the clipped dorsal skin every other day for 3 weeks (10 applications). Twelve days after the last induction patch was removed, the test material was applied as a challenge patch for 48 hours to a previously untested site. One, 7, 24, and 48 hours after removal of the patch, the sites were scored and the skin examined microscopically for evidence of sensitization. Six of the 20 animals reacted to the challenge patch containing 5 percent Butylparaben in olive oil. The mean erythema score was 1.70 (maximum score = 4). Tissue from two of the six animals showed "pathologic aspects" under microscopic examination, and the lesions were considered clearly allergic. In the worst case, spongiosis, squamous crust, and lymphocytic infiltration were observed.[256]

Methylparaben (0.1 percent) was injected intracutaneously every other day for 3 weeks (10 injections) into the dorsal skin of each of 20 guinea pigs. Sites were scored 24 hours postinjection. During the second and third weeks of induction, Methylparaben was incorporated at 0.1 percent in Freund's complete adjuvant and saline. Two weeks after the last induction injection, a challenge injection was admininstered. The site was scored at 24 hours and compared to induction reactions. Ten days later, a 5 percent Methylparaben challenge patch was applied to the skin site, which was scored for irritation 24 hours later and compared to controls. Three of the 20 guinea pigs reacted to the intradermal challenge, whereas four animals reacted to the challenge patch. These frequencies were not considered significant when compared to control values.[257]

The Magnusson–Kligman guinea pig maximization test[258] was used to determine the sensitization potentials of Methylparaben and Ethylparaben. The procedure calls for a complex protocol of induction, dose range, booster, and challenge phases of the experiment. A total of 80 female guinea pigs were used. Freund's complete adjuvant and sodium lauryl sulfate were used to potentiate the allergic response in the guinea pig. Phenylacetaldehyde served as a positive control. The reader is referred to the original article by Magnusson and Kligman[258] for further details of the procedure. Neither Methylparaben nor Ethylparaben showed the potential to elicit contact sensitization in the guinea pig. [259]

A product formulation containing 0.2 percent Methylparaben was tested for contact sensitization using five male and five female guinea pigs. A dose of 0.5 ml was administered topically to the shaved backs of the animals and the application site occluded for 6 hours. Applications were made three times per week for a total of nine. A challenge application was made on an untreated site 14 days after the last induction patch. Slight irritation was observed during the induction phase, but no reactions were observed at challenge.[260]

Special Studies

Mutagenesis

Three different assays, a host-mediated assay, a cytogenic assay, and a dominant lethal assay, were used to evaluate the mutagenicity of Methylparaben in one study.[196] The host-mediated assay consisted of three parts, an acute in vivo test, a subchronic in vivo test, and an in vitro study. In the acute test, 0 to 5000 mg/kg Methylparaben was administered orally to each of 10 mice. Positive and negative controls were used. Animals then received intraperitoneally 2 ml *Salmonella typhimurium* strain TA1530 and 2 ml *Saccharomyces cerevisiae* strain

D-3 indicator organisms. Animals were killed 3 hours later, and peritoneal fluid was extracted, bacterial counts were made, and the number of mutants were recorded. In the subchronic test, each of 10 mice received orally 0 to 3500 mg/kg Methylparaben daily for 5 consecutive days. Within 30 minutes after the last treatment, animals were inoculated with indicator organisms and treated as above. In the in vitro study, 0 to 100 μg/ml Methylparaben were added to plates containing the indicator organisms. After incubation, the number of mutants was recorded. Methylparaben induced no significant increases in mutant or recombinant frequencies with *S. typhimurium* or *S. cerevisiae* in these in vitro or in vivo host-mediated assays.[196]

The cytogenic assay also consisted of acute and subchronic in vivo tests and an in vitro study. In the acute test, groups of 15 rats were given 5 to 5000 mg/kg Methylparaben by gastric intubation. Four hours later, each animal received intraperitoneally 4 mg/kg colcemid to arrest bone marrow cells in C-mitosis. Five animals at each dose level were killed at 6, 24, and 48 hours. Bone marrow was removed and the chromosomes of cells evaluated for abnormalities. Positive and negative controls were used. In the subchronic study, groups of five mice received 0 to 5000 mg/kg Methylparaben daily for 5 consecutive days. Animals were killed 6 hours following the last dosing, and tissue was taken for evaluation as above. In the in vitro study, 1 to 100 μg/ml Methylparaben were added to cultures of human embryonic lung cells in anaphase. Positive and negative controls were used. Chromosomal damage was then evaluated. Methylparaben induced no detectable aberrations in the chromosomes of the rat bone marrow cells in metaphase and induced no significant aberration in the anaphase chromosomes of human lung cells in culture. The investigators noted that fewer mitoses were observed in the bone marrow cells of animals treated with 5000 mg/kg/day for 5 days. They suggested that Methylparaben may interfere with mitosis when administered subchronically at high dosages.[196]

In the dominant lethal assay, groups of 10 male rats received orally 0 to 5000 mg/kg Methylparaben once (acute study) or daily for 5 consecutive days (subchronic study). Positive and negative controls were used. Following treatment, males were mated with two virgin females per week for 7 or 8 weeks. Pregnant females were killed 14 days after separation from treated males, and uteri were examined for deciduomata, late fetal deaths, and total implantations. No dose-response or time-trend patterns that would suggest a dominant lethal effect for Methylparaben were observed. Methylparaben was nonmutagenic under the conditions of the study.[196]

The Ames Test was used to study the mutagenic potential of Propylparaben. *S. typhimurium* strains TA100, TA98, TA1535, and TA1537 were used. Assays were performed with and without Aroclor 1254-induced rat liver microsomal enzymes (S-9). When tested at doses of 10 to 2000 μg/plate, Propylparaben was nonmutagenic both with and without metabolic activation.[261]

The Ames Test was used to evaluate the mutagenic potential of Propylparaben in *S. cerevisiae* stain D-4 and in *S. typhimurium* strains TA1535, TA1537, and TA1538. Assays were performed in the presence and absence of mouse, rat, and monkey liver, lung, and testes homogenates. In plate tests, 0.075 percent Propylparaben was added to cultures. In suspension tests, 0.025 to 0.15 percent Propylparaben was used. Propylparaben was nonmutagenic with and without metabolic activation in all assays.[262]

In a modified Ames Test, Propylparaben in dimethyl sulfoxide (DMSO) was

added to cultures of *S. typhimurium* strains TA100 and TA98, as well as *E. coli* strain D-2. Assays were performed in the presence and absence of PCB-induced rat liver microsomal enzymes. Propylparaben was nonmutagenic in all strains when assayed directly but was mutagenic in strain TA100 under metabolic activation.[263]

Ishizaki et al.[19] reported that when Butylparaben (1 percent) is combined with potassium nitrate or sodium nitrite and irradiated for 5 days, butyl 3-nitro-4-hydroxybenzoate is formed. This reaction product was found to be mutagenic in a "rec-assay" with *B. subtilis.* When tested in the same mutagenic assay, Butylparaben alone was nonmutagenic.

In a poorly documented study, Propylparaben was evaluated for mutagenicity in an in vivo cytogenic assay, an Ames or modified Ames Test, and a bacterial repair test. In the cytogenic assay, mice were given one minimum lethal dose of Propylparaben and killed 6 to 48 hours later. Bone marrow cells chromosomes were examined for aberrations. Mutagenic activity was evaluated in *S. typhimurium* strains TA1535, TA1536, TA1537, and TA1538, and repair testing was performed with bacterial strains H-17, M-45, and WP-2. In some instances, bacterial assays may have been run with and without metabolic activation. In all assays except the repair test, Propylparaben was nonmutagenic.[264]

Chromosomal Aberration Studies

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were studied for their ability to induce chromosomal aberrations in Chinese hamster cells in vitro. Each Paraben at different doses was applied directly to cells; chromosome preparations were made 24 to 48 hours later and aberrations scored. The maximum tolerated doses for Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were 0.50, 0.25, 0.125, and 0.06 mg/ml, respectively. All esters except Methylparaben induced 1 to 3 percent increases in polypoid cell production. Frequency increased as the Paraben alkyl chain length increased. Of the four Parabens tested, Ethylparaben and Methylparaben were judged to induce significant chromosomal aberrations (11.0 and 15.0 percent increases, respectively). Aberrations observed included chromatid breaks, chromatid gaps, chromosomal exchanges, and ring formations.[265]

Matsuoka et al.[266] studied the potential of Methylparaben to induce chromosomal aberrations in Chinese hamster lung cells in vitro. Cells were treated with 0.125 mg/ml Methylparaben in the presence and absence of polychlorinated biphenyl (PCB)-induced rat hepatic cell microsomes (S-9 mix). Chromosome preparations were then made and aberrations were scored. When assayed without S-9 mix, induction of chromosomal aberrations was negative (1 percent). In the presence of S-9 mix, however, aberration incidence increased to 13.0 percent and was judged to be significant. Gaps, breaks, exchanges, and rings were observed. The significance of these effects cannot be assessed.

Carcinogenesis

One hundred male C57BL/6 mice were given 2.5 mg Methylparaben (in tricaprylin) injected subcutaneously into the groin. Five weeks later, injection site skin was excised, minced, and pooled. The resulting mix was injected subcutaneously into each of 25 C57BL/6 males. Eighteen weeks later, animals were killed and examined microscopically for evidence of tumors. Throughout the

study, positive and negative controls were used. Six of the 25 test animals died by the eighth week. By the tenth week, 12 animals had died. Cause of death was not determined. At the injection sites, multiple granulomas with numerous giant cells scattered throughout the tissue were observed. Scar tissue and numerous cysts were present. There were no instances where fibroblasts in granulation or scar tissue suggested malignant transformation. The author concluded that Methylparaben was not carcinogenic under these test conditions.[216]

In a second, more sensitive study, 2.5 mg Methylparaben were injected as a single dose into the tail vein of each of 50 CF-1 strain A and 50 A/Jax female mice. An additional 20 CF1 female mice received intraperitoneal injections of 2.5 mg Methylparaben daily for 7 months. Positive and negative controls were used. All mice were killed at 7 months, and the lungs were examined for the presence of tumors. Methylparaben did not significantly increase pulmonary adenoma formation as compared to controls.[216]

In a cocarcinogenesis study, each of 50 C57BL/6 male mice were given 12.5 μg dibenzo[a,i]pyrene (DBP) in tricaprylin injected subcutaneously. Twenty-four hours later, 2.5 mg Methylparaben was injected in the same site. Additional injections of Methylparaben were made 7 and 14 days later. Positive and negative controls were included. All animals were killed at 29 to 31 weeks. Sites were examined microscopically for tumors. Methylparaben was not cocarcinogenic. However, since the positive control compound (croton oil) had no effect, the authors decided that the test was inconclusive.[216]

Weanling Fischer rats were placed into groups (equal males and females) of 80, 60, 40, and 20 animals and given subcutaneous injections of 3.5, 2.0, 1.1 and 0.6 mg/kg Methylparaben, respectively, twice weekly for 52 weeks. Positive, negative, and vehicle controls were used. All animals were necropsied after they died or were killed for necropsy 26 weeks posttreatment. Of all tumors observed in Methylparaben-treated rats, only mammary fibroadenoma incidence was significantly higher than negative control groups (8 percent incidence for Methylparaben; 1 percent for negative control). The incidence of injection site tumors, pituitary adenomas, uterine polyps, and leukemias did not differ significantly from controls.[217]

In a poorly documented study, Propylparaben was evaluated for carcinogenicity with a transplacental assay and a newborn assay. In the former, pregnant rodents were given orally the maximum dose not causing abortion or early death of neonates. Animals were treated every other day for 5 days during the Days 15 to 19 of gestation. Sucklings were observed for 1 year after birth for tumor development. In the newborn study, four subcutaneous injections of Propylparaben (total dose = LD_{20}) were administered to rodent pups on Days 1, 8, 15, and 22 following birth. Sucklings were observed for 1 year after birth for tumor development. In both studies Propylparaben was noncarcinogenic.[264]

Teratogenesis

The teratogenic effects of Methylparaben were studied in rats, mice, and hamsters. Groups of 21 to 25 pregnant animals were given orally 5.0 to 550 mg/kg (rats, mice) or 3.0 to 300 mg/kg (hamsters) Methylparaben from Day 6 of gestation to Day 10 (hamsters) or 15 (rats, mice). Positive and negative controls were used. Animals were observed for signs of toxicity, and body weight was monitored. On gestation Day 14 (hamsters), 17 (mice), or 20 (rats), all females

were subjected to Caesarean section. Numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. Urogenital tracts of females were examined for abnormalities. All fetuses were examined for visceral, skeletal, and external abnormalities. Oral administration of up to 300 mg/kg Methylparaben for 5 consecutive days in hamsters or up to 550 mg/kg for 10 consecutive days in rats and mice had no effect on nidation or on maternal or fetal survival. The number of visceral, skeletal, and external abnormalities observed in the test group fetuses did not differ significantly from that of negative control groups.[267]

A similar teratologic study was performed on groups of 9 to 11 pregnant rabbits given orally 3.0 to 300 mg/kg Methylparaben daily from Day 6 of gestation to day 18. Positive and negative controls were used. Test animals and fetuses were examined as above. Results indicated that ingestion of up to 300 mg/kg Methylparaben for 13 consecutive days during gestation had no effect on nidation or maternal or fetal survival. The number of visceral, skeletal, and external abnormalities observed in the test group fetuses did not differ significantly from negative control groups.[268]

Ethylparaben was added to the feed of groups of 12 pregnant rats at concentrations of 0.1, 1, or 10 percent between gestation Days 8 and 15. On Day 21 of pregnancy, rats were killed, and the number of fetal implantations, status of maternal visceral organs, fetal body weights, and numbers of skeletal, visceral, and external defects in fetuses were recorded. In addition, two groups of six pregnant rats each were given 0.1 of 10 percent Ethylparaben administered in their feed for 1 week during gestation Days 8 to 15. Neonates were nursed by test dams for 1 month; growth, body weight, and abnormalities were recorded. No apparent teratogenesis or toxicity was observed in 363 fetuses from rats fed up to 10 percent Ethylparaben. At the 10 percent level, many fetuses had cerebral hemorrhages, abnormal enlargement in the ventricles of the brain, and, in some, hydronephrosis and hypo-osteogenesis. Some fetuses at 1 percent Ethylparaben had no blood in the cardiac ventricle; some had intraperitoneal hemorrhages. Incidence of visceral and skeletal abnormalities was considered to be insignificant when compared to that in control animals. Fetuses of rats of the 0.1 percent group had no significant visceral or skeletal defects. Neonates whose mothers had been given 0.1 or 10 percent Ethylparaben for 1 week during gestation grew normally. None had malformations or abnormal behavior. The authors concluded that at concentrations up to 10 percent, Ethylparaben was nonteratogenic.[197]

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were each applied to the backs of 50 humans at concentrations of 5, 7, 10, 12, and 15 percent in propylene glycol. Test compounds were applied daily for 5 days, and patches were then removed and the sites scored. The concentrations of individual Parabens that produced no irritation were Methylparaben, 5 percent; Ethylparaben, 7 percent; Propylparaben, 12 percent; and Butylparaben, 5 percent. Higher concentrations produced some evidence of irritation. In a repeated insult patch test (RIPT), each Paraben at the "no effect" concentration above was ap-

plied to the skin of 50 subjects (25M/25F) for 4 to 8 hours every other day for 3 weeks (10 applications). Following a 3-week rest, the materials were reapplied at induction concentrations for 24 to 48 hours. No sensitization was reported.[22]

An RIPT was used to test the sensitizing potential of mixtures of Methylparaben and Propylparaben in males. The test mixture was applied under occlusion to the subject's arm for 48 hours; the solution was then reapplied. This procedure was repeated for 3 weeks (10 induction applications). At the highest Paraben concentration tested, one group was alternately irritated by topical application of 5 percent sodium lauryl sulfate (SLS) under occlusion for 24 hours, followed by application of Parabens for 48 hours. Five such cycles were used for induction. Following a 2-week rest, the test mixtures were reapplied under 72-hour challenge patches. On one skin site in all subjects, 10 percent SLS was applied for 1 hour before challenge application. At another site, no SLS was used. Results are summarized in Table 5. With a total sensitization of 0.3 percent, the authors concluded that sensitization to Parabens is not a problem in this country where these compounds are used at 0.1 to 0.3 percent in topical medicaments.[254,269]

In 1940, the first case of contact dermatitis caused by Parabens was reported in Denmark. A patient became sensitized to an ointment containing 5 percent Ethylparaben. By 1963, Hjorth and Trolle-Lassen[270] had reported over 140 cases of Paraben sensitivity. The incidence, which appeared to be higher in Denmark than in the US., was ascribed to the use of higher concentrations of Parabens in Denmark than in the US.[271]

Hegyi[272] studied the tendency toward increased incidence in Paraben contact allergy. From 1968 to 1972, a 0.3 percent incidence of Paraben sensitization was reported. From 1973 to 1977, the incidence increased to 1.5 percent. Marzulli and Maibach[269] and Fisher[273] agree that the incidence of Paraben contact sensitization in healthy Americans is low. They concluded that cases of Paraben sensitivity are low considering the extensive use of these materials and that topically applied Parabens do not pose any significant hazard to the public. Evans[274] observed that, in most cases, individuals who are sensitive to Parabens have chronic dermatoses that may be in continual contact with these ingredients.

Fisher[275] coined the term "Paraben Paradox." He observed that 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 sensitization is induced even when these cosmetics contact the thin, delicate membrane of the eyelid. He noted that cosmetics are usually applied to normal

TABLE 5. Paraben Sensitization Results.[254]

*Concentration in Petrolatum (%)**	*No. Sensitized to Challenge*	
	Without SLS	*With SLS*
0.2M + 0.05P	0/102	0/102
1.0M + 0.25P	0/101	0/101
5.0M + 1.25P	1/98	1/98
10.0M + 10.0P	0/74	0/74
10.0M + 10.0P†	0/22	—

*M = Methylparaben; P = Propylparaben.
†SLS induction phase.

TABLE 6. Results of Paraben Patch Tests.

*Ingredient**	*Conc. Tested (%)*	*No. of Subjects*	*Previous Sensitivity or Dermatitis Y/N*	*M/W*	*Procedure*	*Reactants*	*Reference*
Paraben mix	15 in pet.	2061	Y	—	Patch test	44 (2.1%)	280
Paraben mix	15 in pet.	1862	Y	716/1146	Patch test	40 (2.1%)	281
M+P	1	60	Y	14/46	Patch test	7 (11.7%)	277
Paraben mix	14	5799	Y	—	Patch test	(1.13%)	270
E	5	5799	Y	—	Patch test	(1.15%)	270
Paraben mix	15 in pet.	4097	Y	—	24-hour chamber	14 (0.3%)	282
Paraben mix	15	192	Y	—	48-hour chamber	7 (3.6%)	283
M+E+P	15(5 each) in pet.	100	Y	—	Patch test	3 (3%)	273
M+E+P+Bu	12(3 each) in pet.	4000	Y	—	24-hour patch (2000 subjects) 48-hour patch (2000 subjects)	(1.3% in males) (2.3% in females)	284
M+E+P+Bu	12(3 each) in paraffin	1000	Y	477/523	Patch test	6W/4M(1.15% W/0.84% M)	285
Paraben	5 in pet.	30	N	—	Patch test	0	286
Paraben	5 in pet.	273	Y	—	Patch test	2 (0.8%)	287
Paraben	5 in pet.	260	N	—	Patch test	0	287
M+E+P	2 in lanolin	148	Y	—	Patch test	45 (30.4%)	278
M+E+P	15(5 each) in pet.	1200	Y	—	48-hour patch test	38 (3%)	280
M+E+P	30(10 each) in pet.	4825	Y	—	24-hour patch test	91 (1.9%)	288
Parabens	1	210	Y	—	Standard epicut. test	43 (20.5%)	279
Parabens	1	160	N	—	Standard epicut. test	0	279
Paraben mix	15 in paraffin	1312	Y	603/709	48-hour patch test	18M/13F(3.0% M/ 1.86% F)	289
M+E+P	15(5 each) in kaolin	91	Y	—	Patch test	4 (4.4%)	290

*M = Methylparaben; E = Ethylparaben; P = Propylparaben; Bu = Butylparaben.

skin while therapeutics are applied to damaged skin. He concluded that ". . . many women who are allergic to the Parabens can utilize Paraben-containing cosmetics without any reactions providing the skin is normal and not been subjected to a dermatitis in the past." Fisher has also stated that Paraben-sensitive people can usually tolerate injectable solutions containing Parabens.[276]

Table 6 summarizes results of patch tests of Parabens on patients with and without skin problems. Of 27,230 patients with dermatitis, only 2.2 percent were sensitized by patches containing 1 to 30 percent Parabens. These statistics include the three clinical studies.[277-279] The high percentages of reactants resulted from the selection of patients with high sensitivity toward "para-agents," a group of compounds in which Parabens are considered a member. None of the 450 subjects with normal skin developed a sensitivity to Parabens.

Thirty-seven patients with recurrent urticaria were each given orally a tablet containing 100 mg Methylparaben plus 100 mg Propylparaben on Day 1 and a tablet containing 150 mg of each Paraben on Day 2. Five subjects exhibited reactions to Paraben treatment.[291] Sensitization reactions were reported as a result of paste-bandages containing Parabens applied to venous stasis ulcer.[292] Methylparaben and Ethylparaben, in increasing concentrations, were studied for their effect on the oral mucous membrane of 39 subjects. The "toxic limit concentrations" for Methylparaben and Ethylparaben were 5 and 10 percent, respectively. One subject had a reaction of the oral mucous membrane to Methylparaben.[293] Larson[294] has determined that, as a sensitizer, Methylparaben is too small to act as an antigen and, instead, acts as a hapten that binds to tissue protein to form a complex that is antigenic. Wuepper[290] reported cross-reactivity to the Parabens. Four patients with known Paraben sensitivity were patch-tested with Methylparaben, Ethylparaben, Propylparaben, and Butylparaben (5 percent in petrolatum). In addition, three of these patients were patch-tested with 0.1 and 1 percent of each Paraben and 0.1, 1, and 5 percent *p*-hydroxybenzoic acid. These subjects were also given 0.1 ml *p*-hydroxybenzoic acid intradermally. Results revealed cross-reactivity to each of the Paraben esters. All four patients reacted to one or more of the esters at 5 percent; only one patient reacted at 0.1 percent. One patient had positive reactions to intradermal and topical *p*-hydroxybenzoic acid.

A number of product formulations containing various Parabens at concentrations of 0.1 to 0.8 percent have also been tested for human skin irritation. The results and other details of these studies are summarized in Table 7. Single insult occlusive patch tests on three formulations produced no or only minimal irritation.[295-297] A 5-day cumulative irritancy test on a hairdressing showed no irritation.[298] Daily skin patching of seven product formulations for 20 or 21 days produced ratings of "essentially nonirritating" to "moderately irritating."[295,299-304] Controlled use of two eye makeup formulations for 4 weeks produced no irritation.[305,306] Results indicative of irritation from product formulations are difficult to interpret with respect to a single ingredient.

Several product formulations containing the Parabens have been tested for skin sensitization on a total of 3455 human subjects using a variety of test methods. These studies included four Schwartz-Peck prophetic patch tests on product formulations containing both 0.2 percent Methylparaben and 0.1 percent Propylparaben or a 0.2 percent Butylparaben, 25 Draize-Shelanski repeated insult patch tests on product formulations containing 0.1 to 0.8 percent Methyl-, Propyl-, Butyl-, and/or Ethylparaben, and two Kligman maximization tests on product formulations containing both 0.2 percent Methylparaben and

TABLE 7. Clinical Skin Irritation Tests with Product Formulations Containing Parabens.

Test Method	*Material Tested*	*Conc. of Paraben (%)*	*No. of Subjects*	*Results*	*Reference*
24-hour single insult occlusive patch	Unspecified product formulation	0.8—Methylparaben	20	No signs of irritation	296
	Unspecified product formulation	0.8—Methylparaben	20	No signs of irritation	297
	Unspecified product formulation	0.3—Propylparaben	20	PII-0.10 (max = 4.0); minimal irritation in 2 subjects	295
5-day cumulative irritancy (daily occlusive patch)	Hairdressing	0.2—Methylparaben	50	No cumulative irritation reported	298
20-day cumulative irritancy (23-hour occlusive patch 5 days a week for 20 patches)	Facial mask	0.3 – Propylparaben	13	Slightly irritating; total composite score was 50/520 max	307
21-day cumulative irritancy (23-hour occlusive patch for 21 consecutive days)	White cream	0.2—Methylparaben	12	Essentially nonirritating; total composite score was 0.83/630 max	301
	White cream	0.2—Methylparaben	13	Essentially nonirritating; total composite score was 31/630 max	304
	White cream	0.2—Methylparaben 0.2—Propylparaben	11	Slightly irritating; total composite score was 72/630 max	300
	Orange cream	0.2—Methylparaben 0.2—Propylparaben	9	Essentially nonirritating; total composite score was 0/630 max	302
	Lotion	0.2—Methylparaben 0.1—Propylparaben	13	Slightly irritating; total composite score was 141/630 max	299
	Red wax	0.2—Propylparaben 0.1—Butylparaben	9	Essentially nonirritating; total composite score was 2.2/630 max	303
Controlled use (4 weeks of daily use)	Eye makeup	0.2—Methylparaben 0.1—Propylparaben	57	No irritation	305
	Eye makeup	0.2—Butylparaben	56	No irritation	306

TABLE 8. Clinical Skin Sensitization Tests with Product Formulations Containing Parabens.

Test Method	*Material Tested*	*Concentration (%)*	*No. of Subjects*	*Results*	*Reference*
Schwartz–Peck prophetic patch test (open and closed 48-hour patches, repeated after 2 weeks)	Eye makeup	0.2—Methylparaben 0.1—Propylparaben	202	No irritation; no sensitization. Supplemental UV exposure after second insult produced mild reactions in 2 subjects	308
	Lotion	0.2—Methylparaben 0.1—Propylparaben	104	Mild irritation with closed patch in 6 subjects at first exposure and in 2 subjects at second; no evidence of sensitization. Supplemental UV exposure after second insult produced a mild reaction in 1 subject	309
	Lotion	0.2—Methylparaben 0.1—Propylparaben	104	Mild irritation with closed patch in 2 subjects at second exposure. Supplemental UV exposure after second insult produced no reactions	309
	Eye makeup	0.2—Butylparaben	728	Mild irritation with closed patch in 2 subjects at first exposure and in 4 subjects at second. Supplemental UV exposure after second insult showed no photosensitization	310
Draize–Shelanski repeated insult patch test (24- or 48-hour patches 3 days/week for 10 induction patches; challenge patch after 2 week rest)	Eyeshadow	0.8—Methylparaben	87	Isolated transient irritation in 2 subjects; no sensitization	
	Foundation	0.8—Methylparaben	103	Panel consisted of approx. 50% cosmetic "sensitives" with past history of reaction to cosmetic products. Isolated transient irritation in 11 subjects; no confirmed sensitization	311
	Blush	0.8—Methylparaben	198	Mild to moderate irritation in 10 subjects; no confirmed sensitiza-	312

TABLE 8. (Continued.)

Test Method	Material Tested	Concentration (%)	No. of Subjects	Results	Reference
Draize-Shelanski Repeated Insult Patch Test (cont'd.)				tion. Supplemental UV exposure in half of the subjects after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	
	Foundation	0.8—Methylparaben	198	Mild to moderate irritation in 8 subjects. Supplemental UV exposure in half of the subjects showed no photosensitization	312
	Hand lotion	0.2—Methylparaben	103	Isolated transient irritation in 3 subjects; no sensitization	313
	Body scrub	0.2—Methylparaben	91	Doubtful reactions in 2 subjects during induction; no other evidence of irritation on sensitization	314
	Hand cream	0.2—Methylparaben	205	Isolated transient irritation, no sensitization	315
	Unspecified product formulation	0.2—Methylparaben	108	No irritation; no sensitization	316
	Unspecified product formulation	0.2—Methylparaben	108	Isolated transient irritation during induction in 1 subject; mild irritation at challenge on original site, no reaction at challenge on virgin site	317
	Suntan lotion	0.2—Methylparaben	56	No irritation, no sensitization	318
	Unspecified product formulation	0.2—Methylparaben 0.2—Propylparaben	57	Milk reactions in 1 subject at induction patch 10 and at challenge on original site; no reaction at challenge on virgin site	319
	Orange paste	0.2—Methylparaben 0.2—Propylparaben	27	Mild to marked irritation in 2 subjects; no sensitization	320
	Eye makeup	0.2—Methylparaben 01.—Propylparaben	102	No irritation; no sensitization; Supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	308

Lotion	0.2—Methylparaben 0.1—Propylparaben	53	Isolated transient irritation in 3 subjects; no sensitization. Supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	309
	0.2—Methylparaben 0.1—Propylparaben	53	Isolated transient irritation in 5 subjects; no sensitization. Supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	309
Moisturizing facial mask	0.3—Propylparaben	99	Minimal to mild irritation in most subjects; no evidence of sensitization	321
Orange jelly	0.3—Propylparaben	108	No irritation; no sensitization	322
Mascara	0.3—Propylparaben	94	Slight irritation; no sensitization	323
Protective face cream	0.2—Propylparaben	56	Isolated transient irritation in 1 subject; no sensitization	324
Unspecified product formulation	0.2—Propylparaben 0.1—Butylparaben	205	Mild to moderate irritation in 10 subjects; no sensitization	325
Unspecified product formulation	0.2—Propylparaben 0.1—Butylparaben	205	Mild irritation in 1 subject during induction; mild, transient reactions at challenge in 2 subjects on original site and 1 subject on virgin site. Investigators report no significant evidence of sensitization	326
Eyeliner	0.3—Butylparaben	180	No irritation; no sensitization	327
Eye makeup	0.2—Butylparaben	353	Mild to moderate irritation in few subjects; no evidence of sensitization. Supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed few mild reactions but no evidence of photosensitization	310
Moisture milk lotion	0.2—Ethylparaben	111	Mild irritation in 3 subjects; One mild reaction 48 hours after challenge in subject who had not	328

TABLE 8. (Continued.)

Test Method	*Material Tested*	*Concentration (%)*	*No. of Subjects*	*Results*	*Reference*
Draize–Shelanski Repeated Insult Patch Test (cont'd.)				previously reacted. Investigators report no significant evidence of sensitization	
	Night cream	0.2—Ethylparaben	111	Mild irritation in 3 subjects; no reactions indicative of sensitization	328
Kligman maximization test (5 successive 48-hour patches with challenge after 10-day rest; sodium lauryl sulfate pretreatment before induction and challenge)	Unspecified product formulation	0.2—Methylparaben 0.1—Propylparaben	25	No sensitization	329
	Unspecified product formulation	0.2—Methylparaben 0.1—Propylparaben	25	No sensitization	329

0.1 percent Propylparaben. The results and other details of these studies are summarized in Table 8. Of the 3455 subjects reported in Table 8, there were no reactions indicative of sensitization.

Case Reports

Paraben hypersensitivity has been reported in a number of cases. In many, sensitization followed topical application of Paraben medicaments to broken skin.[276,286,289,330-335] Other cases of sensitivity from Parabens in anesthetic solutions injected intravenously are reported.[336-338]

Eye Irritation

Aqueous solutions of 0.10 to 0.30 percent Methylparaben instilled in the eyes of humans produced moderate hyperemia, slight lacrimation, and slight burning. All symptoms disappeared within 1 minute. These results were confirmed when instillation of these solutions several times daily into the eyes of more than 100 subjects produced no irritation.[218]

Toxicity

One patient ingested 500 mg Methylparaben and one patient ingested 200 mg daily for 28 days, then 500 mg daily for 4 days. Two patients ingested 1000 mg daily for 29 days, then 2000 mg daily for 28 days. No toxicity to Methylparaben was reported.[215]

In 1972, Saiki et al.[339] reported a case in which a patient developed paraplegia following intrathecal chemotherapy. They suggested that Methylparaben, contained in the chemotherapy agents, may have caused damage to the spinal nerve roots within the subarachnoid space, accounting for the neurologic deficit.

Photocontact Sensitization

Each of four products containing 0.2 percent Methylparaben and/or 0.2 percent Propylparaben were tested for evidence of photo-induced contact sensitization in 27 to 30 subjects.[318-320,324] The volar forearm was designated as the site of test material applications. One forearm was irradiated and the other served as a nonirradiated control site. Abour 0.2 ml of the test material was applied under an occlusive patch for 24 hours. The irradiated test site was subjected to nonerythrogenic ultraviolet radiation for 15 minutes at a distance of 10 to 12 cm from the source, receiving a UV light dose of 4400 $\mu W/cm^2$. The light source consisted of four GE F40 BL black light lamps of a wavelength in the UV-A range with a peak at 360 nm. These procedures were repeated 3 days a week until 10 treatments had been given and then twice again after a 10- to 14-day rest period. Each of the product formulations produced mild reactions with and without irradiation, but there were no reactions indicative of photocontact sensitization.

In addition, six of the Draize–Shelanski repeated insult patch tests summarized in Table 8 used supplemental ultraviolet light exposure after the first, fourth, seventh, tenth, and challenge patches as noted in Table C.[308-310,312] Test sites were irradiated for 1 minute at a distance of 12 inches from the source. The light source consisted of the Hanovia Tannette Mark I Lamp, which has a continuous emission spectrum from 300 to 370 nm and an output of no more than 150 watts. The formulations tested in these studies contained Methyl- Propyl-,

and/or Butylparaben at concentrations of 0.1 to 0.8 percent. Of the 607 subjects thus treated, none had reactions indicative of photosensitization.

Phototoxicity

Four product formulations, each containing 0.2 percent Methylparaben and/or 0.2 percent Propylparaben, were tested for human phototoxicity.[318-320,324] The volar forearms of 10 to 12 subjects were scrubbed with alcohol and tape-stripped to remove several layers of cornified epithelium. About 0.2 ml of the test material was applied and occluded for 24 hours. The test site on one forearm was subjected to nonerythemogenic ultraviolet light for 15 minutes at a distance of 10 to 12 cm from the source, receiving a UVA light dose of 4,400 $\mu W/cm^2$. The light source consisted of four GE F40 BL black light lamps of a wavelength in the UV-A range with a peak at 360 nm. One subject in each of two of the tested groups showed mild irritation at both control and irradiated sites. There were no reactions indicative of phototoxicity.

In addition, four of the Schwartz-Peck Prophetic Patch Tests summarized in Table 8 used a single supplemental UV light exposure after the second patch, as noted in Table 8.[308-310] Test sites were irradiated for 1 minute at a distance of 12 inches from the source. The light source consisted of the Hanovia Tannette Mark I Lamp already described. The formulations tested in these studies contained either 0.2 percent Butylparaben or both 0.2 percent Methylparaben and 0.1 percent Propylparaben. Of the 1034 subjects thus tested, only 3 had mild skin reactions.

Industry Complaint Experience

Complaint experience data are available on a body scrub product, two suntan lotions, a hand lotion, and a bubble bath, each containing 0.2 percent Methylparaben. There were three safety-related complaints (one each listed under "allergy," "burning sensation," and "pimple rash") with an estimated 18.4 million total uses of these products.[340-344]

Complaint experience data on a protective face cream containing 0.2 percent Propylparaben shows three safety-related complaints in 3 years with 0.4 million uses. Two of these were listed as "allergy" and one as "burning sensation."[345]

There were 35 safety-related complaints for a mascara containing both 0.2 percent Methylparaben and 0.1 percent Propylparaben with 4.6 million units sold: 20 "burning/stinging," 11 "irritated skin," and 4 "allergic reaction."[346] An aftershave lotion also containing 0.2 percent Methylparaben and 0.1 percent Propylparaben had one safety-related complaint with 0.17 million units sold.[347]

Complaint experience data on a mascara containing 0.2 percent Butylparaben shows 36 complaints with 2.3 million units sold; 33 of these were listed as "irritating/burning," 2 as "itching," and 1 "swelling."[348]

SUMMARY

The Parabens are esters of *p*-hydroxybenzoic acid (PHBA). They are prepared by esterification of PHBA with the corresponding alcohol in the presence of a catalyst. Parabens are generally oil soluble and poorly soluble in

water. Water solubility decreases as the ester chain length increases. These compounds are stable in air and resist hydrolysis in acid solutions and under conditions of sterilization. In alkaline solutions, Parabens hydrolyze to PHBA and the corresponding alcohol. Paraben interactions with gelatin, sodium lauryl sulfate, polysorbates, PEGs, cellulose esters, and PVP have been reported. Micellar interactions bind Parabens to such nonionic sufactants as sodium lauryl sulfate.

Parabens are used as preservatives in over 13,200 cosmetic formulations at concentrations almost exclusively less than 5 percent. They are most commonly used at concentrations up to 1 percent. Parabens preserve fats, proteins, oils, and gums in cosmetics. Products containing Parabens contact all surfaces of the body as well as ocular, oral, and vaginal mucosae. Duration of application may be continuous and may extend over a period of years. Certain Parabens are also used as preservatives in foods (up to 0.1 percent as GRAS ingredient), pharmaceuticals (as inactive or safe and effective OTC ingredients), and other products.

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

The antimicrobial activity of the Parabens increases with increasing ester chain length. They are more active against fungi than bacteria and more active against gram-positive than gram-negative bacteria. Their effect is more microbiostatic than microbiocidal. Parabens are effective within a pH range of 4 to 8. Parabens act as microbiostatic agents by increasing cell wall permeability and thereby disrupting transport. Parabens also alter cellular respiration, electron transport, and oxidative enzyme systems of microbes. Both the ester-linkage and the para-hydroxy group of the Paraben molecule have been implicated as active sites.

The Parabens inhibit and potentiate many enzyme systems. They also compete with bilirubin for binding sites on serum albumin. These substances inhibit growth of cultures of animal and human cells and reduce biosynthesis of RNA and DNA in cultures of fibroblasts. Parabens have varying anticonvulsive, vasodilating, analgesic, and anesthetic effects in animals.

Acute toxicity studies in animals indicate that Parabens are practically nontoxic by various routes of administration. Methylparaben (100 and 10 percent), Propylparaben (10 percent), and Ethylparaben (100 and 10 percent) were, at most, mildly irritating when applied to rabbit skin. Methylparaben and Ethylparaben at 100 percent concentration were slightly irritating when instilled into the eyes of rabbits. Subchronic and chronic oral studies indicate that Parabens are practically nontoxic. Practically all animal sensitization tests indicate that the Parabens are nonsensitizing.

Numerous mutagenicity studies, including the Ames Test, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the Parabens are nonmutagenic. Methylparaben was noncarcinogenic when injected subcutaneously in mice or rats when administered intravaginally in rats and was not co-

carcinogenic when injected subcutaneously in mice. Propylparaben was non-carcinogenic in a study of transplacental carcinogenesis. Methylparaben was nonteratogenic in rabbits, rats, mice and hamsters, and Ethylparaben was non-teratogenic in rats.

Parabens are practically nonirritating and nonsensitizing in the 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 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. A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses up to 2 g/kg/day. A primary eye irritation study in humans showed Methylparaben to be nonirritating at concentrations up to 0.3 percent. Photocontact sensitization and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methyl-, Propyl-, and/or Butylparaben gave no evidence for significant photoreactivity. Industry complaint experience data showed low to moderate numbers of safety-related complaints with the incidence depending on the product.

DISCUSSION

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.

CONCLUSION

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

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Final Report on the Safety Assessment of Benzylparaben

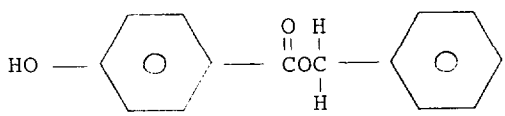
The safety of this ingredient has not been documented and substantiated. The Cosmetic Ingredient Review Expert Panel cannot conclude that Benzylparaben is safe for use in cosmetic products until such time that the appropriate safety data have been obtained and evaluated. The data that were available are documented in the report as well as the types of data that are required before a safety evaluation may be undertaken.

INTRODUCTION

Benzylparaben was originally included in the CIR group review of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben.[1] The CIR Expert Panel noted the difference in chemical structure and the lack of safety test data on Benzylparaben and deleted that ingredient from the group review. CIR issued a public announcement that Benzylparaben would be reviewed as a separate cosmetic ingredient.

CHEMISTRY

Benzylparaben (CAS No. 94-18-9) is an ester of benzyl alcohol and *p*-hydroxybenzoic acid and conforms to the following structure[2]:



The properties of Benzylparaben are shown in Table 1.

Reactivity

Benzylparaben can form salts with strong alkalies. The compound is subject to hydrolysis by both acids and alkalies.[3]

TABLE 1. Physical Properties[2,3]

Molecular weight		228.25
Description		White, crystalline, odorless powder
Solubility	Water	0.01 g/100 ml
	Ethanol	72.0 g/100 ml
	Propylene glycol	13 g/100 ml
Assay		99–100%
Acidity		<0.01 mEq/200 mg
Melting range °C		110–112
Sulfated ash		0.1% maximum

Method of Manufacture

Benzylparaben can be prepared by esterification of *p*-hydroxybenzoic acid with benzyl alcohol.[3] It can also be prepared by conversion of benzyl chloride with sodium *p*-hydroxybenzoic acid.[4]

PURPOSE AND USE IN COSMETICS

Benzylparaben is used in cosmetic products as a preservative.[5] In general use since 1943, it is more commonly used in Europe than in the United States.

The Food and Drug Administration (FDA), in its computer tabulation of voluntary filing of product formulations, reported six uses of Benzylparaben in deodorant and moisturizing skin care preparations. One deodorant contained concentrations in the 0.1–1.0% range, and the remaining five uses were reported as below 0.1%.[6] The EEC reports Benzylparaben to be in general and widespread use as a preservative in conjunction with other paraben esters. Maximum concentration in use is 0.1% as the acid.[2]

The advantage of Benzylparaben use as a preservative is its effectiveness over a wide pH range. It is more effective against fungi and gram-positive bacteria than against gram-negative bacteria. Its disadvantages are low water solubility, partition in favor of the oily phase, and inactivation by ions and proteins.[5]

BIOLOGY

Antimycotic and Antibacterial Effects

A comparative study on the bactericidal effects of Benzylparaben has been published.[7]

Benzylparaben was effective in prevention of growth of *Epidermophyton interdigitale* and *Microsporum audouni* in a substrate concentration of 1:10,000.[8]

The use of 0.025 mg of Benzylparaben/ml in agar medium was effective in inhibiting the growth of *Cryptococcus neoformans*.[9]

Pseudomonas species of bacteria are inhibited by 0.002–0.008% Benzylparaben. The development of aerobic sporogenic agents, such as *Bacillus subtilis*, can be inhibited with 0.002–0.008% Benzylparaben.[10]

Metabolism

Two grams of Benzylparaben were consumed by each of two human volunteers per day for 5 days. Their urine was analyzed for metabolic products. Approximately 6% of the administered compound was eliminated unchanged, and approximately 87% was eliminated as the sulfate conjugate of the ester. Small quantities of the ester were also hydrolyzed to *p*-hydroxybenzoic acid and benzyl alcohol, the latter being oxidized to benzoic acid. The latter two were excreted either unchanged or as their glycine conjugates, *p*-hydroxyhippuric acid and hippuric acid. The investigators reported these percentages as approximations due to the isolation and analytical procedures used in the study.[11]

ANIMAL TOXICOLOGY

Acute Studies

Oral Toxicity

No deaths or toxic signs were reported when up to 10 g/kg of Benzylparaben was given by oral intubation to groups of slc-ddy mice.[12] No deaths occurred when 5 g/kg of Benzylparaben was given to groups of Charles River CD rats.[13]

Two guinea pigs were fed 2 g of Benzylparaben per day; no injurious effects to the animals were noted. The duration and method of dosing were unspecified.[11]

Guinea pigs fed 1 g of Benzylparaben per day for 19 days had no signs of toxicity.[7] See also reference 12.

Spasmolytic action was observed in cerebral vessels of cats after intravertebral injection of 5 mg/kg of Benzylparaben.[14] Intravenous injection of Benzylparaben to dogs and cats caused no variation in blood sugar concentration of the animals.[15] Intravenous injection of 0.7 g/kg of Benzylparaben to dogs produced no ill effects.[16]

Skin Irritation

The Primary Irritation Index (PII) of 500 mg of Benzylparaben when applied under occlusive patches to intact and abraded skin of six female New Zealand rabbits was 0.11 ± 0.08 (control: 0.09 ± 0.09).[2] In another study, Benzylparaben was neither an irritant nor a corrosive agent when 0.5 g of the pure ingredient was applied under semiocclusive conditions to the abraded skin of rabbits.[13]

Ocular Irritation

No adverse ocular responses were observed at 1, 24, 48, or 72 h after the instillation of 0.1 g of Benzylparaben into the conjunctival sac of three New Zealand rabbits.[13]

CLINICAL ASSESSMENT OF SKIN SENSITIZATION

The principal patch tests of the North American Contact Dermatitis Group from 1970–1976 were performed using a 15% paraben mixture containing 3% each of methyl, ethyl, propyl, butyl, and benzyl esters. Positive results were noted in 3% of 1200 patients, 3.5% of 3000, 3.7% of 1900, and 2% of 900–2000 patients, respectively.[17]

Cronin[18] reported irritation in 10 of 1000 eczematous patients patch tested with a 15% paraben mixture similar to the Rudner (1978) study.[17] Scoring was performed at 2 and 4 days after application of the mixture using A1 test patches and zinc oxide strapping.[18]

Four thousand eczematous patients in five European clinics were tested with a series of medicaments including the 15% mixed paraben ester solution previously described.[17] Positive reactions for parabens were 1.3% of males, 2.3% of females, and 2% total.[19]

Nine of 465 patients with dermatitis were sensitized to a mixed paraben ointment. The reactive patients were sensitized as well to a number of other products.[20]

Romaguera and Grimalt reported 57 of 4600 (1.24%) positive reactions to a 15% mixed paraben ointment containing 3% of each of the esters.[21] The above studies are summarized in Table 2.

TABLE 2. Patient Sensitivity to Mixed Paraben Esters

Allergen	*Total patients tested*	*Percent positive*	*Reference*
Paraben mixture	1200	3	17
(3% each of methylparaben,	3000	3.5	17
ethylparaben, propylparaben,	1900	3.7	17
butylparaben, and Benzyl-	900–2000	2.7	17
paraben)	1000	1	18
	4000	2	19
	465	1.5	20

Hjorth and Trolle-Lassen[22] reported on the sensitivity and cross-sensitivity of eczematous patients to paraben esters. Preliminary tests were conducted using routine patch tests with a mixture comprised of 10% methylparaben, 2% ethylparaben, and 2% propylparaben in equal parts Aquaphor and water. Fifteen cases positive to this mixture were assayed for Benzylparaben sensitivity, and 7/15 were sensitive to both 1 and 5% Benzylparaben solutions (Table 3). About two thirds of the patients sensitive to one of the paraben esters also reacted to one or several other esters (Table 4).

Allergic contact dermatitis to Benzylparaben and cross-sensitizations with other parabens is possible but is considered to be rare.[2]

TABLE 3. Sensitivity to Benzylparaben in 15 Paraben-sensitive Persons[22]

Benzylparaben[a] (%)	No. of cases	
	Positive	Negative
5	7	8
1	7	8
0.5	4	11
0.1	2	13

[a]In equal parts Aquaphor and water.

TABLE 4. Cross-sensitivity between Paraben Esters[a][22]

			Methylparaben		Ethylparaben		Propylparaben	
			Positive	Negative	Positive	Negative	Positive	Negative
		No. of cases	21	11	27	5	22	9
Ethyl-paraben	Positive	27	18	9				
	Negative	5	3	2				
Propyl-paraben	Positive	22	15	7	20	2		
	Negative	9	5	4	7	2		
Benzyl-paraben	Positive	14	10	4	12	2	13	1
	Negative	17	16	7	15	2	9	8

Note: One case was not tested with propyl paraben or benzyl paraben.
[a]Thirty-two cases tested with 5% paraben esters in petrolatum or in equal parts Eucerin and water.

SUMMARY

Benzylparaben is an ester of benzyl alcohol and *p*-hydroxybenzoic acid used in cosmetics as a preservative. It is often used in conjunction with other paraben esters.

Metabolism of Benzylparaben is by sulfate conjugation of the parent compound. Excretion is in the urine. Small amounts of the ester are excreted unmetabolized or hydrolyzed to the benzyl alcohol and *p*-hydroxybenzoic acid.

Benzylparaben was not considered an acute toxic agent to mice or rats and was neither an eye nor skin irritant when tested in rabbits. Intravenous injections of Benzylparaben to dogs and cats caused no variation in blood sugar, circulation, and respiration.

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.

DISCUSSION

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, 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.

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.

CONCLUSION

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

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Final Report on the Safety Assessment of Isobutylparaben and Isopropylparaben[1]

Abstract: Isobutylparaben and Isopropylparaben are esters with a phenol ring structure intended for use as preservatives in cosmetics. In 1993, only Isobutylparaben was reported to be in use. When administered to rabbits via a stomach tube, Isobutylparaben was metabolized primarily to *p*-hydroxybenzoic acid; <1% was recovered unchanged. Spleen, thymus, hepatic parenchyma, and lymph node atrophy was observed in short-term feeding studies in mice with Isobutylparaben at a concentration of 1.25%. All mice died at concentrations of 5 and 10%. Isobutylparaben at a concentration of 0.6% produced no adverse effects. Mutagenesis was not observed with either ingredient in Ames tests, but both positive and negative results were seen in chromosome abberation assays of Chinese hamster ovary cells in culture. In chronic mouse-feeding studies, 0.15, 0.3, and 0.6% Isobutylparaben caused no increase in neoplasias or decrease in time to neoplasm development. Data on Methylparaben, Ethylparaben, Propylparaben, and Butylparaben considered relevant to the assessment of Isobutylparaben and Isopropylparaben were summarized. Subchronic and chronic studies indicate that these other parabens are practically nontoxic, nonmutagenic, and noncarcinogenic. They also do not irritate or sensitize normal skin. Tests of patients with damaged skin, however, do show that a few of these individuals can be sensitized. These data support the recommendation that all six of these parabens should not be used on damaged skin. Given the absence of any adverse effects, including irritation and sensitization, in persons with normal skin, the conclusion that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben are safe as cosmetic ingredients was reaffirmed. By extension, because of their similarities, the conclusion was reached that Isobutylparaben and Isopropylparaben are safe as cosmetic ingredients in the present practices of use. **Key Words:** Isobutylparaben—Isopropylparaben—Cosmetic use—Rabbits—Mice—Toxicity—Mutagenicity—Carcinogenicity.

Isobutylparaben and Isopropylparaben are used in cosmetic formulations as preservatives. This report is a summary of data available to the Cosmetic Ingredient Review (CIR) concerning the chemistry, cosmetic use, toxicity, mutagenicity, and carcinogenicity of these compounds. The CIR Expert Panel has reviewed the safety of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. The summary from that report is also included herein. Abstracts published in the 10 years since publication of the Final Report on Parabens have described additional case reports of allergic reactions to Parabens or to products containing

[1] Reviewed by the Cosmetic Ingredient Review Expert Panel.

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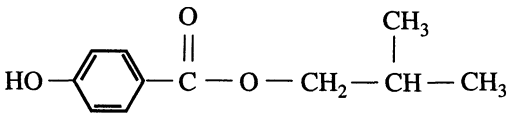
Parabens: contact dermatitis to bandages containing Propylparaben (Lindner et al., 1989); allergic reactions to Parabens, particularly Methylparaben, found in medical supplies such as anesthetics, barium enemas, and mandibular block injections (Ivy, 1983; Wahl, 1983; Javors et al., 1984; Schwartz et al., 1984; Fine and Dingman, 1988); and one report of facial contact urticaria to Methylparaben in cosmetics (Kojima, 1992). In addition, there has been a report that Methylparaben is mildly ciliotoxic to male Wistar rats at an inhaled concentration of 1.18 m*M* (Jain and Po, 1993) and a report of intercellular vacuolization and thickening of the endothelial layer in rabbit corneal endothelium 1 day following subconjunctival administration of solutions containing Methylparaben and Propylparaben (Weinreb et al., 1986). The four parabens, especially Butylparaben, have been shown to exhibit spermicidal activity, possibly through impairment of sperm membrane function (Song et al., 1989).

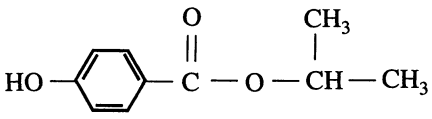
CHEMISTRY

Definition and Structure

Isobutylparaben (CAS No. 4247-02-3) and Isopropylparaben (CAS No. 4191-73-5) are esters of *p*-hydroxybenzoate that conform to the following formulas (Nikitakis et al., 1991):



Isobutylparaben



Isopropylparaben

Other names for Isobutylparaben are benzoic acid, 4-hydroxy-, 2-methylpropyl ester; 4-hydroxybenzoic acid, 2-methylpropyl ester; isobutyl *p*-hydroxybenzoate; isobutyl parahydroxybenzoate. Other names for Isopropylparaben are benzoic acid, 4-hydroxy-, 1-methylethyl ester; 4-hydroxybenzoic acid, 1-methylethyl ester; 1-methylethyl-4-hydroxybenzoate (Nikitakis et al., 1991).

Chemical and Physical Properties

Isobutylparaben has a molecular weight of 194.25. Isopropylparaben has a molecular weight of 180.22 (Registry of Toxic Effects of Chemical Substances, 1993).

Analytic Methods

Detection of Isopropylparaben and Isobutylparaben can be made by high-performance liquid chromatography (Kitada et al., 1980; Terada and Sakabe, 1985; Shiroma and Oshiro, 1986; Maeda et al., 1987).

USE

Cosmetic

Isobutylparaben and Isopropylparaben are used in cosmetic formulations as preservatives (Nikitakis, 1988). The product formulation data submitted to the Food and Drug Administration (FDA) in 1993 reported that Isobutylparaben was used in a total of 83 cosmetic product formulations (Table 1) and that no formulations contained Isopropylparaben (FDA, 1993). Cosmetic products containing either Isobutylparaben or Isopropylparaben may be applied to or come in contact with skin, eyes, hair, nails, and mucous membranes. Product formulations containing either Isobutylparaben or Isopropylparaben may be applied as many as several times a day and may remain in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

International

Isobutylparaben (as isobutyl parahydro benzoate, isobutyl paraben, or isobutyl *p*-hydroxybenzoate) and Isopropylparaben (as isopropyl *p*-hydroxybenzoate) are approved for use in Japan in cosmetic formulations (Rempe and Santucci, 1992). Isobutylparaben and Isopropylparaben are included in the list of allowed preservatives in cosmetic products by the European Economic Commission under the category of 4-hydroxybenzoic acid and its salts and esters. Maximum concentra-

TABLE 1. *Cosmetic product formulation data for Isobutylparaben*

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Mascara	247	3
Bath capsules	7	1
Eyebrow pencil	113	2
Eyeliner	253	1
Other eye makeup preparations	160	1
Other hair preparations	418	1
Lipstick	937	1
Other makeup preparations	418	1
Other shaving preparations	157	1
Face and neck (except shaving preps)	148	12
Paste masks (mud packs)	293	4
Skin fresheners	246	1
Suntan gels, creams, and liquids	290	5
Indoor tanning preparations	53	1
Other suntan preparations	65	3
Other fragrance preparations	177	3
Face powders	313	2
Foundations	398	5
Cleansing	854	7
Body and hand (except shaving)	1,229	4
Moisturizing	933	12
Night	263	2
Other skin care preparations	848	10
1993 Totals	—	83

tions set were 0.4% for any single ester and 0.8% for mixtures of esters (European Economic Community, 1990).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Four male rabbits weighing between 2.25 and 3.50 kg were given a 12% solution in the form of Na salt of either 800 mg/kg or 400 mg/kg of Isobutylparaben via a stomach tube. A 24-h urine sample was collected and analyzed via paper chromatography. Between 25 and 33% of the Isobutylparaben dose was metabolized to free *p*-hydroxybenzoic acid, 16–31% became *p*-hydroxybenzoic acid conjugated with glycine, and 7–17% was recovered as *p*-hydroxybenzoic acid conjugated with one of the following three acids: ester-type glucuronic acid, ether-type glucuronic acid, or sulfuric acid. In total, between 77 and 85% of the Isobutylparaben was recovered as one of the above-mentioned forms of *p*-hydroxybenzoic acid. Only between 0.2 and 0.9% of Isobutylparaben was detected in the urine as the unchanged alkyl ester. No explanation was offered as to why ~20% of the initial dose was not recovered (Tsukamoto and Terada, 1964).

ANIMAL TOXICOLOGY

Acute Toxicity

The subcutaneous LD_{50} of Isobutylparaben in mice was 2,600 mg/kg (details not available) (Registry of Toxic Effects of Chemical Substances, 1993).

Short-term Toxicity

Groups of 10 male and 10 female ICR/Jcl mice were administered 0.6, 1.25, 2.5, 5, and 10% Isobutylparaben in the feed for 6 weeks (Inai et al., 1985). A group of 20 males and 20 females served as a control. All mice of the 5 and 10% Isobutylparaben dose groups died during the first 2 weeks of the study. Body weight gain percentages for mice of the 1.25 and 2.5% Isobutylparaben groups were ~10% of the control group. Body weight gain for mice of the 0.6% dose group was about the same as control. Upon microscopic examination, atrophy of the spleen, thymus, and lymph nodes was observed in groups dosed with ≥1.25% Isobutylparaben. Multifocal degeneration and necrosis of the hepatic parenchyma was also noted in these groups. No significant lesions were found in mice dosed with 0.6% Isobutylparaben.

Chronic Toxicity

Groups of 50 male and 50 female 8-week-old ICR/Jcl mice were administered 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks (Inai et al., 1985). A group of 50 males and 50 females served as a control and were fed the basal diet. Body weights were measured once a week for the first 6 weeks, once every other week for the next 24 weeks, and once every 4 weeks for the remainder of the study. Feed consumption was measured once a week for the first 30 weeks, once

every other week for the next 20 weeks, and once every 4 weeks for the remainder of the study. Animals found moribund during the study were killed and necropsied. Animals surviving to the end of the study (nine females in the test group) were killed and necropsied. There was no significant difference between groups in the amount of feed consumed; the intake of Isobutylparaben was four times greater by mice in the 0.6% dosing group than in the 0.15% group.

Data were compiled from animals surviving the study for ≥78 weeks (30% of males and 57% of females in the dosing group and 24% of males and 44% of females in the control group). There were no significant differences in the incidence of neoplasms between the treated mice and the controls or between groups given different doses of Isobutylparaben. Among treated male mice, the most frequently observed neoplasms were lung adenomas and adenocarcinomas. A high incidence of hematopoietic neoplasms was found in males in the 0.6% group and in treated females. There was a low incidence of neoplasms at other sites in females. Amyloidosis was noted in 58% of dosed males and 33% of dosed females compared with 25% of control males and 10% of control females. While no information is available concerning the incidence of amyloidosis in historical controls, it is known that spontaneous amyloidosis is common in mice, particularly in some inbred strains and in older mice (Rigdon and Schadewald, 1972; Soret et al., 1977; Conner et al., 1983).

GENOTOXICITY

A chromosomal abberation assay was performed using a Chinese hamster fibroblast cell line. Cells treated with 0.03% Isobutylparaben in ethanol (dose volume equal to 1.0% of total volume) had no chromosomal aberrations after 48 h (Ishidate and Odashima, 1977). At a concentration of 1 mg/plate in Dimethyl Sulfoxide, Isopropylparaben and Isobutylparaben were negative in Ames tests using *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98. A chromosomal aberration assay was also performed using a Chinese hamster fibroblast cell line. 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, and both had a 1% incidence of structural chromosomal aberrations (Ishidate et al., 1984). Kawachi (1976) (in Odashima, 1980) reported that Isobutylparaben was positive in a chromosomal aberration assay but negative in an Ames test and a *rec* assay (details not available).

CARCINOGENICITY

Groups of 50 male and 50 female ICR/Jcl mice were administered 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks (Inai et al., 1985). A group of 50 males and 50 females served as a control. Animals found moribund during the study were killed and necropsied. A microscopic examination was performed on all neoplasms. No changes in either neoplasm incidence or time to neoplasm development were observed in dosed mice compared with controls.

SUMMARY OF ISOBUTYLPARABEN/ISOPROPYLPARABEN

Isobutylparaben and Isopropylparaben are esters of *p*-hydroxybenzoate and are used as preservatives. In 1993, Isobutylparaben was reported to be used in 83 cosmetic formulations (at unknown concentrations), and Isopropylparaben was not in use. When male rabbits were administered either 800 mg/kg or 400 mg/kg of Isobutylparaben via a stomach tube, 77–85% of the ingredient was recovered as a form of *p*-hydroxybenzoic acid; 20% was not recovered. Isobutylparaben had a subcutaneous LD_{50} of 2,600 mg/kg in mice.

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. Mice were orally dosed with 0.15, 0.3, and 0.6% Isobutylparaben in the feed for 102 weeks. 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. Chinese hamster fibroblast cell lines treated with 0.03% Isobutylparaben had no chromosomal aberrations after 48 h.

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

SUMMARY OF METHYLPARABEN, ETHYLPARABEN, PROPYLPARABEN, AND BUTYLPARABEN

The CIR review of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben (Elder, 1984) concluded that the parabens are esters of *p*-hydroxybenzoic acid (PHBA). They are prepared by esterification of PHBA with the corresponding alcohol in the presence of a catalyst. Parabens are generally oil soluble and poorly soluble in water. Water solubility decreases as the ester chain length increases. These compounds are stable in air and resist hydrolysis in acid solutions and under conditions of sterilization. In alkaline solutions, Parabens hydrolyze to PHBA and the corresponding alcohol. Paraben interactions with gelatin, sodium lauryl sulfate, polysorbates, polyethylene glycols, cellulase esters, and polyvinylpyrrolidone have been reported. Micellar interactions bind Parabens to such nonionic surfactants as sodium lauryl sulfate.

Parabens are used as preservatives in >13,200 cosmetic formulations at concentrations almost exclusively <5%. They are most commonly used at concentrations <1%. Parabens preserve fats, proteins, oils, and gums in cosmetics. Products containing Parabens contact all surfaces of the body as well as ocular, oral, and vaginal mucosae. Duration of application may be continuous and may extend over a period of years. Certain Parabens are also used as preservatives in

foods (≤0.1% as GRAS ingredient), pharmaceuticals (as inactive or safe and effective OTC ingredients), and other products.

Parabens are quickly absorbed from the blood and gastrointestinal tract, hydrolyzed to *p*-hydroxybenzoic acid, and conjugated; the conjugate is excreted in the urine. 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 5–72 h as *p*-hydroxybenzoic acid or its conjugates. Parabens appear to be rapidly absorbed through intact skin.

The antimicrobial activity of the Parabens increases with increasing ester chain length. They are more active against fungi than bacteria and more active against gram-positive than gram-negative bacteria. Their effect is more microbiostatic than microbicidal. Parabens are effective within a pH range of 4–8. Parabens act as microbiostatic agents by increasing cell wall permeability and thereby disrupting transport. Parabens also alter cellular respiration, electron transport, and oxidative enzyme systems of microbes. Both the ester linkage and the para-hydroxy group of the Paraben molecule have been implicated as active sites.

The Parabens inhibit and potentiate many enzyme systems. They also compete with bilirubin for binding sites on serum albumin. These substances inhibit growth of cultures of animal and human cells and reduce biosynthesis of RNA and DNA in cultures of fibroblasts. Parabens have varying anticonvulsive, vasodilating, analgesic, and anesthetic effects in animals.

Acute toxicity studies in animals indicate that Parabens are practically nontoxic by various routes of administration. Methylparaben (10 and 100%), Propylparaben (10%), and Ethylparaben (10 and 100%) were, at most, mildly irritating when applied to rabbit skin. Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits. Subchronic and chronic oral studies indicate that Parabens are practically nontoxic. Almost all animal sensitization tests indicate that the Parabens are nonsensitizing.

Numerous mutagenicity studies, including the Ames test, dominant lethal assay, host-mediated assay, and cytogenic assays, indicate that the Parabens are nonmutagenic. Methylparaben was noncarcinogenic when injected subcutaneously in mice or rats or when administered intravaginally in rats. It was not cocarcinogenic when injected subcutaneously in mice. Propylparaben was noncarcinogenic in a study of transplacental carcinogenesis. Methylparaben was nonteratogenic in rabbits, rats, mice, and hamsters. Ethylparaben was nonteratogenic in rats.

Parabens are practically nonirritating and nonsensitizing in a 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 <3% of such individuals. Of 27,230 patients with chronic skin problems, 2.2% were sensitized by preparations of Parabens at concentrations of

1–30%. Many patients sensitized to Paraben-containing medications can wear cosmetics containing these ingredients with no adverse effects. Skin irritation and sensitization tests on product formulations containing from 0.1 to 0.8% of one or two of the Parabens showed no evidence of significant irritation or sensitization potential for these ingredients. A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses ≤2 g/kg/day. A primary eye irritation study in humans showed Methylparaben to be nonirritating at concentrations ≤0.3%. Photocontact sensitization and phototoxicity tests on product formulations containing 0.1–0.8% Methyl-, Propyl-, and/or Butylparaben gave no evidence of significant photoreactivity. Industry complaint experience data showed low to moderate numbers of safety-related complaints, with the incidence depending on the product.

DISCUSSION

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.

CONCLUSION

From the available information, the Panel concludes that Isobutylparaben and Isopropylparaben are safe as cosmetic ingredients in the present practices of use.

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The above material is also available by searching paraben at the following URL:

http://www.cir-safety.org/ingredients

2017 VCRP Data for Parabens

01A - Baby Shampoos	METHYLPARABEN	3
01B - Baby Lotions, Oils, Powders, and Creams	METHYLPARABEN	23
01C - Other Baby Products	METHYLPARABEN	14
02A - Bath Oils, Tablets, and Salts	METHYLPARABEN	23
02B - Bubble Baths	METHYLPARABEN	33
02D - Other Bath Preparations	METHYLPARABEN	68
03A - Eyebrow Pencil	METHYLPARABEN	72
03B - Eyeliner	METHYLPARABEN	479
03C - Eye Shadow	METHYLPARABEN	844
03D - Eye Lotion	METHYLPARABEN	162
03E - Eye Makeup Remover	METHYLPARABEN	62
03F - Mascara	METHYLPARABEN	377
03G - Other Eye Makeup Preparations	METHYLPARABEN	191
04A - Cologne and Toilet waters	METHYLPARABEN	60
04B - Perfumes	METHYLPARABEN	24
04C - Powders (dusting and talcum, excluding aftershave talc)	METHYLPARABEN	101
04D - Sachets	METHYLPARABEN	2
04E - Other Fragrance Preparation	METHYLPARABEN	46
05A - Hair Conditioner	METHYLPARABEN	469
05B - Hair Spray (aerosol fixatives)	METHYLPARABEN	9
05C - Hair Straighteners	METHYLPARABEN	19
05D - Permanent Waves	METHYLPARABEN	6
05E - Rinses (non-coloring)	METHYLPARABEN	14
05F - Shampoos (non-coloring)	METHYLPARABEN	381
05G - Tonics, Dressings, and Other Hair Grooming Aids	METHYLPARABEN	394
05H - Wave Sets	METHYLPARABEN	14
05I - Other Hair Preparations	METHYLPARABEN	349
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	METHYLPARABEN	157
06B - Hair Tints	METHYLPARABEN	12
06C - Hair Rinses (coloring)	METHYLPARABEN	10
06D - Hair Shampoos (coloring)	METHYLPARABEN	11
06E - Hair Color Sprays (aerosol)	METHYLPARABEN	3
06F - Hair Lighteners with Color	METHYLPARABEN	2
06G - Hair Bleaches	METHYLPARABEN	19
06H - Other Hair Coloring Preparation	METHYLPARABEN	58
07A - Blushers (all types)	METHYLPARABEN	289
07B - Face Powders	METHYLPARABEN	394
07C - Foundations	METHYLPARABEN	327
07D - Leg and Body Paints	METHYLPARABEN	29
07E - Lipstick	METHYLPARABEN	309
07F - Makeup Bases	METHYLPARABEN	88
07G - Rouges	METHYLPARABEN	8
07H - Makeup Fixatives	METHYLPARABEN	8
07I - Other Makeup Preparations	METHYLPARABEN	219
08A - Basecoats and Undercoats	METHYLPARABEN	3
08B - Cuticle Softeners	METHYLPARABEN	18
08C - Nail Creams and Lotions	METHYLPARABEN	7
08E - Nail Polish and Enamel	METHYLPARABEN	20
08F - Nail Polish and Enamel Removers	METHYLPARABEN	6
08G - Other Manicuring Preparations	METHYLPARABEN	23
09A - Dentifrices	METHYLPARABEN	11
09B - Mouthwashes and Breath Fresheners	METHYLPARABEN	4
09C - Other Oral Hygiene Products	METHYLPARABEN	6
10A - Bath Soaps and Detergents	METHYLPARABEN	370
10B - Deodorants (underarm)	METHYLPARABEN	30
10C - Douches	METHYLPARABEN	3
10E - Other Personal Cleanliness Products	METHYLPARABEN	264
11A - Aftershave Lotion	METHYLPARABEN	131
11C - Mens Talcum	METHYLPARABEN	1
11D - Preshave Lotions (all types)	METHYLPARABEN	2
11E - Shaving Cream	METHYLPARABEN	39
11F - Shaving Soap	METHYLPARABEN	1

11G - Other Shaving Preparation Products	METHYLPARABEN	50
12A - Cleansing	METHYLPARABEN	653
12B - Depilatories	METHYLPARABEN	8
12C - Face and Neck (exc shave)	METHYLPARABEN	1005
12D - Body and Hand (exc shave)	METHYLPARABEN	1248
12E - Foot Powders and Sprays	METHYLPARABEN	10
12F - Moisturizing	METHYLPARABEN	2346
12G - Night	METHYLPARABEN	266
12H - Paste Masks (mud packs)	METHYLPARABEN	235
12I - Skin Fresheners	METHYLPARABEN	105
12J - Other Skin Care Preps	METHYLPARABEN	552
13A - Suntan Gels, Creams, and Liquids	METHYLPARABEN	52
13B - Indoor Tanning Preparations	METHYLPARABEN	120
13C - Other Suntan Preparations	METHYLPARABEN	26
		13797

02B - Bubble Baths	SODIUM METHYLPARABEN	9
02D - Other Bath Preparations	SODIUM METHYLPARABEN	1
03B - Eyeliner	SODIUM METHYLPARABEN	14
03C - Eye Shadow	SODIUM METHYLPARABEN	3
03D - Eye Lotion	SODIUM METHYLPARABEN	4
03E - Eye Makeup Remover	SODIUM METHYLPARABEN	5
03F - Mascara	SODIUM METHYLPARABEN	9
03G - Other Eye Makeup Preparations	SODIUM METHYLPARABEN	15
04E - Other Fragrance Preparation	SODIUM METHYLPARABEN	1
05A - Hair Conditioner	SODIUM METHYLPARABEN	2
05B - Hair Spray (aerosol fixatives)	SODIUM METHYLPARABEN	1
05D - Permanent Waves	SODIUM METHYLPARABEN	1
05F - Shampoos (non-coloring)	SODIUM METHYLPARABEN	42
05G - Tonics, Dressings, and Other Hair Grooming Aids	SODIUM METHYLPARABEN	9
05H - Wave Sets	SODIUM METHYLPARABEN	1
05I - Other Hair Preparations	SODIUM METHYLPARABEN	13
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	SODIUM METHYLPARABEN	65
06B - Hair Tints	SODIUM METHYLPARABEN	1
06C - Hair Rinses (coloring)	SODIUM METHYLPARABEN	2
06D - Hair Shampoos (coloring)	SODIUM METHYLPARABEN	4
06F - Hair Lighteners with Color	SODIUM METHYLPARABEN	2
07C - Foundations	SODIUM METHYLPARABEN	1
07I - Other Makeup Preparations	SODIUM METHYLPARABEN	11
09C - Other Oral Hygiene Products	SODIUM METHYLPARABEN	1
10A - Bath Soaps and Detergents	SODIUM METHYLPARABEN	12
10C - Douches	SODIUM METHYLPARABEN	3
10E - Other Personal Cleanliness Products	SODIUM METHYLPARABEN	3
11E - Shaving Cream	SODIUM METHYLPARABEN	1
12A - Cleansing	SODIUM METHYLPARABEN	33
12B - Depilatories	SODIUM METHYLPARABEN	2
12C - Face and Neck (exc shave)	SODIUM METHYLPARABEN	61
12D - Body and Hand (exc shave)	SODIUM METHYLPARABEN	18
12E - Foot Powders and Sprays	SODIUM METHYLPARABEN	3
12F - Moisturizing	SODIUM METHYLPARABEN	23
12G - Night	SODIUM METHYLPARABEN	8
12H - Paste Masks (mud packs)	SODIUM METHYLPARABEN	12
12I - Skin Fresheners	SODIUM METHYLPARABEN	6
12J - Other Skin Care Preps	SODIUM METHYLPARABEN	28
13B - Indoor Tanning Preparations	SODIUM METHYLPARABEN	4
		434

01A - Baby Shampoos	ETHYLPARABEN	1
01B - Baby Lotions, Oils, Powders, and Creams	ETHYLPARABEN	12
01C - Other Baby Products	ETHYLPARABEN	2
02A - Bath Oils, Tablets, and Salts	ETHYLPARABEN	6
02B - Bubble Baths	ETHYLPARABEN	9
02D - Other Bath Preparations	ETHYLPARABEN	33
03A - Eyebrow Pencil	ETHYLPARABEN	15
03B - Eyeliner	ETHYLPARABEN	75
03C - Eye Shadow	ETHYLPARABEN	224
03D - Eye Lotion	ETHYLPARABEN	95
03E - Eye Makeup Remover	ETHYLPARABEN	25
03F - Mascara	ETHYLPARABEN	186
03G - Other Eye Makeup Preparations	ETHYLPARABEN	96
04A - Cologne and Toilet waters	ETHYLPARABEN	2
04B - Perfumes	ETHYLPARABEN	1
04C - Powders (dusting and talcum, excluding aftershave talc)	ETHYLPARABEN	12
04E - Other Fragrance Preparation	ETHYLPARABEN	14
05A - Hair Conditioner	ETHYLPARABEN	64
05B - Hair Spray (aerosol fixatives)	ETHYLPARABEN	3
05C - Hair Straighteners	ETHYLPARABEN	5
05E - Rinses (non-coloring)	ETHYLPARABEN	2
05F - Shampoos (non-coloring)	ETHYLPARABEN	172
05G - Tonics, Dressings, and Other Hair Grooming Aids	ETHYLPARABEN	75
05H - Wave Sets	ETHYLPARABEN	3
05I - Other Hair Preparations	ETHYLPARABEN	136
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	ETHYLPARABEN	90
06B - Hair Tints	ETHYLPARABEN	1
06D - Hair Shampoos (coloring)	ETHYLPARABEN	5
06F - Hair Lighteners with Color	ETHYLPARABEN	2
06H - Other Hair Coloring Preparation	ETHYLPARABEN	17
07A - Blushers (all types)	ETHYLPARABEN	35
07B - Face Powders	ETHYLPARABEN	89
07C - Foundations	ETHYLPARABEN	135
07D - Leg and Body Paints	ETHYLPARABEN	3
07E - Lipstick	ETHYLPARABEN	97
07F - Makeup Bases	ETHYLPARABEN	31
07G - Rouges	ETHYLPARABEN	43
07H - Makeup Fixatives	ETHYLPARABEN	1
07I - Other Makeup Preparations	ETHYLPARABEN	77
08A - Basecoats and Undercoats	ETHYLPARABEN	1
08B - Cuticle Softeners	ETHYLPARABEN	12
08C - Nail Creams and Lotions	ETHYLPARABEN	2
08E - Nail Polish and Enamel	ETHYLPARABEN	15
08F - Nail Polish and Enamel Removers	ETHYLPARABEN	3
08G - Other Manicuring Preparations	ETHYLPARABEN	12
09B - Mouthwashes and Breath Fresheners	ETHYLPARABEN	1
10A - Bath Soaps and Detergents	ETHYLPARABEN	152
10B - Deodorants (underarm)	ETHYLPARABEN	12
10C - Douches	ETHYLPARABEN	2
10E - Other Personal Cleanliness Products	ETHYLPARABEN	106
11A - Aftershave Lotion	ETHYLPARABEN	39
11D - Preshave Lotions (all types)	ETHYLPARABEN	1
11E - Shaving Cream	ETHYLPARABEN	11
11F - Shaving Soap	ETHYLPARABEN	1
11G - Other Shaving Preparation Products	ETHYLPARABEN	17
12A - Cleansing	ETHYLPARABEN	274
12B - Depilatories	ETHYLPARABEN	6
12C - Face and Neck (exc shave)	ETHYLPARABEN	464
12D - Body and Hand (exc shave)	ETHYLPARABEN	433
12E - Foot Powders and Sprays	ETHYLPARABEN	7
12F - Moisturizing	ETHYLPARABEN	589
12G - Night	ETHYLPARABEN	148
12H - Paste Masks (mud packs)	ETHYLPARABEN	98
12I - Skin Fresheners	ETHYLPARABEN	25
12J - Other Skin Care Preps	ETHYLPARABEN	215
13A - Suntan Gels, Creams, and Liquids	ETHYLPARABEN	20

13B - Indoor Tanning Preparations	ETHYLPARABEN	64
13C - Other Suntan Preparations	ETHYLPARABEN	11
		4635

03D - Eye Lotion	SODIUM ETHYLPARABEN	1
03E - Eye Makeup Remover	SODIUM ETHYLPARABEN	1
03F - Mascara	SODIUM ETHYLPARABEN	2
03G - Other Eye Makeup Preparations	SODIUM ETHYLPARABEN	8
07C - Foundations	SODIUM ETHYLPARABEN	1
07I - Other Makeup Preparations	SODIUM ETHYLPARABEN	2
10C - Douches	SODIUM ETHYLPARABEN	2
12C - Face and Neck (exc shave)	SODIUM ETHYLPARABEN	4
12D - Body and Hand (exc shave)	SODIUM ETHYLPARABEN	2
12F - Moisturizing	SODIUM ETHYLPARABEN	4
12I - Skin Fresheners	SODIUM ETHYLPARABEN	1
12J - Other Skin Care Preps	SODIUM ETHYLPARABEN	5
13B - Indoor Tanning Preparations	SODIUM ETHYLPARABEN	2
		35

02A - Bath Oils, Tablets, and Salts	ISOPROPYLPARABEN	1
02D - Other Bath Preparations	ISOPROPYLPARABEN	1
03B - Eyeliner	ISOPROPYLPARABEN	7
03C - Eye Shadow	ISOPROPYLPARABEN	20
03D - Eye Lotion	ISOPROPYLPARABEN	4
03F - Mascara	ISOPROPYLPARABEN	20
03G - Other Eye Makeup Preparations	ISOPROPYLPARABEN	3
04A - Cologne and Toilet waters	ISOPROPYLPARABEN	1
04B - Perfumes	ISOPROPYLPARABEN	14
04E - Other Fragrance Preparation	ISOPROPYLPARABEN	4
05A - Hair Conditioner	ISOPROPYLPARABEN	15
05F - Shampoos (non-coloring)	ISOPROPYLPARABEN	3
05G - Tonics, Dressings, and Other Hair Grooming Aids	ISOPROPYLPARABEN	7
07A - Blushers (all types)	ISOPROPYLPARABEN	15
07B - Face Powders	ISOPROPYLPARABEN	7
07C - Foundations	ISOPROPYLPARABEN	9
07E - Lipstick	ISOPROPYLPARABEN	31
07F - Makeup Bases	ISOPROPYLPARABEN	1
07G - Rouges	ISOPROPYLPARABEN	2
07I - Other Makeup Preparations	ISOPROPYLPARABEN	12
08B - Cuticle Softeners	ISOPROPYLPARABEN	2
08C - Nail Creams and Lotions	ISOPROPYLPARABEN	1
08G - Other Manicuring Preparations	ISOPROPYLPARABEN	3
10A - Bath Soaps and Detergents	ISOPROPYLPARABEN	5
10E - Other Personal Cleanliness Products	ISOPROPYLPARABEN	21
11A - Aftershave Lotion	ISOPROPYLPARABEN	1
12A - Cleansing	ISOPROPYLPARABEN	4
12B - Depilatories	ISOPROPYLPARABEN	1
12C - Face and Neck (exc shave)	ISOPROPYLPARABEN	3
12D - Body and Hand (exc shave)	ISOPROPYLPARABEN	18
12F - Moisturizing	ISOPROPYLPARABEN	71
12G - Night	ISOPROPYLPARABEN	2
12H - Paste Masks (mud packs)	ISOPROPYLPARABEN	1
12J - Other Skin Care Preps	ISOPROPYLPARABEN	6

13A - Suntan Gels, Creams, and Liquids	ISOPROPYLPARABEN	4
13B - Indoor Tanning Preparations	ISOPROPYLPARABEN	3
		323

01A - Baby Shampoos	PROPYLPARABEN	3
01B - Baby Lotions, Oils, Powders, and Creams	PROPYLPARABEN	24
01C - Other Baby Products	PROPYLPARABEN	12
02A - Bath Oils, Tablets, and Salts	PROPYLPARABEN	28
02B - Bubble Baths	PROPYLPARABEN	25
02D - Other Bath Preparations	PROPYLPARABEN	46
03A - Eyebrow Pencil	PROPYLPARABEN	82
03B - Eyeliner	PROPYLPARABEN	484
03C - Eye Shadow	PROPYLPARABEN	703
03D - Eye Lotion	PROPYLPARABEN	108
03E - Eye Makeup Remover	PROPYLPARABEN	43
03F - Mascara	PROPYLPARABEN	302
03G - Other Eye Makeup Preparations	PROPYLPARABEN	152
04A - Cologne and Toilet waters	PROPYLPARABEN	4
04B - Perfumes	PROPYLPARABEN	8
04C - Powders (dusting and talcum, excluding aftershave talc)	PROPYLPARABEN	67
04D - Sachets	PROPYLPARABEN	1
04E - Other Fragrance Preparation	PROPYLPARABEN	49
05A - Hair Conditioner	PROPYLPARABEN	230
05B - Hair Spray (aerosol fixatives)	PROPYLPARABEN	3
05C - Hair Straighteners	PROPYLPARABEN	12
05D - Permanent Waves	PROPYLPARABEN	2
05E - Rinses (non-coloring)	PROPYLPARABEN	9
05F - Shampoos (non-coloring)	PROPYLPARABEN	216
05G - Tonics, Dressings, and Other Hair Grooming Aids	PROPYLPARABEN	222
05H - Wave Sets	PROPYLPARABEN	6
05I - Other Hair Preparations	PROPYLPARABEN	144
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	PROPYLPARABEN	118
06B - Hair Tints	PROPYLPARABEN	11
06C - Hair Rinses (coloring)	PROPYLPARABEN	6
06D - Hair Shampoos (coloring)	PROPYLPARABEN	9
06F - Hair Lighteners with Color	PROPYLPARABEN	2
06G - Hair Bleaches	PROPYLPARABEN	1
06H - Other Hair Coloring Preparation	PROPYLPARABEN	30
07A - Blushers (all types)	PROPYLPARABEN	251
07B - Face Powders	PROPYLPARABEN	340
07C - Foundations	PROPYLPARABEN	249
07D - Leg and Body Paints	PROPYLPARABEN	21
07E - Lipstick	PROPYLPARABEN	621
07F - Makeup Bases	PROPYLPARABEN	61
07G - Rouges	PROPYLPARABEN	5
07H - Makeup Fixatives	PROPYLPARABEN	6
07I - Other Makeup Preparations	PROPYLPARABEN	245
08A - Basecoats and Undercoats	PROPYLPARABEN	3
08B - Cuticle Softeners	PROPYLPARABEN	21
08C - Nail Creams and Lotions	PROPYLPARABEN	5
08E - Nail Polish and Enamel	PROPYLPARABEN	17
08F - Nail Polish and Enamel Removers	PROPYLPARABEN	3
08G - Other Manicuring Preparations	PROPYLPARABEN	18
09A - Dentifrices	PROPYLPARABEN	5
09B - Mouthwashes and Breath Fresheners	PROPYLPARABEN	2
09C - Other Oral Hygiene Products	PROPYLPARABEN	3
10A - Bath Soaps and Detergents	PROPYLPARABEN	267
10B - Deodorants (underarm)	PROPYLPARABEN	24
10C - Douches	PROPYLPARABEN	3

10E - Other Personal Cleanliness Products	PROPYLPARABEN	178
11A - Aftershave Lotion	PROPYLPARABEN	63
11D - Preshave Lotions (all types)	PROPYLPARABEN	2
11E - Shaving Cream	PROPYLPARABEN	34
11F - Shaving Soap	PROPYLPARABEN	1
11G - Other Shaving Preparation Products	PROPYLPARABEN	38
12A - Cleansing	PROPYLPARABEN	451
12B - Depilatories	PROPYLPARABEN	18
12C - Face and Neck (exc shave)	PROPYLPARABEN	631
12D - Body and Hand (exc shave)	PROPYLPARABEN	991
12E - Foot Powders and Sprays	PROPYLPARABEN	8
12F - Moisturizing	PROPYLPARABEN	1896
12G - Night	PROPYLPARABEN	189
12H - Paste Masks (mud packs)	PROPYLPARABEN	172
12I - Skin Fresheners	PROPYLPARABEN	54
12J - Other Skin Care Preps	PROPYLPARABEN	430
13A - Suntan Gels, Creams, and Liquids	PROPYLPARABEN	52
13B - Indoor Tanning Preparations	PROPYLPARABEN	84
13C - Other Suntan Preparations	PROPYLPARABEN	18
		10642

01C - Other Baby Products	SODIUM PROPYLPARABEN	1
02B - Bubble Baths	SODIUM PROPYLPARABEN	3
02D - Other Bath Preparations	SODIUM PROPYLPARABEN	1
03B - Eyeliner	SODIUM PROPYLPARABEN	2
03C - Eye Shadow	SODIUM PROPYLPARABEN	3
03D - Eye Lotion	SODIUM PROPYLPARABEN	2
03E - Eye Makeup Remover	SODIUM PROPYLPARABEN	1
03F - Mascara	SODIUM PROPYLPARABEN	5
03G - Other Eye Makeup Preparations	SODIUM PROPYLPARABEN	8
05F - Shampoos (non-coloring)	SODIUM PROPYLPARABEN	2
05I - Other Hair Preparations	SODIUM PROPYLPARABEN	1
06C - Hair Rinses (coloring)	SODIUM PROPYLPARABEN	1
07C - Foundations	SODIUM PROPYLPARABEN	1
07I - Other Makeup Preparations	SODIUM PROPYLPARABEN	5
10A - Bath Soaps and Detergents	SODIUM PROPYLPARABEN	3
10C - Douches	SODIUM PROPYLPARABEN	3
12A - Cleansing	SODIUM PROPYLPARABEN	11
12C - Face and Neck (exc shave)	SODIUM PROPYLPARABEN	26
12D - Body and Hand (exc shave)	SODIUM PROPYLPARABEN	13
12E - Foot Powders and Sprays	SODIUM PROPYLPARABEN	3
12F - Moisturizing	SODIUM PROPYLPARABEN	10
12G - Night	SODIUM PROPYLPARABEN	3
12H - Paste Masks (mud packs)	SODIUM PROPYLPARABEN	10
12I - Skin Fresheners	SODIUM PROPYLPARABEN	1
12J - Other Skin Care Preps	SODIUM PROPYLPARABEN	19
13B - Indoor Tanning Preparations	SODIUM PROPYLPARABEN	3
		141

01A - Baby Shampoos	ISOBUTYLPARABEN	1
01B - Baby Lotions, Oils, Powders, and Creams	ISOBUTYLPARABEN	2
01C - Other Baby Products	ISOBUTYLPARABEN	2
02A - Bath Oils, Tablets, and Salts	ISOBUTYLPARABEN	5
02B - Bubble Baths	ISOBUTYLPARABEN	3

02D - Other Bath Preparations	ISOBUTYLPARABEN	26
03A - Eyebrow Pencil	ISOBUTYLPARABEN	7
03B - Eyeliner	ISOBUTYLPARABEN	39
03C - Eye Shadow	ISOBUTYLPARABEN	42
03D - Eye Lotion	ISOBUTYLPARABEN	34
03E - Eye Makeup Remover	ISOBUTYLPARABEN	11
03F - Mascara	ISOBUTYLPARABEN	84
03G - Other Eye Makeup Preparations	ISOBUTYLPARABEN	46
04A - Cologne and Toilet waters	ISOBUTYLPARABEN	3
04B - Perfumes	ISOBUTYLPARABEN	15
04C - Powders (dusting and talcum, excluding aftershave talc)	ISOBUTYLPARABEN	4
04E - Other Fragrance Preparation	ISOBUTYLPARABEN	11
05A - Hair Conditioner	ISOBUTYLPARABEN	34
05E - Rinses (non-coloring)	ISOBUTYLPARABEN	1
05F - Shampoos (non-coloring)	ISOBUTYLPARABEN	65
05G - Tonics, Dressings, and Other Hair Grooming Aids	ISOBUTYLPARABEN	28
05H - Wave Sets	ISOBUTYLPARABEN	1
05I - Other Hair Preparations	ISOBUTYLPARABEN	36
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	ISOBUTYLPARABEN	23
06D - Hair Shampoos (coloring)	ISOBUTYLPARABEN	4
06H - Other Hair Coloring Preparation	ISOBUTYLPARABEN	15
07A - Blushers (all types)	ISOBUTYLPARABEN	25
07B - Face Powders	ISOBUTYLPARABEN	24
07C - Foundations	ISOBUTYLPARABEN	63
07D - Leg and Body Paints	ISOBUTYLPARABEN	2
07E - Lipstick	ISOBUTYLPARABEN	73
07F - Makeup Bases	ISOBUTYLPARABEN	6
07G - Rouges	ISOBUTYLPARABEN	2
07H - Makeup Fixatives	ISOBUTYLPARABEN	1
07I - Other Makeup Preparations	ISOBUTYLPARABEN	54
08A - Basecoats and Undercoats	ISOBUTYLPARABEN	1
08B - Cuticle Softeners	ISOBUTYLPARABEN	12
08C - Nail Creams and Lotions	ISOBUTYLPARABEN	2
08E - Nail Polish and Enamel	ISOBUTYLPARABEN	10
08F - Nail Polish and Enamel Removers	ISOBUTYLPARABEN	3
08G - Other Manicuring Preparations	ISOBUTYLPARABEN	13
10A - Bath Soaps and Detergents	ISOBUTYLPARABEN	103
10B - Deodorants (underarm)	ISOBUTYLPARABEN	7
10C - Douches	ISOBUTYLPARABEN	2
10E - Other Personal Cleanliness Products	ISOBUTYLPARABEN	85
11A - Aftershave Lotion	ISOBUTYLPARABEN	23
11D - Preshave Lotions (all types)	ISOBUTYLPARABEN	1
11E - Shaving Cream	ISOBUTYLPARABEN	2
11F - Shaving Soap	ISOBUTYLPARABEN	1
11G - Other Shaving Preparation Products	ISOBUTYLPARABEN	11
12A - Cleansing	ISOBUTYLPARABEN	117
12B - Depilatories	ISOBUTYLPARABEN	3
12C - Face and Neck (exc shave)	ISOBUTYLPARABEN	262
12D - Body and Hand (exc shave)	ISOBUTYLPARABEN	235
12E - Foot Powders and Sprays	ISOBUTYLPARABEN	2
12F - Moisturizing	ISOBUTYLPARABEN	296
12G - Night	ISOBUTYLPARABEN	60
12H - Paste Masks (mud packs)	ISOBUTYLPARABEN	45
12I - Skin Fresheners	ISOBUTYLPARABEN	14
12J - Other Skin Care Preps	ISOBUTYLPARABEN	140
13A - Suntan Gels, Creams, and Liquids	ISOBUTYLPARABEN	12
13B - Indoor Tanning Preparations	ISOBUTYLPARABEN	34
13C - Other Suntan Preparations	ISOBUTYLPARABEN	3
		2291

12I - Skin Fresheners	SODIUM ISOBUTYLPARABEN	1

01A - Baby Shampoos	BUTYLPARABEN	2
01B - Baby Lotions, Oils, Powders, and Creams	BUTYLPARABEN	7
01C - Other Baby Products	BUTYLPARABEN	3
02A - Bath Oils, Tablets, and Salts	BUTYLPARABEN	7
02B - Bubble Baths	BUTYLPARABEN	7
02D - Other Bath Preparations	BUTYLPARABEN	27
03A - Eyebrow Pencil	BUTYLPARABEN	48
03B - Eyeliner	BUTYLPARABEN	358
03C - Eye Shadow	BUTYLPARABEN	296
03D - Eye Lotion	BUTYLPARABEN	62
03E - Eye Makeup Remover	BUTYLPARABEN	32
03F - Mascara	BUTYLPARABEN	127
03G - Other Eye Makeup Preparations	BUTYLPARABEN	76
04A - Cologne and Toilet waters	BUTYLPARABEN	3
04B - Perfumes	BUTYLPARABEN	15
04C - Powders (dusting and talcum, excluding aftershave talc)	BUTYLPARABEN	20
04E - Other Fragrance Preparation	BUTYLPARABEN	18
05A - Hair Conditioner	BUTYLPARABEN	62
05C - Hair Straighteners	BUTYLPARABEN	5
05E - Rinses (non-coloring)	BUTYLPARABEN	4
05F - Shampoos (non-coloring)	BUTYLPARABEN	128
05G - Tonics, Dressings, and Other Hair Grooming Aids	BUTYLPARABEN	56
05H - Wave Sets	BUTYLPARABEN	2
05I - Other Hair Preparations	BUTYLPARABEN	64
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	BUTYLPARABEN	23
06B - Hair Tints	BUTYLPARABEN	3
06D - Hair Shampoos (coloring)	BUTYLPARABEN	5
06H - Other Hair Coloring Preparation	BUTYLPARABEN	17
07A - Blushers (all types)	BUTYLPARABEN	99
07B - Face Powders	BUTYLPARABEN	152
07C - Foundations	BUTYLPARABEN	133
07D - Leg and Body Paints	BUTYLPARABEN	7
07E - Lipstick	BUTYLPARABEN	297
07F - Makeup Bases	BUTYLPARABEN	38
07G - Rouges	BUTYLPARABEN	39
07H - Makeup Fixatives	BUTYLPARABEN	1
07I - Other Makeup Preparations	BUTYLPARABEN	105
08A - Basecoats and Undercoats	BUTYLPARABEN	2
08B - Cuticle Softeners	BUTYLPARABEN	16
08C - Nail Creams and Lotions	BUTYLPARABEN	3
08E - Nail Polish and Enamel	BUTYLPARABEN	11
08F - Nail Polish and Enamel Removers	BUTYLPARABEN	3
08G - Other Manicuring Preparations	BUTYLPARABEN	13
10A - Bath Soaps and Detergents	BUTYLPARABEN	149
10B - Deodorants (underarm)	BUTYLPARABEN	10
10C - Douches	BUTYLPARABEN	2
10E - Other Personal Cleanliness Products	BUTYLPARABEN	112
11A - Aftershave Lotion	BUTYLPARABEN	30
11D - Preshave Lotions (all types)	BUTYLPARABEN	1
11E - Shaving Cream	BUTYLPARABEN	11
11F - Shaving Soap	BUTYLPARABEN	1
11G - Other Shaving Preparation Products	BUTYLPARABEN	13
12A - Cleansing	BUTYLPARABEN	220
12B - Depilatories	BUTYLPARABEN	4
12C - Face and Neck (exc shave)	BUTYLPARABEN	378
12D - Body and Hand (exc shave)	BUTYLPARABEN	350
12E - Foot Powders and Sprays	BUTYLPARABEN	5
12F - Moisturizing	BUTYLPARABEN	518

12G - Night	BUTYLPARABEN	90
12H - Paste Masks (mud packs)	BUTYLPARABEN	91
12I - Skin Fresheners	BUTYLPARABEN	21
12J - Other Skin Care Preps	BUTYLPARABEN	195
13A - Suntan Gels, Creams, and Liquids	BUTYLPARABEN	29
13B - Indoor Tanning Preparations	BUTYLPARABEN	48
13C - Other Suntan Preparations	BUTYLPARABEN	11
		4685

03E - Eye Makeup Remover	SODIUM BUTYLPARABEN	1
12I - Skin Fresheners	SODIUM BUTYLPARABEN	1
12J - Other Skin Care Preps	SODIUM BUTYLPARABEN	1
13B - Indoor Tanning Preparations	SODIUM BUTYLPARABEN	2
		5

There were no reported uses in the 2017 VCRP for:

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

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: December 12, 2016

SUBJECT: Concentration of Use by FDA Product Category: Parabens

Concentration of Use by FDA Product Category – Parabens*

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

Ingredient	**Product Category**	**Maximum Concentration of Use**
Sodium Methylparaben	Eyebrow pencils	0.25-0.28%
Sodium Methylparaben	Eyeliners	0.3%
Sodium Methylparaben	Eye shadows	0.4%
Sodium Methylparaben	Eye lotions	0.000012-0.014%
Sodium Methylparaben	Eye makeup removers	0.0009%
Sodium Methylparaben	Mascara	0.4%
Sodium Methylparaben	Hair conditioners	0.000047-0.3%
Sodium Methylparaben	Hair sprays Pump spray	 0.00002%
Sodium Methylparaben	Hair straighteners	0.014%
Sodium Methylparaben	Rinses (noncoloring)	0.0004%
Sodium Methylparaben	Shampoos (noncoloring)	0.012-0.4%
Sodium Methylparaben	Tonics, dressings and other hair grooming aids	0.00022-0.3%
Sodium Methylparaben	Other hair preparations (noncoloring)	0.3%
Sodium Methylparaben	Hair dyes and colors	0.4%
Sodium Methylparaben	Hair rinses (coloring)	0.3%
Sodium Methylparaben	Blushers	0.3%
Sodium Methylparaben	Face powders	0.00013%
Sodium Methylparaben	Makeup bases	0.4%
Sodium Methylparaben	Cuticle softeners	0.000046%
Sodium Methylparaben	Bath soaps and detergents	0.25%
Sodium Methylparaben	Aftershave lotions	0.00025%
Sodium Methylparaben	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000005-0.00013%
Sodium Methylparaben	Face and neck products Not spray	 0.00026-0.3%
Sodium Methylparaben	Body and hand products Not spray	 0.00016-0.0065%
Sodium Methylparaben	Moisturizing products	

	Not spray	0.0007%
Sodium Methylparaben	Night products Not spray	 0.00001%
Sodium Methylparaben	Paste masks and mud packs	0.000005-0.02%
Sodium Methylparaben	Other skin care preparations	0.009-0.3%
Sodium Methylparaben	Suntan products Not spray	 0.05%
Sodium Methylparaben	Indoor tanning products	0.013%
Sodium Ethylparaben	Eye lotions	0.0036%
Sodium Ethylparaben	Hair straighteners	0.0036%
Sodium Ethylparaben	Hair rinses (coloring)	0.0036%
Sodium Ethylparaben	Cuticle softeners	0.000012%
Sodium Ethylparaben	Face and neck products Not spray	 0.0036%
Sodium Ethylparaben	Other skin care preparations	0.062%
Sodium Paraben	Other skin care preparations	0.008%
Sodium Propylparaben	Eyebrow pencils	0.28%
Sodium Propylparaben	Eyeliners	0.12-0.15%
Sodium Propylparaben	Eye shadows	0.19%
Sodium Propylparaben	Eye lotions	0.0051%
Sodium Propylparaben	Eye makeup removers	0.0004%
Sodium Propylparaben	Mascara	0.2%
Sodium Propylparaben	Shampoos (noncoloring)	0.000015%
Sodium Propylparaben	Hair rinses (coloring)	0.0051%
Sodium Propylparaben	Makeup bases	0.19%
Sodium Propylparaben	Cuticle softeners	0.000017%
Sodium Propylparaben	Dentifrices	0.1%
Sodium Propylparaben	Face and neck products Not spray	 0.0051%
Sodium Propylparaben	Other skin care preparations	0.004-0.042%
Methylparaben	Baby lotions, oils and creams Not powder	 0.13-0.4%
Methylparaben	Bath oils, tablets and salts	0.3-0.5%
Methylparaben	Bubble baths	0.21-0.31%
Methylparaben	Bath capsules	0.4%
Methylparaben	Other bath preparations	0.21-0.41%
Methylparaben	Eyebrow pencils	0.0000089-0.5%
Methylparaben	Eyeliners	0.0026-0.4%
Methylparaben	Eye shadows	0.004-0.44%
Methylparaben	Eye lotions	0.0025-0.4%
Methylparaben	Eye makeup removers	0.000002-0.4%
Methylparaben	Mascara	0.0036-0.8%
Methylparaben	Other eye makeup preparations	0.000025-0.4%
Methylparaben	Colognes and toilet waters	0.0021-0.4%
Methylparaben	Perfumes	0.000016-0.4%
Methylparaben	Powders (dusting and talcum)	0.21-0.4%

Methylparaben	Sachets	0.4%
Methylparaben	Other fragrance preparations	0.15-0.41%
Methylparaben	Hair conditioners	0.0016-0.41%
Methylparaben	Hair sprays Aerosol Pump spray	 0.05-0.4% 0.039-0.4%
Methylparaben	Hair straighteners	0.0015-0.44%
Methylparaben	Permanent waves	0.0002-0.31%
Methylparaben	Rinses (noncoloring)	0.15-0.2%
Methylparaben	Shampoos (noncoloring)	0.0075-0.9%
Methylparaben	Tonics, dressings and other hair grooming aids Not spray	0.0024-0.5% 0.2-0.4%
Methylparaben	Wave sets	0.21%
Methylparaben	Other hair preparations (noncoloring) Spray	0.004-0.31% 0.16%
Methylparaben	Hair dyes and colors	0.0012-0.22%
Methylparaben	Hair tints	0.00025-0.36%
Methylparaben	Hair rinses (coloring)	0.2-0.35%
Methylparaben	Hair shampoos (coloring)	0.2%
Methylparaben	Hair lighteners with color	0.05%
Methylparaben	Hair bleaches	0.0000016-0.05%
Methylparaben	Other hair coloring preparations	0.0004-0.4%
Methylparaben	Blushers	0.004-0.4%
Methylparaben	Face powders	0.004-0.4%
Methylparaben	Foundations	0.15-0.47%
Methylparaben	Leg and body paints	0.2-0.4%
Methylparaben	Lipstick	0.0016-0.35%
Methylparaben	Makeup bases	0.08-0.4%
Methylparaben	Rouges	0.3%
Methylparaben	Makeup fixatives	0.0035-0.4%
Methylparaben	Other makeup preparations	0.15-0.4%
Methylparaben	Basecoats and undercoats (manicuring preparations)	0.000075-0.31%
Methylparaben	Cuticle softeners	0.4-0.41%
Methylparaben	Nail creams and lotions	0.0015-0.3%
Methylparaben	Nail polish and enamel	0.0024-0.2%
Methylparaben	Nail polish and enamel removers	0.0000012-0.00005%
Methylparaben	Other manicuring preparations	0.12-0.4%
Methylparaben	Dentifrices	0.000032-0.3%
Methylparaben	Mouthwashes and breath fresheners	0.05%
Methylparaben	Bath soaps and detergents	0.000001-0.4%
Methylparaben	Deodorants Not spray Aerosol Pump spray	 0.15-0.4% 0.00012% 0.000075%
Methylparaben	Feminine hygiene deodorants	0.4%

Methylparaben	Other personal cleanliness products	0.00021-0.3%
Methylparaben	Aftershave lotions	0.00055-0.41%
Methylparaben	Beard softeners	0.4%
Methylparaben	Shaving cream	0.016-0.4%
Methylparaben	Other shaving preparations	0.0022-0.4%
Methylparaben	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.001-0.5%
Methylparaben	Depilatories	0.00003-0.34%
Methylparaben	Face and neck products Not spray Spray	 0.0013-0.4% 0.036-0.3%
Methylparaben	Body and hand products Not spray Spray	 0.001-0.6% 0.0000043-0.2%
Methylparaben	Foot products	0.25-0.6%
Methylparaben	Moisturizing products Not spray Spray	 0.0012-0.4% 0.088%
Methylparaben	Night products Not spray Spray	 0.15-0.4% 0.23%
Methylparaben	Paste masks and mud packs	0.00016-0.4%
Methylparaben	Skin fresheners	0.4%
Methylparaben	Other skin care preparations	0.002-0.29%
Methylparaben	Suntan products Not spray Spray	 0.16-0.4% 0.083%
Methylparaben	Indoor tanning preparations	0.2-0.4%
Methylparaben	Other suntan preparations	0.17-0.4%
Ethylparaben	Baby lotions, oils and creams Not powder	 0.032-0.2%
Ethylparaben	Bath oils, tablets and salts	0.015-0.1%
Ethylparaben	Other bath preparations	0.005%
Ethylparaben	Eyebrow pencils	0.1-0.4%
Ethylparaben	Eyeliners	0.00032-0.45%
Ethylparaben	Eye shadows	0.0024-0.65%
Ethylparaben	Eye lotions	0.005-0.25%
Ethylparaben	Eye makeup removers	0.000002-0.2%
Ethylparaben	Mascara	0.0012-0.6%
Ethylparaben	Other eye makeup preparations	0.0000035-0.13%
Ethylparaben	Colognes and toilet waters	0.06%
Ethylparaben	Perfumes	0.0004-0.15%
Ethylparaben	Powders (dusting and talcum)	0.05-0.2%
	Other fragrance preparations Not spray	 0.2%
Ethylparaben	Hair conditioners	0.00001-0.15%

Ethylparaben	Hair sprays Aerosol Pump spray	 0.000031-0.0024% 0.0032-0.06%
Ethylparaben	Hair straighteners	0.00054-0.2%
Ethylparaben	Permanent waves	0.0006-0.16%
Ethylparaben	Rinses (noncoloring)	0.00001%
Ethylparaben	Shampoos (noncoloring)	0.003-0.06%
Ethylparaben	Tonics, dressings and other hair grooming aids Not spray	0.00059-0.2% 0.04-0.3%
Ethylparaben	Wave sets	0.0000008-0.00078%
Ethylparaben	Other hair preparations (noncoloring)	0.015-0.07%
Ethylparaben	Hair dyes and colors	0.000048-0.048%
Ethylparaben	Hair tints	0.048-0.2%
Ethylparaben	Hair rinses (coloring)	0.000004-0.2%
Ethylparaben	Hair shampoos (coloring)	0.000065%
Ethylparaben	Hair color sprays	0.00005%
Ethylparaben	Blushers	0.048-0.5%
Ethylparaben	Face powders	0.0057-0.5%
Ethylparaben	Foundations	0.0001-0.4%
Ethylparaben	Lipstick	0.00002-0.3%
Ethylparaben	Makeup bases	0.02-0.3%
Ethylparaben	Rouges	0.3%
Ethylparaben	Makeup fixatives	0.00057%
Ethylparaben	Other makeup preparations	0.00042-0.05%
Ethylparaben	Basecoats and undercoats (manicuring preparations)	0.00005-0.12%
Ethylparaben	Cuticle softeners	0.00000032-0.2%
Ethylparaben	Nail creams and lotions	0.2%
Ethylparaben	Nail polish and enamel	0.00003-0.001%
Ethylparaben	Nail polish and enamel removers	0.00005%
Ethylparaben	Other manicuring preparations	0.12%
Ethylparaben	Dentifrices	0.000008-0.054%
Ethylparaben	Mouthwashes and breath fresheners	0.1%
Ethylparaben	Bath soaps and detergents	0.000034-0.06%
Ethylparaben	Deodorants Not spray Pump spray	 0.5% 0.00005%
Ethylparaben	Other personal cleanliness products	0.0074-0.05%
Ethylparaben	Aftershave lotions	0.000084-0.2%
Ethylparaben	Beard softeners	0.06%
Ethylparaben	Shaving cream	0.004-0.2%
Ethylparaben	Other shaving preparations	0.08%
Ethylparaben	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.033-0.5%
Ethylparaben	Depilatories	0.000035-0.0004%
Ethylparaben	Face and neck products	

	Not spray or powder	0.0002-0.04%
	Not spray	0.0002-0.48%
	Spray	0.009-0.14%
Ethylparaben	Body and hand products	
	Not spray or powder	0.044-0.048%
	Not spray	0.0004-0.35%
Ethylparaben	Foot powders and sprays	0.06-0.15%
Ethylparaben	Moisturizing products	
	Not spray	0.00028-0.5%
Ethylparaben	Night products	
	Not spray	0.00038-0.21%
Ethylparaben	Paste masks and mud packs	0.00004-0.2%
Ethylparaben	Other skin care preparations	0.000008-0.2%
Ethylparaben	Suntan products	
	Not spray	0.04-0.2%
	Aerosol	0.22%
	Pump spray	0.022%
Ethylparaben	Indoor tanning preparations	0.04%
Ethylparaben	Other suntan preparations	0.15%
	Aerosol	0.043%
Propylparaben	Baby lotions, oils and creams	
	Not powder	0.15%
Propylparaben	Bath oils, tablets and salts	0.0001-0.1%
Propylparaben	Bubble baths	0.11-0.3%
Propylparaben	Bath capsules	0.1%
Propylparaben	Other bath preparations	0.1-0.19%
Propylparaben	Eyebrow pencils	0.0015-0.38%
Propylparaben	Eyeliners	0.0019-0.3%
Propylparaben	Eye shadows	0.0019-0.4%
Propylparaben	Eye lotions	0.0001-0.21%
Propylparaben	Eye makeup removers	0.000002-0.15%
Propylparaben	Mascara	0.0017-0.7%
Propylparaben	Other eye makeup preparations	0.0000014-0.19%
Propylparaben	Colognes and toilet waters	0.00000014-0.25%
Propylparaben	Perfumes	0.0001-0.25%
Propylparaben	Powders (dusting and talcum)	0.05-0.28%
Propylparaben	Sachets	0.3%
Propylparaben	Other fragrance preparations	0.31%
	Not spray	0.2%
Propylparaben	Hair conditioners	0.00009-0.25%
Propylparaben	Hair sprays	
	Aerosol	0.000018-0.3%
	Pump spray	0.0000055-0.25%
Propylparaben	Hair straighteners	0.00027-0.25%
Propylparaben	Permanent waves	0.00002-0.21%
Propylparaben	Rinses (noncoloring)	0.0005-0.2%

Propylparaben	Shampoos (noncoloring)	0.0036-0.25%
Propylparaben	Tonics, dressings and other hair grooming aids Not spray	0.0003-0.25% 0.008-0.4%
Propylparaben	Wave sets	0.000012-0.0009%
Propylparaben	Other hair preparations (noncoloring)	0.000015-0.12%
Propylparaben	Hair dyes and colors	0.0004-0.057%
Propylparaben	Hair tints	0.00005-0.1%
Propylparaben	Hair rinses (coloring)	0.000002-0.25%
Propylparaben	Hair shampoo (coloring)	0.2%
Propylparaben	Hair color sprays	0.2%
Propylparaben	Hair bleaches	0.00000026%
Propylparaben	Other hair coloring preparations	0.0005-0.15%
Propylparaben	Blushers	0.0015-0.3%
Propylparaben	Face powders	0.0018-0.3%
Propylparaben	Foundations	0.0017-0.3%
Propylparaben	Lipstick	0.0019-0.3%
Propylparaben	Makeup bases	0.04-0.4%
Propylparaben	Rouges	0.096%
Propylparaben	Makeup fixatives	0.0018-0.25%
Propylparaben	Other makeup preparations	0.002-0.4%
Propylparaben	Basecoats and undercoats (manicuring preparations)	0.000025-0.11%
Propylparaben	Cuticle softeners	0.001-0.2%
Propylparaben	Nail creams and lotions	0.000045-0.1%
Propylparaben	Nail polish and enamel	0.0000003-0.15%
Propylparaben	Other manicuring preparations	0.073-0.15%
Propylparaben	Dentifrices	0.000004-0.05%
Propylparaben	Bath soaps and detergents	0.000014-0.25%
Propylparaben	Deodorants Not spray Aerosol Pump spray	 0.025-0.15% 0.000058% 0.000025%
Propylparaben	Feminine hygiene deodorants	0.02%
Propylparaben	Other personal cleanliness products	0.0016-0.25%
Propylparaben	Aftershave lotions	0.000042-0.2%
Propylparaben	Beard softeners	0.25%
Propylparaben	Shaving cream	0.00003-0.25%
Propylparaben	Other shaving preparations	0.15%
Propylparaben	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.001-0.3%
Propylparaben	Depilatories	0.000015-0.1%
Propylparaben	Face and neck products Not spray Spray	 0.0001-0.3% 0.00000091-0.0044%
Propylparaben	Body and hand products Not spray	 0.0019-0.3%

	Spray	0.00000076-0.1%
Propylparaben	Foot powders and sprays	0.2-0.25%
Propylparaben	Moisturizing products	
	Not spray	0.00014-0.3%
	Spray	0.024%
Propylparaben	Night products	
	Not spray	0.001-0.25%
Propylparaben	Paste masks or mud packs	0.00002-0.3%
Propylparaben	Other skin care preparations	0.000002-0.3%
Propylparaben	Suntan products	
	Not spray	0.000015-0.21%
	Aerosol	0.012%
	Pump spray	0.012%
Propylparaben	Indoor tanning preparations	0.065-0.19%
Propylparaben	Other suntan preparations	0.02-0.23%
Isopropylparaben	Eye shadows	0.19%
Isopropylparaben	Hair conditioners	0.000005-0.00004%
Isopropylparaben	Hair sprays	
	Pump spray	0.00004%
Isopropylparaben	Hair straighteners	0.22%
Isopropylparaben	Shampoos (noncoloring)	0.00004%
Isopropylparaben	Tonics, dressings and other hair grooming aids	0.00004%
Isopropylparaben	Blushers	0.18%
Isopropylparaben	Lipstick	0.12%
Isopropylparaben	Nail polish and enamel	0.00012%
Isopropylparaben	Aftershave lotions	0.32%
Isopropylparaben	Shaving cream	0.031%
Isopropylparaben	Face and neck products	
	Not spray	0.32%
Isopropylparaben	Moisturizing products	
	Not spray	0.32%
Isopropylparaben	Suntan products	
	Not spray	0.32%
	Aerosol	0.012%
	Pump spray	0.022%
Isopropylparaben	Indoor tanning preparations	0.24%
Isopropylparaben	Other suntan preparations	0.023%
Butylparaben	Bath oils, tablets and salts	0.00002-0.1%
Butylparaben	Other bath preparations	0.005%
Butylparaben	Eyebrow pencils	0.05-0.2%
Butylparaben	Eye liners	0.00015-0.4%
Butylparaben	Eye shadows	0.0014-0.3%
Butylparaben	Eye lotions	0.0003-0.2%
Butylparaben	Eye makeup removers	0.000002-0.1%
Butylparaben	Mascara	0.0012-0.5%
Butylparaben	Other eye makeup preparations	0.0000033-0.05%

Butylparaben	Colognes and toilet waters	0.05%
Butylparaben	Perfumes	0.1%
Butylparaben	Other fragrance preparations Not spray	 0.1%
Butylparaben	Hair conditioners	0.000072-0.2%
Butylparaben	Hair sprays Aerosol Pump spray	 0.00006% 0.00000011-0.0024%
Butylparaben	Hair straighteners	0.00047-0.17%
Butylparaben	Permanent waves	0.0000005-0.0008%
Butylparaben	Rinses (noncoloring)	0.0000096%
Butylparaben	Shampoos (noncoloring)	0.00002-0.2%
Butylparaben	Tonics, dressings and other hair grooming aids	0.00059-0.22%
Butylparaben	Wave sets	0.000001-0.000012%
Butylparaben	Other hair preparations (noncoloring)	0.00014-0.04%
Butylparaben	Hair dyes and colors	0.000006-0.00031%
Butylparaben	Hair tints	0.03-0.05%
Butylparaben	Hair rinses (coloring)	0.0000005-0.00091%
Butylparaben	Hair color sprays	0.0005%
Butylparaben	Blushers	0.0014-0.2%
Butylparaben	Face powders	0.0057-0.3%
Butylparaben	Foundations	0.000015-0.32%
Butylparaben	Lipstick	0.0000026-0.2%
Butylparaben	Makeup bases	0.00001-0.24%
Butylparaben	Rouges	0.3%
Butylparaben	Makeup fixatives	0.00056%
Butylparaben	Basecoats and undercoats (manicuring preparations)	0.000025%
Butylparaben	Cuticle softeners	0.05%
Butylparaben	Nail creams and lotions	0.000005-0.1%
Butylparaben	Nail polish and enamel	0.00000006%
Butylparaben	Other manicuring preparations	0.07%
Butylparaben	Dentifrices	0.000008%
Butylparaben	Bath soaps and detergents	0.017-0.15%
Butylparaben	Deodorants Pump spray	 0.000025%
Butylparaben	Other personal cleanliness products	0.0069%
Butylparaben	Aftershave lotions	0.00002-0.25%
Butylparaben	Shaving cream	0.023-0.2%
Butylparaben	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.024-0.33%
Butylparaben	Depilatories	0.0000004%
Butylparaben	Face and neck products Not spray Spray	 0.0002-0.24% 0.00002-0.087%
Butylparaben	Body and hand products	

	Not spray	0.0001-0.2%
Butylparaben	Moisturizing products	
	Not spray	0.00028-0.24%
Butylparaben	Night products	
	Not spray	0.00013-0.00015%
Butylparaben	Paste masks and mud packs	0.00004-0.2%
Butylparaben	Other skin care preparations	0.000001-0.3%
Butylparaben	Suntan products	
	Not spray	0.1-0.24%
	Aerosol	0.022%
	Pump spray	0.022%
Butylparaben	Indoor tanning preparations	0.18%
Butylparaben	Other suntan preparations	0.012-0.05%
Isobutylparaben	Bath oils, tablets and salts	0.000012%
Isobutylparaben	Other bath preparations	0.005%
Isobutylparaben	Eyeliners	0.0075-0.03%
Isobutylparaben	Eye shadows	0.14%
Isobutylparaben	Eye lotions	0.00001-0.015%
Isobutylparaben	Mascara	0.00000006-0.004%
Isobutylparaben	Other eye makeup preparations	0.000044%
Isobutylparaben	Other fragrance preparations	
	Not spray	0.0000006%
Isobutylparaben	Hair conditioners	0.0000098-0.0015%
Isobutylparaben	Hair sprays	
	Aerosol	0.0019%
	Pump spray	0.00004%
Isobutylparaben	Hair straighteners	0.00035-0.17%
Isobutylparaben	Permanent waves	0.0004%
Isobutylparaben	Rinses (noncoloring)	0.0000048-0.00004%
Isobutylparaben	Shampoos (noncoloring)	0.0000016-0.008%
Isobutylparaben	Tonics, dressings and other hair grooming aids	0.00002-0.013%
Isobutylparaben	Wave sets	0.0000004%
Isobutylparaben	Other hair preparations (noncoloring)	0.02%
Isobutylparaben	Hair tints	0.000036%
Isobutylparaben	Hair rinses (coloring)	0.00008%
Isobutylparaben	Blushers	0.14%
Isobutylparaben	Face powders	0.0029-0.0086%
Isobutylparaben	Foundations	0.000006-0.055%
Isobutylparaben	Lipstick	0.000008-0.09%
Isobutylparaben	Makeup bases	0.000016%
Isobutylparaben	Rouges	0.015%
Isobutylparaben	Dentifrices	0.000004%
Isobutylparaben	Bath soaps and detergents	0.008%
Isobutylparaben	Other personal cleanliness products	0.0035%
Isobutylparaben	Aftershave lotions	0.00001-0.25%
Isobutylparaben	Shaving cream	0.023%

Isobutylparaben	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00002-0.23%
Isobutylparaben	Face and neck products Not spray Spray	 0.0001-0.24% 0.0044%
Isobutylparaben	Body and hand products Not spray	 0.0000007-0.02%
Isobutylparaben	Moisturizing products Not spray	 0.00014-0.24%
Isobutylparaben	Night products Not spray	 0.00015%
Isobutylparaben	Paste masks and mud packs	0.00002-0.0074%
Isobutylparaben	Other skin care preparations	0.015-0.3%
Isobutylparaben	Suntan products Not spray Spray	 0.24% 0.012%
Isobutylparaben	Indoor tanning preparations	0.18%
Isobutylparaben	Other suntan preparations Spray	0.02% 0.023%

*Ingredients included in the title of the table but not in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2016

Table prepared December 12, 2016