NEW DATA

Parabens

CIR EXPERT PANEL MEETING

MARCH 5-6, 2012

Parabens – New Data

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Memorandum

To: CIR Expert Panel Members and Liaisons

From: Alan Andersen, Director, CIR

Date: February 10, 2012

Subject: Parabens

After the December, 2011 meeting, the Council asked the Panel to re-examine (see attached December 15, 2011 memo) its recent review of Parabens, and, if needed, re-review the use of Parabens as ingredients for use in cosmetics and personal care products.

The Council based its request on: (1) the 22 March 2011 revised opinion on parabens issued by the European Commission's Scientific Committee on Consumer Safety (SCCS) and (2) the 10 October, 2011 SCCS clarification on opinion SCCS/1348/10 in the light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age. Both documents are attached.

A copy of the CIR amended safety assessment of parabens is attached.

Do the positions taken in Europe and the data on which they are based provide a sufficient basis to consider an early re-review of parabens? If not, no further action need be taken. If a re-review is warranted, CIR would prepare a re-review package that updates the available data for consideration at a future meeting.

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Final Amended Report on the Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben as used in Cosmetic Products[1]

Parabens is the name given to a group of *p*-hydroxybenzoic acid (PHBA) esters used in over 22,000 cosmetics as preservatives at concentrations up to 0.8% (mixtures of parabens) or up to 0.4% (single paraben). The group includes Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben. Industry estimates of the daily use of cosmetic products that may contain parabens were 17.76 g for adults and 378 mg for infants. Parabens in cosmetic formulations applied to skin penetrate the stratum corneum in inverse relation to the ester chain length. Carboxylesterases hydrolyze parabens in the skin. Parabens do not accumulate in the body. Serum concentrations of parabens, even after intravenous administration, quickly decline and remain low. Acute toxicity studies in animals indicate that parabens are not significantly toxic by various routes of administration. Subchronic and chronic oral studies indicate that parabens are practically nontoxic. 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 did increase chromosomal aberrations in a Chinese Hamster ovary cell assay. 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, but Isobutylparaben and Butylparaben were noncarcinogenic in a mouse chronic feeding study. Methylparaben was noncarcinogenic when injected subcutaneously in mice or rats, or when administered intravaginally in rats, and 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, and Ethylparaben was nonteratogenic in rats. Parabens, even at levels that produce maternal toxicity, do not produce fetal anomalies in animal studies. Parabens have been extensively studied to evaluate male reproductive toxicity. In one in vitro study, sperm were not viabile at 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; and decreased sperm number and motile activity in F_1 offspring of rats maternally exposed to 100 mg/kg day^{-1} were reported. Decreased sperm numbers and activity were reported in F_1 offspring of female rats given Butylparaben (in DMSO) by subcutaneous injection at 100 or 200 mg/kg day^{-1}, but there were no abnormalities in the reproductive organs. Methylparaben was studied using rats at levels in the diet up to an estimated mean dose of 1141.1 mg/kg day^{-1} with no adverse testicular effects. Butylparaben was studied using rats at levels in the diet up to an 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 does bind to estrogen receptors in isolated rat uteri, but with an affinity orders of magnitude less than natural estradiol. Relative binding (diethylstilbesterol binding affinity set at 100) to the human estrogen receptors α and β increases as a function of chain length from not detectable for Methylparaben to 0.267 $\pm$ 0.027 for human estrogen receptor α and 0.340 $\pm$ 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. PHBA at 5 mg/kg day^{-1} subcutaneously (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 at least 1000$\times$ less when compared to natural estradiol. The same pattern was reported for Ethylparaben, Propylparaben, and Butylparaben when potency was compared to natural estradiol. In two studies, Isobutylparaben did produce an estrogenic response in the uterotrophic assay, but the potency was at least 240,000$\times$ less than estradiol. In one study, Benzylparaben produced an estrogenic response in the uterotrophic assay, but the potency was at least 330,000$\times$ 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 produced by estradiol. Parabens are practically nonirritating and nonsensitizing in the population with normal skin. Paraben sensitization has occurred and continues to be reported in the case literature, but principally when exposure involves damaged or broken skin. Even when patients with chronic dermatitis are patch-tested to a parabens mix,

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

parabens generally induce sensitization in less than 4% of such individuals. Many patients sensitized to paraben-containing medications can wear cosmetics containing these ingredients with no adverse effects. 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. As reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel, the 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 concentrations used in cosmetics. Although parabens do penetrate the stratum corneum, metabolism of parabens takes place within viable skin, which is likely to result in only 1% unmetabolized parabens available for absorption into the body. The Expert Panel did consider data in the category of endocrine disruption, including male reproductive toxicity and various estrogenic activity studies. The CIR Expert Panel compared exposures to parabens resulting from use of cosmetic products to a no observed adverse effect level (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 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.

INTRODUCTION

A safety assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben was published in 1984 with the conclusion that these ingredients are safe as cosmetic ingredients in the present practices of use (Elder 1984). In 1986, it was reported that the available data were insufficient to support the safety of Benzylparaben as used in cosmetic products (Elder 1986). A safety assessment of Isobutylparaben and Isopropylparaben was reported in 1995 (Andersen 1995) with the conclusion that these ingredients are safe as cosmetic ingredients in the present practices of use. The generic term "parabens" will be used to encompass Benzylparaben, Butylparaben, Ethylparaben, Isobutylparaben, Isopropylparaben, Methylparaben, and Propylparaben.

New studies since 1984 have been reported on the use of parabens in cosmetics; parabens' skin penetration, cytotoxicity, vasodilation effects, and carcinogenesis; and clinical testing of parabens—all areas considered in the original safety assessments. Not addressed in the original safety assessments were new studies reporting a link between parabens and endocrine disruption. These data were sufficient to reopen consideration of the safety of these ingredients in cosmetics and prepare this amended safety assessment.

Because the available data suggest that biological effects of parabens are related to the alkyl chain length, the order of ingredients in the report title and the presentation within each section has been organized from the shortest to the longest/largest; i.e., Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben.

In addition, a safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate has been completed (Andersen 2001). These data may be relevant because Benzyl Alcohol and Benzoic Acid are metabolites of Benzylparaben. A summary of that safety assessment is provided.

CHEMISTRY

Structure and Terminology

According to the *International Cosmetic Ingredient Dictionary and Handbook* published by the Cosmetic, Toiletry, and Fragrance Association (CTFA), parabens are esters of *p*-hydroxybenzoic acid (PHBA) with various alcohols and conform to the structure shown in Figure 1 (Gottschalck and McEwen 2004).

Benzylparaben is the ester of benzyl alcohol and *p*-hydroxybenzoic acid and conforms to the structure shown in Figure 2 (Gottschalck and McEwen 2004).

Other technical names and CAS numbers for each of the parabens are given in Table 1.

Parabens are provided to the cosmetics industry under the trade names listed in Table 2. Parabens are included in trade name mixtures supplied to the cosmetics industry as shown in Table 3.

Physical and Chemical Properties

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 (Neidig and Burrell 1944; Shiralkar et al. 1978; Lide 1993; Nikitakis and McEwen 1990).

Table 4 summarizes other physical and chemical properties of parabens.

Manufacturing Process

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 (Informatics 1972). Benzylparaben can also be prepared by reacting benzyl chloride with sodium *p*-hydrobenzoic acid (Schneider 1957).

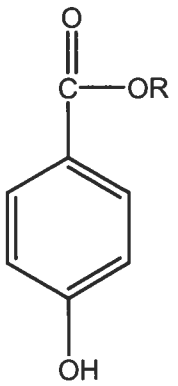

FIGURE 1
Paraben chemical structure. R = alkyl chains that are methyl (CH_3) for Methylparaben, ethyl (C_2H_5) for Ethylparaben, propyl (C_3H_7) for Propylparaben, isopropyl (C_3H_7) for Isopropylparaben, butyl (C_4H_9) for Butylparaben, and isobutyl (C_4H_9) for Isobutylparaben.

Analytical Methods

Chromatography, especially high-performance liquid chromatography (HPLC), is used presently for determinations of parabens in foods, cosmetics, and pharmaceuticals. Parabens may be determined directly, or they may be chemically modified and the derivative subsequently identified.

Table 5 lists analytical methods for Paraben determination.

Grom Chromatography GmbH (2004) provides a HPLC separation column specific to the analysis of preservatives. The eluent is 0.05 M NaH_5PO_4 at a flow rate of 0.8 ml/min at 14 mPa and 21°C. Detection is made at 235 nm. Figure 3 shows the obtainable separation of parabens.

Reactivity/Stability

Parabens are stable in air and are resistant to hydrolysis in hot and cold water, as well as in acidic solutions, although the Cosmetic, Toiletry, and Fragrance Association (1981) did state that Benzylparaben is subject to acid hydrolysis. Resistance to hydrolysis increases as the size of alkyl sidechain increases. The rate of hydrolysis is pH-dependent. 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.

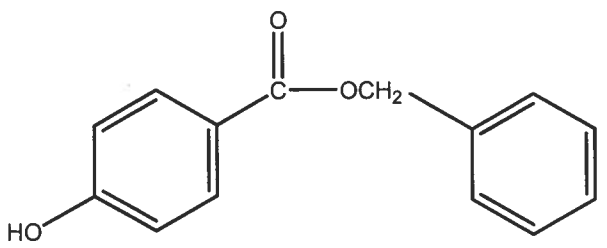

FIGURE 2
Benzylparaben chemical structure.

TABLE 1
Technical names and CAS numbers for parabens (Gottschalck and McEwen 2004).

Methylparaben (CAS no. 99-76-3)
Benzoic Acid, 4-Hydroxy-, Methyl Ester
p-Carbomethoxyphenol
4-Hydroxybenzoic Acid, Methyl Ester
p-Methoxycarbonylphenol
Methyl-4-Hydroxybenzoate
Methyl p-hydroxybenzoate
Methyl Parahydroxybenzoate
Parahydroxybenzoate Ester
Ethylparaben (CAS no. 120-47-8)
Benzoic Acid, 4-Hydroxy-, Ethyl Ester
Ethyl p-hydroxy benzoate
Ethyl 4-Hydroxybenzoate
Ethyl p-Hydroxybenzoate
Ethyl Parahydroxybenzoate
4-Hydroxybenzoic Acid, Ethyl Ester
Parahydroxybenzoate Ester
Propylparaben (CAS no. 94-13-3)
Benzoic Acid, 4-Hydroxy-, Propyl Ester
4-Hydroxybenzoic Acid, Propyl Ester
Parahydroxybenzoate Ester
Propyl p-hydroxybenzoate
Propyl p-Hydroxybenzoate
Propyl Parahydroxybenzoate
Isopropylparaben (CAS no. 4191-73-5)
Benzoic Acid, 4-Hydroxy-, 1-Methylethyl Ester
p-Hydroxybenzoic Acid, Isopropyl Ester
4-Hydroxybenzoic Acid, 1-Methylethyl Ester
Isopropyl p-Hydroxybenzoate
1-Methylethyl-4-Hydroxybenzoate
Parahydroxybenzoic Acid, Isopropyl Ester
Butylparaben (CAS no. 94-26-8)
Benzoic Acid, 4-Hydroxy-, Butyl Ester
Butyl 4-Hydroxybenzoate
Butyl p-Hydroxybenzoate
Butyl p-hydroxy benzoate
Butyl Parahydroxybenzoate
Parahydroxybenzoate Ester
Isobutylparaben (CAS no. 4247-02-3)
Benzoic Acid, 4-Hydroxy-, 2-Methylpropyl Ester
4-Hydroxybenzoic Acid, 2-Methylpropyl Ester
Isobutyl p-Hydroxybenzoate
Isobutyl Parahydroxybenzoate
Parahydroxybenzoic Acid, Isobutyl Ester
Benzylparaben (CAS no. 94-18-8)
Benzoic Acid, 4-Hydroxy-, Phenylmethyl Ester
Benzyl p-Hydroxybenzoate
Benzyl Parahydroxybenzoate
4-Hydroxybenzoic Acid, Benzyl Ester
Phenylmethyl 4-Hydroxybenzoate

TABLE 2
Trade name products containing parabens (Gottschalck and McEwen 2004).

Trade Name	Supplier
Methylparaben	
Aseptoform	Greeff
Botanistat MP	Botanigenics
CoSept M	Costec
Jeen Methyl Paraben	Jeen
Lexgard M	Inolex
Methyl-4-Hydroxybenzoate	Merck KGaA
Methylparaben NF	RITA
Methylparaben NF-PC	Protameen
Methyl Parasept	Tenneco
Nipagin M	Clariant GmbH, Personal Care
NS 3550	Nutri-Shield
Paridol M	Dekker
S&M Methylparaben	Schulke & Mayr
Solbrol M	Bayer AG
Unisept M	Universal Preserv-A-Chem
Ethylparaben	
CoSept E	Costec
Ethyl-4-Hydroxybenzoate	Merck KGaA
Ethyl Paraben NF	Jeen
Ethylparaben NF-PC	Protameen
Ethyl Parasept	Tenneco
Nipagin A	Clariant GmbH, Personal Care
S&M Ethylparaben	Schulke & Mayr
Solbrol A	Bayer AG
Unisept E	Universal Preserv-A-Chem
Propylparaben	
Botanistat PP	Botanigenics
CoSept P	Costec
Jeen Propyl Paraben	Jeen
Lexgard P	Inolex
Nipasol M	Clariant GmbH, Personal Care
Paridol P	Dekker
Propyl Aseptoform	Greef
Propylparaben NF	RITA
Propylparaben NF-PC	Protameen
Propyl Parasept	Tenneco
Solbrol M	Bayer AG
S&M Methylparaben	Schulke & Mayr
Unisept P	Universal Preserv-A-Chem
Butylparaben	
Butyl Paraben NF	Jeen
Butylparaben NF-PC	Protameen
Butyl Parasept	Tenneco
CoSept B	Costec
Lexgard B	Inolex
Methyl-4-Hydroxybenzoate	Merck KGaA
Nipabutyl	Clariant GmbH, Personal Care
Paridol B	Dekker
Unisept B	Universal Preserv-A-Chem
Benzylparaben	
Nipabenzyl	Clariant
Nipabenzyl	Clariant GmbH, Personal Care
Unisept BZ	Universal Preserv-A-Chem

Parabens are resistant to hydrolysis under usual conditons of sterilization (autoclaving) and also resist saponification (Aalto et al. 1953; Benmaman and Sorby 1965; Raval and Parrott 1967; Chichester and Tanner 1968; McDavid 1974).

Interaction with Other Cosmetic Ingredients

Bolle and Mirimanoff (1950) reported that 2% Tween 81, Tween 60, and Arlacel 83 interfered with the preservative properties of 0.1% Methylparaben (Tween is a trade name for a nonionic surfactant and emulsifier, and Arlacel is a trade name for an emulsifier). De Navarre (1956) observed that 1% Tween (2, 4, 6, or 8) improved the preservative effect of 0.1% Methylparaben, whereas 2% Tween inhibited the effect of 0.2% Methylparaben. At 2%, an oleyl alcohol ethylene oxide adduct (Emulphor OW-870) also interfered with 0.2% Paraben. Ishizaki et al. (1978) reported that 0.7% Tween 80 inactivated Butylparaben.

According to De Navarre (1957), 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.

The interaction of fatty acid esters of sucrose and Parabens was studied by Valdez et al. (1968). 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 (1973), if a 5% nonionic surfactant is added to Paraben-containing water-oil emulsion, as much as 75% of the total preservative will migrate to the nonionic surfactant micelle, leaving only 25% to distribute between the oil and water phases of the emulsion.

Goto and Endo (1979) studied the hydrogen bonding of the parabens to sodium lauryl sulfate (SLS) micelles. These authors 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.

Rosen and Berke (1973) reported that parabens are bound by various macromolecules (such as methylcellulose and gelatin), nonionic emulsifiers (especially those containing polyethylene glycol [PEG] groups), and proteins.

Diffusion from Formulations

Esposito et al. (2003) examined the diffusion of Methylparaben, Ethylparaben, and Propylparaben from a water-in-oil emulsion, and oil-in-water emulsion, and two hydrophillic gels, described as typical topical formulation bases. For the water/oil emulsion, the parabens were dissolved in boiling water and

TABLE 3
Parabens contained in trade name mixtures (Gottschalck and McEwen 2004).

		Contains:						
Trade name mixture	Supplier	Methyl-	Ethyl-	Propyl-	Isopropyl-	Butyl-	Isobutyl-	Benzyl-
AEC Cosflor Blend 017 Moisture Factor WSS	A & E Connock	+						
AEC Moisture Factor HV	A & E Connock	+						
AEC Papaya Extract	A & E Connock	+						
AEC Pineapple Extract	A & E Connock	+						
Bactecar 125S	Phytocos	+	+	+		+		
Bactiphen 2506 G	Grau	+	+	+		+		
Chenynol	Chemyunion	+	+	+		+		
Compositum	Vevy	+	+	+				
Conservateur GD500	Phytocos	+	+	+		+		
Conservateur GD700	Phytocos	+	+	+		+		
CoSept PEP	RTD Hall Star	+	+	+		+	+	
Cosmocil AF	Zeneca	+		+				
Covalip LL 48	LCW			+				
Dekaben	Dekker	+	+	+		+		
Dekaben P	Dekker	+	+	+		+		
Dekacydol	Dekker	+		+		+		
Dermocide L	Fabriquimica	+		+				
Dragocid Forte 2/027045	Synrise	+		+				
Elastase Inhibitor-3	Arval	+	+	+				
Elestab 305	Laboratoires Serobiologiques	+		+				
Elestab 388	Laboratoires Serobiologiques	+						
Elestab 4112	Laboratoires Serobiologiques	+						
Elestab 4121	Laboratoires Serobiologiques					+		
Elestab 4150	Laboratoires Serobiologiques					+		
Elestab FL	Laboratoires Serobiologiques	+						
Elestab 50	Laboratoires Serobiologiques	+						
Erase	Degussa Care Specialties			+				
Euxyl K 300	Schulke & Mayr	+	+	+		+	+	
Fenlight	Sinerga	+		+				
Fenossiparaben	Sinerga	+	+	+		+		
Germaben II	Sutton	+		+				
Germaben II-E	Sutton	+		+				
Germazide MPB	Collaborative Labs	+						
Gramben II	Sinerga	+		+				
Killitol II	Collaborative Labs	+		+				
Liposerve DUP	Lipo	+		+				
Liposerve PP	Lipo	+	+	+		+	+	
LiquiPar Oil	Sutton				+	+	+	
Liquipar PE	Sutton				+	+	+	
Microcare DMP	Acti-Chem	+		+				
Microcare IMP	Acti-Chem	+		+				
Microcare PM	Acti-Chem	+	+	+				
Microcare PM5	Acti-Chem	+	+	+		+		
Mikrokill 300	Arch Personal Care Products	+		+				

(Continued on next page)

TABLE 3
Parabens contained in trade name mixtures (Gottschalck and McEwen 2004). (*Continued*)

Trade name mixture	Supplier	Contains: Methyl-	Ethyl-	Propyl-	Isopropyl-	Butyl-	Isobutyl-	Benzyl-
Neo Dragocide Powder 2/060100	Symrise	+		+				
Neo Dragocid Liquidr 2/060110	Symrise	+		+				
Nipacide A	Clariant GmbH, Personal Care	+				+	+	
Nipaguard BPX	Clariant GmbH, Personal Care	+		+				
Nipaguard MPS	Clariant GmbH, Personal Care	+		+				
Nipaguard PDU	Clariant GmbH, Personal Care	+		+				
Nipasept	Clariant GmbH, Personal Care	+	+	+				
Nipastat	Clariant GmbH, Personal Care	+	+	+		+	+	
Ocean Collagen B-03	Air Water	+	+					
Ocean Collagen B-05	Air Water	+	+					
Paragon	McIntyre	+						
Paragon II	McIntyre	+		+				
Paragon III	McIntyre	+		+				
Paragon MEPB	McIntyre	+	+	+		+		
Paraoxiben	Vevy	+	+	+		+		
Phenagon IPBC	McIntyre							+
Phenonip	Clariant GmbH, Personal Care	+	+	+		+		
Phenova	Crodarom	+	+	+		+		
Pongamia Complex	Greentech	+	+	+		+	+	
RonaCare ASC III	Merck KGaA	+	+	+		+		
RonaCare VTA	Merck KGaA	+	+	+		+		
Saccaluronate CC	LCW	+	+	+		+		
Saccaluronate LC	LCW	+	+	+		+		
Self Tanning Complex	Greentech	+	+			+	+	
Sepicide HB	SEPPIC	+	+	+		+		
Sepicide HB2	SEPPIC	+	+	+		+	+	
Sepicide WP1	SEPPIC	+	+	+		+	+	
Talcoseptic C	Vevy	+	+	+		+		
Undebenzofene C	Vevy	+	+	+		+		
Uniphen P-23	Induchem	+	+	+		+		

slowly added to the oil phase at 70°C under vigorous stirring. The oil/water emulsion was prepared in the reverse manner. The resulting emulsions were then cooled to room temperature. Gels were prepared by dissolving the parabens in water as above, followed by adding either Permulen® TR2 or Carbopol® 940 and allowing the mixture to swell at room temperature overnight. Triethanolamine was added as a neutralizer to each gel.

Diffusion of parabens from the four formulations was measured using a Franz diffusion cell in which a synthetic membrane was mounted. A 60 mM phosphate buffer, pH 7.4, was used as the receptor fluid. Parabens levels in the receptor fluid were measured using HPLC, which allows for separation and measurement of each paraben.

For both the water/oil and the oil/water emulsions, diffusion of parabens into the receptor fluid was directly proportional to the solubility of the paraben in water. For the two gels, the reverse was true. Table 6 presents the normalized fluxes for each paraben from each formulation.

The authors suggested that the differences between the results for water/oil versus oil/water could be explained by the solubility of Methylparaben in the water phase and the partitioning of Ethylparaben and Propylparaben to the internal disperse phase of the oil/water emulsion or the continuous oil phase of the water/oil emulsion. The data on diffusion from the gels suggested to the authors that the lipophilic components of the gel matrix could be a means to hold parabens in a formulation (Esposito et al. 2003).

TABLE 4
Physical and chemical properties of parabens.

Property	Methyl-	Ethyl-	Propyl-	Isopropyl-	Butyl-	Isobutyl-	Benzyl-	Reference
Molecular weight	152.16	166.18	180.21	180.22	194.23	194.25	228.25	Sokol 1952; CTFA 1981x; Lide 1993; Registry of Toxic Effects of Chemical Substances (RTECS) 1993
Melting point (°C)	131	116–18	96.2–98	—	68–69	—	110–112	Greenberg et al. 1954; Lide 1993
	125–128	115–118	95–98	—	68–72	—		Nikitakis and McEwen 1990
Boiling point (°C)	270–280	297–298	—	—	—	—	—	Lide 1993
Density	—	—	1.0630	—	—	—	—	Lide 1993
Refractive index	1.5250	1.5050	1.5050	—		—	—	Reimers 1941; Lide 1993
UV abs_{max} in water (nm)	—	256	256	—	256	—	—	Nagasawa et al. 1969
ε(extinction coefficient)	—	1.5×10^{-2}	1.5×10^{-2}	—	1.55×10^{-2}	—	—	
pKa	8.17	8.22	8.35	—	8.37	—	—	Dymicky and Huhtanen 1979
Inorganic $impurities_{max}$								
As	1 ppm	—	1 ppm	—	1 ppm	—	—	Nikitakis and McEwen 1990
Pb	10 ppm		10 ppm	—	10 ppm	—	—	Nikitakis and McEwen 1990
Ash	0.1%	0.1%	0.1%	—	0.1%	—	0.1%	Nikitakis and McEwen 1990
Residue on $ignition_{man}$	0.05%	0.05%	0.05%	—	0.05%	—	—	Nikitakis and McEwen 1990
Loss on $drying_{max}$	0.5%	0.5%	0.5%	—	0.5%	—	—	Nikitakis and McEwen 1990
$Acidity_{max}$ (mEq/750 mg)	0.02 mEq per 750 mg	0.02 mEq per 750 mg	0.02 mEq per 750 mg	—	0.02 mEq per 750 mg	—	<0.01 mEq per 200 mg	Nikitakis and McEwen 1990
Octanol/water partition coefficient (log *P*)	1.87	—	—	—	3.46	—	—	Fasano 2004
	1.66	2.19	2.71	2.91	3.24	3.4	3.56	Golden et al. 2005
Solubility in:								
alcohol	very soluble	very soluble	soluble	—	soluble	—	72.0 g per 100 ml	Greenberg et al. 1954; CTFA 1981x, Lide 1993
water	slightly soluble	slightly soluble	insoluble	—	insoluble	—	0.01 g per 100 ml	Greenberg et al. 1954; CTFA 1981x, Lide 1993
ether	very soluble	very soluble	soluble	—	soluble	—	—	Greenberg et al. 1954; Lide 1993, Budavari 1989
acetone	very soluble	soluble	soluble	—	soluble	—	—	Greenberg et al. 1954; Lide 1993, Budavari 1989
benzene	slightly soluble	—	—	—	—	—	—	Nikitakis and McEwen 1990
carbon tetrachloride	slightly soluble	—	—	—	—	—	—	Nikitakis and McEwen 1990
propylene glycol	—	—	—	—	—	—	13 g per 100 ml	CTFA 1981x
glycerin	slightly soluble	slightly soluble	—	—	slightly soluble	—	—	National Academy of Sciences 1996, United States Pharmacopeial Convention 1995

TABLE 5
Analytical methods for parabens determination.

Method	References
Thin-layer chromatography (TLC)	Talukar and Datta 1969; Gossele 1971; Lemieszek-Chodorowska and Snycerski 1971; Sarsunova 1973; Thielemann 1975; Valdehita et al. 1979
TLC/ultraviolet spectroscopy	Tiscornia and Stacchini 1964; Ludwig and Freimuth 1965, Nagasawa et al. 1969; Tammilehto and Buchi 1969; Ficicchia and DelMastro 1977
High-performance liquid chromatography	Kitada et al. 1980; Terada and Sakabe 1985; Shiromea and Oshiro 1986; Maeda et al. 1987; Talukar and Datta 1969; Lemieszek-Chodorowska and Snycerski 1971; Fujiwara et al. 1971; Gossele 1971; Sarsunova 1973; Fitzpatrick et al. 1975; Laurent and Bourdon 1975; Thielemann 1975; Wilson 1975; Caude and Le 1976; Clarke and Rashid 1977; Cox et al. 1977; Tymes 1977; Yost et al. 1977; Austin and Mather 1978; Brown et al. 1978a; Sauermann et al. 1978, Lee 1979; Leuenberger et al. 1979; Valdehita et al. 1979
Gas chromatography (GC)	Iguchi et al. 1963; Nishimoto and Uyeta 1965; Vogel and Deshusses 1965; Gupta and Lundberg 1977; Jensen 1977; Hopp 1978
GC with flame ionization	Narafu et al. 1969; Toyoda et al. 1977
Reversed phase TLC/UV spectroscopy	Rangone and Ambrosio 1970
Saponification/bromometric titration	Reimers 1938; Valencien and Deshusses 1939
Densitometry/TLC/UV spectroscopy	Schriftman 1968; Macioci and Fiotek 1975
UV spectroscopy	Montes 1956
Microrefractive index determination	Reimers 1941
Gel electrophoresis	Moore and Stretton 1978
Etherification	Lach and Sawardeker 1965
Isotachophoresis	Rubach et al. 1980
Saponification	Schoorl 1941
Saponification/TLC	Lambion et al. 1968
Ion-exchange chromatography	Fujiwara et al. 1971; Laurent and Bourdon 1975
Partition chromatography/UV spectroscopy	Sheppard and Wilson 1975
Fluorescence	Lee 1979
Partition chromatography/GC	Wilson 1972
Microbiological assay (*Candida albicans*)	Siegel 1953
Nuclear magnetic resonance (NMR) spectrometry	Shibah et al. 1970
Colorimetric test	Edwards et al. 1936; Stevenson et al. 1938; Deshusses 1945
Fractional sublimation/polarimetry	Fischer 1934
Column chromatography/gas liquid chromatography	Daenens and Laruelle 1973; Weisenberg et al. 1977
Column chromatography/UV spectroscopy	Batchelder et al. 1972
Sublimation/UV spectroscopy	Trifiro 1960
Trimethyl silyl ether conversion/GC	Donato 1965
Microdetermination of refractive index	Reimers 1940
High-speed gel permeation chromatography	Attebery 1975
Mass spectroscopy	Tatematsu et al. 1970
Extraction/TLC/colorimetric test	Peereboom and Beekes 1964; Engst et al. 1969
TLC/paper chromatography	Thielemann 1977
Paper chromatography/UV spectroscopy	Hoyem 1962; Fellegiova 1963; Guthenberg and Beckman 1963
Spectrophotometric assay	Wahbi et al. 1977
Paper electrophoresis	Fukuda et al. 1969
Polyamide TLC	Chiang 1969, Clemens 1969; Wang and Chou 1970
Liquid chromatography	Cantwell 1976; King et al. 1980

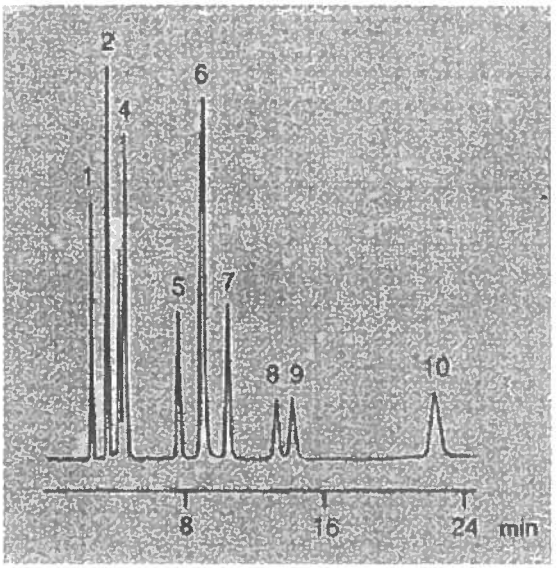

FIGURE 3
Separation of parabens obtainable using high-performance liquid chromatography. (Grom Chromatography GmbH 2004).

USE

Cosmetic

Parabens function as preservatives in cosmetics (Gottschalck and McEwen 2004). According to the Cosmetic, Toiletry, and Fragrance Association (CTFA), formulations may contain mixtures of parabens (up to 0.8%) or may contain a single paraben (up to 0.4%), and industry estimates of the daily use of cosmetic products that may contain parabens were 17.76 g for adults and 378 mg for infants (CTFA 2005).

Methylparaben

Industry has reported to the Food and Drug Administration (FDA) that Methylparaben was used in 8786 products across a wide range of product categories (FDA 2006).

Table 7 presents the available information on frequency of use and concentration of use of Methylparaben. FDA has also provided the number of products in each category so that the reader may determine what portion of reported products contain Methylparaben (FDA 2006). For example, Methylparaben is used in 4 of the 38 baby shampoos reported to FDA.

In 1981, industry provided information on broad concentration ranges in each product category and most such uses were in the >0.1% to 1% range, although one product was reported in the 10% to 25% range (Elder 1984). An industry survey conducted by the CTFA found that the concentration of use of Methylparaben ranged from 0.0003% to 1% (CTFA 2003).

TABLE 6
Diffusion of parabens from different topical formulations (Esposito et al. 2003).

	Normalized paraben fluxes[a]		
Formulation	Methyl-	Ethyl-	Propyl-
Water/oil	6.96	6.74	2.16
Oil/water	9.74	2.80	0.74
Permulen® TR2	1.34	2.54	2.67
Carbopol® 940	7.9	16.94	18.44

[a]Normalized flux (cm/h × 10^3) = flux (μg/cm^{2}h) divided by saturation concentration (mg/ml).

Ethylparaben

Industry reported to the Food and Drug Administration (FDA) that Ethylparaben was used in 2679 products across a wide range of product categories (FDA 2002), compared to 139 in 1981 (Elder 1984). Broad concentration ranges reported in each product category in 1981 were ≤0.1% and >0.1% to 1% (Elder 1984). An industry survey conducted by CTFA in 2003 found that the concentration of use of Ethylparaben ranged from 0.0002% to 0.98% (CTFA 2003).

Table 8 presents the available information on frequency of use and concentration of use of Ethylparaben.

Propylparaben

Industry reported to the Food and Drug Administration (FDA) that Propylparaben was used in 7118 products across a wide range of product categories (FDA 2006). This is a decrease over the 5868 products with Propylparaben reported to FDA in 1981 (Elder 1984). In the concentration of use data reported to FDA in 1981, industry provided information on broad concentration ranges at which the ingredient was used in each product category—such uses were primarily in the ≤0.1% and the >0.1% to 1% ranges, but one product was reported in the >10% to 25% range (Elder 1984). An industry survey conducted

TABLE 7
Current and historical uses and concentrations of Methylparaben in cosmetic products'.

Product category (number of products in category) (FDA 2006)	1981 uses (Elder 1984)	2006 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Baby products				
Baby shampoos (38)	12	4	≤0.1–1%	—
Baby lotions, oils, powders, and creams (67)	13	33	≤0.1–1%	0.2–0.4%
Other baby products (64)	4	23	≤0.1–1%	0.2%
Bath preparations				
Oils, tablets, and salts (207)	36	29	≤0.1–1%	0.3–0.5%
Soaps and detergents (594)	34	161	≤0.1–1%	0.001–0.4%
Bubble baths (256)	142	42	≤0.1–1%	0.15–0.35%
Capsules (5)	3	2	≤0.1–1%	—
Other bath preparations (276)	73	140	≤0.1–5%	0.0003 – 0.4%
Eye makeup preparations				
Eyebrow pencil (124)	14	77	>0.1–1%	0.1–0.35%
Eyeliner (639)	114	485	≤0.1–5%	0.13–0.6%
Eye shadow (1061)	883	613	≤0.1–5%	0.15–0.5%
Eye lotion (32)	9	20	≤0.1–5%	0.12–0.45%
Eye makeup remover (114)	33	67	≤0.1–5%	0.07–0.4%
Mascara (308)	227	213	≤0.1–5%	0.25–0.54%
Other eye makeup preparations (229)	73	135	≤0.1–5%	0.15–0.4%
Fragrance preparations				
Colognes and toilet waters (948)	44	24	≤0.1–1%	0.2–0.3%
Perfumes (326)	28	13	≤0.1–1%	0.15–0.35%
Fragrance powders (324)	152	91	≤0.1–5%	0.2–0.4%
Sachets (28)	77	17	≤0.1–1%	0.2%
Other fragrance preparations (187)	53	63	≤0.1–1%	0.2–0.3%
Noncoloring hair preparations				
Conditioners (715)	163	331	≤0.1–5%	0.1–0.4%
Sprays/aerosol fixatives (294)	6	10	≤0.1–1%	0.1–0.25%
Straighteners (63)	6	11	≤0.1–1%	0.15–0.18%
Permanent waves (169)	28	31	≤0.1–5%	0.3%
Rinses (46)	39	17	≤0.1–1%	0.1–0.2%
Shampoos (1022)	364	381	≤0.1–1%	0.1–0.4%
Tonics, dressings, and other hair-grooming aids (623)	56	199	≤0.1–1%	0.14–0.3%
Wave sets (59)	52	20	≤0.1–5%	—
Other hair preparations (464)	20	133	≤0.1–1%	0.2%
Hair-coloring preparations				
Dyes and colors (1600)	7	158	≤0.1–1%	0.2–0.3%
Tints (56)	—	2	—	0.2–0.35%
Rinses (15)	—	1	—	—
Shampoos (27)	4	10	>0.1–1%	-
Color sprays (4)	—	1	—	—
Lighteners with color (14)	—	4	—	0.05%
Bleaches (103)	2	1	≤0.1%	0.05–0.13%
Other hair-coloring preparations (73)	5	20	≤0.1–1%	0.2–0.32%
Makeup preparations				
Blushers (459)	274	338	≤0.1–25%	0.17–0.6%
Face powders (447)	186	282	≤0.1–5%	0.1–0.5%
Foundations (530)	301	296	≤0.1–5%	0.16–0.7%

(Continued on next page)

TABLE 7
Current and historical uses and concentrations of Methylparaben in cosmetic products'. *(Continued)*

Product category (number of products in category) (FDA 2006)	1981 uses (Elder 1984)	2006 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Leg and body paints (10)	—	6	—	0.26%
Lipstick (1681)	144	286	≤0.1–5%	0.15–1.0%
Makeup bases (273)	419	189	≤0.1–1%	0.1–0.3%
Rouges (115)	34	13	≤0.1–1%	0.2–0.3%
Makeup fixatives (37)	6	15	≤0.1–1%	0.2%
Other makeup preparations (304)	61	148	≤0.1–5%	0.2–0.43%
Nail care products				
Basecoats and undercoats (43)	1	1	>0.1–1%	—
Cuticle softeners (20)	15	13	≤0.1–1%	0.17–0.4%
Nail creams and lotions (13)	10	9	>0.1–1%	—
Nail polish and enamel (398)	—	5	—	0.12–0.4%
Nail polish and enamel remover (39)	1	—	≤0.1%	0.002%
Other manicuring preparations (58)	9	9	≤0.1–1%	0.006–0.31%
Oral hygiene products				
Dentifrices (54)	17	12	≤0.1–1%	0.07–0.15%
Mouthwashes (57)	—	1	—	—
Other oral hygiene products (10)	1	2	>0.1–1%	—
Personal cleanliness products				
Underarm deodorants (281)	28	35	≤0.1–5%	0.0008–0.3%
Douches (8)	4	3	≤0.1–1%	—
Feminine hygiene deodorants (7)	2	—	≤0.1%	0.17%
Other personal cleanliness products (390)	41	73	≤0.1–1%	0.1–0.46%
Shaving preparations				
Aftershave lotions (260)	38	77	≤0.1–1%	0.16–0.4%
Beard softeners (0)	1	—	>1–1%	—
Men's talcum (8)	3	3	≤0.1–1%	—
Preshave lotion (20)	3	1	≤0.1–1%	0.15%
Shaving creams (135)	46	50	≤0.1–1%	0.12–0.3%
Other shaving preparations (64)	13	24	≤0.1–1%	0.2%
Skin care preparations				
Cleansing creams, lotions, liquids, and pads (1009)	421	533	≤0.1–1%	0.16–0.4%
Depilatories (49)	3	4	≤0.1–1%	0.25%
Face and neck skin care preparations (546)	—	317	—	0.2–0.44%
Body and hand skin care preparations (992)	556[a]	631	≤0.1–5%[a]	0.15–0.4%
Foot powders and sprays (43)	2	20	≤0.1%	0.2–0.3%
Moisturizers (1200)	532	787	≤0.1–5%	0.07–0.4%
Night skin care preparations (229)	135	167	≤0.1–1%	0.1–0.5%
Paste masks/mud packs (312)	123	183	≤0.1–1%	0.15–0.3%
Fresheners (212)	117	94	≤0.1–5%	0.1–0.3%
Other skin care preparations (915)	4	443	≤0.1–5%	0.1–0.46%
Hormone skin care preparations[b]	8	—	≤0.1–1%	—
Skin lighteners[b]	22	—	≤0.1–1%	—
Wrinkle removers[b]	20	—	≤0.1–1%	—
Suntan preparations				
Suntan gels, creams, and liquids (138)	68	63	≤0.1–1%	0.15–0.4%
Indoor tanning preparations (74)	10	50	≤0.1–1%	0.07–0.25%
Other suntan preparations (41)	12	21	≤0.1–1%	0.2–0.3%
Total Methylparaben uses/ranges	6467	8786	≤0.1–25%	0.0003–1.0%

[a]In 1981, face and neck skin care preparations and body and hand skin care preparations were grouped in one category.
[b]This category no longer exists.

TABLE 8
Current and historical use and concentrations of Ethylparaben in cosmetic products.

Product category (number of products in category) (FDA 2002)	1981 uses (Elder 1984)	2005 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Baby products				
Baby shampoos (38)	—	1	—	—
Baby lotions, oils, powders, and creams (67)	—	12	—	—
Other baby products (64)	—	2	—	—
Bath preparations				
Oils, tablets, and salts (207)	—	6	—	0.02%
Soaps and detergents (594)	—	21	—	0.04%
Bubble baths (256)	5	13	≤0.1%	0.00004–0.06%
Capsules (5)	—	1	—	—
Other bath preparations (276)	—	31	—	0.03–0.15%
Eye makeup preparations				
Eyebrow pencil (124)	—	5	—	0.4%
Eyeliner (639)	—	23	—	0.03–0.4%
Eye shadow (1061)	4	295	≤0.1–1%	0.06–0.49%
Eye lotion (32)	—	7	—	0.03–0.11%
Eye makeup remover (114)	—	17	—	0.03–0.3%
Mascara (308)	1	127		0.00002–0.4%
Other eye makeup preparations (229)	1	69	>1–1%	0.04–0.2%
Fragrance preparations				
Colognes and toilet waters (948)	—	2	—	0.02–0.2%
Perfumes (326)	—	—	—	0.17%
Fragrance powders (324)	4	3	≤0.1%	0.07–0.08%
Other fragrance preparations (187)	—	17	—	0.03%
Noncoloring hair preparations				
Conditioners (715)	—	33	—	0.001–0.3%
Sprays/aerosol fixatives (294)	—	4	—	0.1%
Rinses (46)	—	1	—	0.2%
Shampoos (1022)	—	108	—	0.03–0.2%
Tonics, dressings, and other hair-grooming aids (623)	—	28	—	0.04–0.6%
Wave sets (59)	5	1	≤0.1–1%	—
Other hair preparations (464)	—	53	—	0.001%
Hair-coloring preparations				
Dyes and colors (1600)	—	88	—	—
Lighteners (14)	—	2	—	—
Tints (56)	—	1	—	0.2%
Other hair-coloring preparations (73)	—	1	—	0.2%
Makeup preparations				
Blushers (459)	1	84	≤0.1%	0.04–0.3%
Face powders (447)	2	119	≤0.1%	0.04–0.5%
Foundations (530)	8	150	≤0.1%	0.001–0.5%
Leg and body paints (10)	—	—	—	0.04%
Lipstick (1681)	2	72	≤0.1%	0.0002–0.2%
Makeup bases (273)	2	35	>0.1–1%	0.00006–0.35%
Rouges (115)	—	17	—	0.001–0.2%
Makeup fixatives (37)	—	6	—	0.001–0.1%
Other makeup preparations (304)	1	41	>0.1–1%	0.1–0.45%

(Continued on next page)

TABLE 8
Current and historical use and concentrations of Ethylparaben in cosmetic products.

Product category (number of products in category) (FDA 2002)	1981 uses (Elder 1984)	2005 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Nail care products				
Cuticle softeners (19)	—	3	—	0.2%
Nail creams and lotions (15)	25	2	>0.1–1%	0.15%
Nail polish and enamel (123)	—	4	—	0.01%
Other manicuring preparations (55)	—	1	—	0.06%
Personal cleanliness products				
Underarm deodorants (281)	—	10	—	0.002–0.1%
Douches (8)	—	2	—	—
Other personal cleanliness products (390)	1	24	≤0.1%	0.0002–0.12%
Shaving preparations				
Aftershave lotions (260)	1	3	≤0.1%	0.03–0.2%
Preshave lotions (20)	—	—	—	0.04–0.98%
Shaving creams (135)	—	3	—	0.0001–0.08%
Other shaving preparations (64)	—	2	—	0.02–0.036%
Skin care preparations				
Cleansing creams, lotions, liquids, and pads (1009)	13	181	≤0.1–1%	0.0006–0.54%
Depilatories (49)	—	—	—	0.1%
Face and neck skin care preparations (546)	31[a]	169	≤0.1–1%[a]	0.03–0.3%
Body and hand skin care preparations (992)		153		0.001–0.4%
Foot powders and sprays (43)	—	8	—	0.0004%
Moisturizers (1200)	9	268	≤0.1–1%	0.001–0.3%
Night skin care preparations (229)	7	64	≤0.1–1%	0.0001–0.25%
Paste masks/mud packs (312)	13	76	≤0.1–1%	0.0009–0.22%
Fresheners (212)	1	14	≤0.1%	0.05%
Other skin care preparations (915)	1	139	≤0.1–1%	0.0005–0.35%
Suntan preparations				
Suntan gels, creams, and liquids (138)	1	22	>0.1–1%	0.04–0.2%
Indoor tanning preparations (74)	—	24	—	0.04–0.4%
Other suntan preparations (41)	—	11	—	0.04–0.25%
Total Ethylparaben uses/ranges	139	2679	≤0.1–1%	0.00002–0.98%

[a]In 1981, face and neck skin care preparations and body and hand skin care preparations were combined in one category.

by the CTFA in 2003 found that the current concentration of use of Propylparaben ranged from 0.00002% to 0.7% (CTFA 2003).

Table 9 presents the available information on frequency of use and concentration of use of Propylparaben.

Isopropylparaben

Industry reported to the FDA that Isopropylparaben was used in 48 products across a wide range of product categories (FDA 2006). Only one use had been reported in 1993 (Andersen 1995). A survey conducted by CTFA found that the current concentration of use of Isopropylparaben ranged from 0.00001% to 0.3% (CTFA 2004a).

Table 10 gives the available use data for Isopropylparaben.

Butylparaben

Industry reported to the FDA that Butylparaben was used in 3001 products across a wide range of product categories (FDA 2002). This is an increase over the 704 products with Butylparaben reported to FDA in 1981 (Elder 1984). In the concentration of use data reported to FDA in 1981, industry provided information on broad concentration ranges at which the ingredient was used in each product category—such uses were primarily in the ≤0.1% range (Elder 1984). An industry survey conducted by CTFA in 2003 found that the current concentration of use of Butylparaben ranged from 0.00004% to 0.54% (CTFA 2003).

Table 11 presents the available use data for Butylparaben.

TABLE 9
Current and historical use and concentrations of Propylparaben in cosmetic products.

Product category (number of products in category) (FDA 2006)	1981 uses (Elder 1984)	2006 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Baby products				
Baby shampoos (38)	8	4	≤0.1–1%	0.1%
Baby lotions, oils, powders, and creams (67)	10	31	≤0.1–1%	0.2%
Other baby products (64)	4	21	≤0.1%	0.05%
Bath preparations				
Oils, tablets, and salts (207)	25	37	≤0.1–1%	0.3%
Soaps and detergents (594)	26	97	≤0.1–1%	0.02–0.1%
Bubble baths (256)	95	31	≤0.1–1%	0.04–0.2%
Capsules (5)	3	2	≤0.1%	—
Other bath preparations (276)	42	70	≤0.1–5%	0.1–0.3%
Eye makeup preparations				
Eyebrow pencil (124)	17	83	>0.1–1%	0.13–0.2%
Eyeliner (639)	106	477	≤0.1–1%	0.05–0.4%
Eye shadow (1061)	857	541	≤0.1–1%	0.1–0.5%
Eye lotion (32)	5	14	≤0.1–1%	0.1–0.37%
Eye makeup remover (114)	36	45	≤0.1–5%	0.05–0.15%
Mascara (308)	191	190	≤0.1–5%	0.1–0.32%
Other eye makeup preparations (229)	100	127	≤0.1–1%	0.02–0.4%
Fragrance preparations				
Colognes and toilet waters (948)	22	3	≤0.1–1%	0.2–0.3%
Perfumes (326)	14	8	≤0.1–1%	0.1–0.3%
Fragrance powders (324)	105	58	≤0.1–1%	0.1–0.2%
Sachets (28)	48	8	0.1–1%	0.15%
Other fragrance preparations (187)	37	41	≤0.1–1%	0.3%
Noncoloring hair preparations				
Conditioners (715)	100	183	≤0.1–5%	0.03–0.2%
Sprays/aerosol fixatives (294)	3	9	≤0.1–1%	0.1%
Straighteners (61)	6	7	≤0.1%	0.05%
Permanent waves (169)	23	3	≤0.1%	0.05%
Rinses (46)	28	15	≤0.1–1%	0.03%
Shampoos (1022)	190	227	≤0.1–1%	0.04–0.4%
Tonics, dressings, and other hair-grooming aids (623)	48	112	≤0.1–1%	0.04–0.5%
Wave sets (59)	14	5	≤0.1–1%	—
Other hair preparations (464)	13	58	≤0.1–1%	—
Hair-coloring preparations				
Dyes and colors (1600)	1	129	≤0.1–1%	0.2%
Tints (56)	—	2	—	0.1–0.25%
Rinses (15)	—	1	—	—
Shampoos (27)	3	1	≤0.1%	—
Color sprays (4)	—	1	—	—
Lighteners (14)	—	2		
Bleaches (103)	—	—	—	0.04–0.5%
Other hair-coloring preparations (73)	3	14	≤0.1–1%	0.1–0.2%
Makeup preparations				
Blushers (459)	284	308	≤0.1–1%	0.1–0.6%
Face powders (447)	179	250	≤0.1–5%	0.1–0.7%

(Continued on next page)

TABLE 9
Current and historical use and concentrations of Propylparaben in cosmetic products. (*Continued*)

Product category (number of products in category)	1981 uses	2006 uses	1981 concentrations	2003 concentrations
Foundations (530)	316	325	≤0.1–5%	0.05–0.4%
Leg and body paints (10)	—	2	—	0.1–0.16%
Lipstick (1681)	357	520	≤0.1–1%	0.1–0.62%
Makeup bases (273)	429	193	≤0.1–1%	0.02–0.25%
Rouges (115)	68	9	≤0.1–1%	0.15–0.2%
Makeup fixatives (37)	5	7	≤0.1–1%	-
Other makeup preparations (304)	130	165	≤0.1–1%	0.1–0.4%
Nail care products				
Basecoats and undercoats (43)	2	1	≤0.1%	—
Cuticle softeners (20)	13	9	≤0.1–1%	0.2%
Nail creams and lotions (13)	12	8	≤0.1–5%	0.2–0.3%
Nail polish and enamel (398)	1	4	≤0.1%	0.1–0.4%
Other manicuring preparations (58)	8	5	≤0.1–1%	0.002–0.3%
Oral hygiene products				
Dentifrices (54)	11	5	≤0.1%	0.03–0.15%
Mouthwashes and breath fresheners (57)	—	1	—	0.05%
Other oral hygiene (10)	—	1	—	—
Personal cleanliness products				
Underarm deodorants (281)	17	29	≤0.1–1%	0.002–0.2%
Douches (8)	2	3	≤0.1%	—
Other personal cleanliness products (390)	39	65	≤0.1–1%	0.1–0.4%
Shaving preparations				
Aftershave lotions (260)	21	26	≤0.1–1%	0.03–0.2%
Beard softeners (0)	1	—	≤0.1%	—
Men's talcum (8)	2	—	≤0.1%	—
Preshave lotion (20)	2	1	≤0.1%	0.01–0.1%
Shaving creams (135)	34	45	≤0.1–1%	0.1%
Other shaving preparations (64)	8	18	≤0.1–1%	0.01–0.15%
Skin care preparations				
Cleansing creams, lotions, liquids, and pads (1009)	350	403	≤0.1–5%	0.03–0.3%
Depilatories (49)	3	4	>0.1–1%	0.15%
Face and neck skin care preparations (546)		215		0.03–0.35%
Body and hand skin care preparations (992)	467[a]	478	≤0.1–25%	0.1–0.4%
Foot powders and sprays (43)	1	13	≤0.1%	0.1–0.2%
Moisturizers (1200)	481	591	≤0.1–5%	0.05–0.35%
Night skin care preparations (229)	111	135	≤0.1–1%	0.001–0.3%
Paste masks/mud packs (312)	64	141	≤0.1–1%	0.1–0.3%
Fresheners (212)	32	35	≤0.1–5%	0.05%
Other skin care preparations (915)	104	324	≤0.1–1%	0.00002–0.2%
Hormone skin care preparations[b]	5	—	≤0.1–1%	—
Skin lighteners[b]	15	—	≤0.1–1%	—
Wrinkle removers[b]	16	—	≤0.1–1%	—
Suntan preparations				
Suntan gels, creams, and liquids (138)	77	61	≤0.1–1%	0.1–0.3%
Indoor tanning preparations (74)	7	42	≤0.1–1%	0.02–0.15%
Other suntan preparations (41)	11	19	≤0.1–1%	0.02–0.2%
Total Propylparaben uses/ranges	5868	7118	≤0.1–25%	0.00002–0.7%

[a]In 1981, face and neck skin care preparations and body and hand skin care preparations were all in one category.
[b]This category no longer exists.

TABLE 10
Current and historical uses and concentrations of Isopropylparaben in cosmetic products.

Product category (number of products in category) (FDA 2006)	1993 uses (Andersen 1995)	2006 uses (FDA 2006)	1993 concentrations (Andersen 1995)	2003 concentrations (CTFA 2004a)
Bath preparations				
Oils, tablets, and salts (207)	—	1	—	—
Soaps and detergents (594)	—	—	—	0.03–0.1%
Other bath preparations (276)	—	—	—	0.005%
Eye makeup preparations				
Eyeliner (639)	—	6	—	0.2%
Eye shadow (1061)	—	1	—	0.06–0.2%
Eye makeup remover (114)	—	1	—	—
Mascara (308)	1	2	—	0.2%
Other eye makeup preparations (229)	—	—	—	0.06%
Fragrance preparations				
Fragrance powders (324)	—	2	—	—
Other fragrance preparations (187)	—	1	—	—
Noncoloring hair preparations				
Conditioners (715)	—	4	—	—
Sprays/aerosol fixatives (294)	—	1	—	—
Tonics, dressings, and other hair-grooming aids (623)	—	1	—	0.001%
Makeup preparations				
Blushers (459)	—	2	—	0.00001%
Face powders (447)	—	3	—	0.00001–0.00002%
Foundations (530)	—	2	—	0.00001%
Lipstick (1681)	—	1	—	0.2%
Rouges (115)	—	1	—	—
Other makeup preparations (304)	—	4	—	—
Nail care products				
Other manicuring preparations (58)	—	—	—	0.1%
Shaving preparations				
Aftershave lotions (260)	—	1	—	—
Other shaving preparations (64)	—	—	—	0.1%
Skin care preparations				
Cleansing creams, lotions, etc. (1009)	—	3		
Face and neck skin care preparations (546)		1		0.1%
Body and hand skin care preparations (992)	—	5	—	0.1–0.2%
Moisturizers (1200)	—	2	—	—
Foot powders and sprays (43)	—	—	—	0.2%
Night skin care preparations (229)	—	—	—	0.0005%
Paste masks/mud packs (312)	—	—	—	0.2%
Suntan preparations				
Suntan gels, creams and liquids (138)	—	3	—	—
Indoor tanning preparations (74)	—	—	—	0.3%
Total Isopropylparaben uses/ranges	1	48	—	0.00001–0.3%

TABLE 11
Current and historical use and concentrations of Butylparaben in cosmetic products.

Product category (number of products in category) (FDA 2002)	1981 uses (Elder 1984)	2005 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Baby products				
Baby shampoos (38)	12	2	≤0.1%	—
Baby lotions, oils, powders, and creams (67)	—	21	—	0.05%
Other baby products (64)	—	5	—	—
Bath preparations				
Oils, tablets, and salts (207)	8	8	≤0.1%	0.02%
Soaps and detergents (594)	—	22	—	0.06–0.11%
Bubble baths (256)	10	10	≤0.1–1%	0.00004–0.06%
Capsules (5)	—	1	—	—
Other bath preparations (276)	4	22	≤0.1%	0.03–0.07%
Eye makeup preparations				
Eyebrow pencil (124)	11	60	≤0.1%	0.05–0.1%
Eyeliner (639)	8	398	≤0.1–1%	0.05–0.2%
Eye shadow (1061)	42	199	≤0.1–1%	0.05–0.3%
Eye lotion (32)	—	7	—	0.02–0.21%
Eye makeup remover (114)	18	27	≤0.1–1%	0.07–0.15%
Mascara (308)	14	80	≤0.1–1%	0.00002–0.21%
Other eye makeup preparations (229)	18	41	≤0.1–1%	0.05–0.15%
Fragrance preparations				
Colognes and toilet waters (948)	4	3	≤0.1%	0.02%
Perfumes (326)	11	—	≤0.1%	0.1–0.2%
Fragrance powders (324)	14	20	≤0.1%	0.07%
Sachets (28)	16	—	≤0.1–1%	—
Other fragrance preparations (187)	4	19	≤0.1–1%	0.03%
Noncoloring hair preparations				
Conditioners (715)	7	49	≤0.1–1%	0.02–0.25%
Sprays/aerosol fixatives (294)	—	4	—	0.0004%
Rinses (46)	1	4	≤0.1%	—
Shampoos (1022)	6	108	≤0.1%	0.01–0.25%
Tonics, dressings, and other hair-grooming aids (623)	9	40	≤0.1–1%	0.06–0.2%
Wave sets (59)	6	– – –	≤0.1–1%	—
Other hair preparations (464)	—	39	—	0.03–0.1%
Hair coloring preparations				
Dyes and colors (1600)	—	23	—	0.03%
Tints (56)	—	1	—	—
Color sprays (4)	—	1	—	—
Other hair-coloring preparations (73)	1	3	≤0.1%	—
Makeup preparations				
Blushers (459)	4	35	≤0.1–1%	0.07–0.2%
Face powders (447)	—	67	—	0.07–0.14%
Foundations (530)	46	96	≤0.1–1%	0.06–0.2%
Leg and body paints (10)	—	—	—	0.09%
Lipstick (1681)	44	218	≤0.1–1%	0.0008–0.1%
Makeup bases (273)	10	51	≤0.1–1%	0.00006–0.1%
Rouges (115)	1	10	≤0.1–1%	0.05–0.08%
Makeup fixatives (37)	3	5	≤0.1%	0.07–0.08%

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TABLE 11
Current and historical use and concentrations of Butylparaben in cosmetic products. *(Continued)*

Product category (number of products in category) (FDA 2002)	1981 uses (Elder 1984)	2005 uses (FDA 2006)	1981 concentrations (Elder 1984)	2003 concentrations (CTFA 2003)
Other makeup preparations (304)	20	75	≤0.1–1%	0.1–0.2%
Nail care products				
Basecoats and undercoats (43)	—	1	—	—
Cuticle softeners (20)	1	3	≤0.1%	—
Nail creams and lotions (13)	2	1	≤0.1%	0.1%
Nail polish and enamel (398)	—	14	—	0.01–0.2%
Other manicuring preparations (58)	2	2	≤0.1–1%	0.003–0.07%
Oral hygiene products				
Dentifrices (54)	—	1	—	—
Personal cleanliness products				
Underarm deodorants (281)	2	10	≤0.1–1%	0.002%
Douches (8)	—	1	—	—
Other personal cleanliness products (390)	3	29	≤0.1%	0.00004–0.09%
Shaving preparations				
Aftershave lotions (260)	1	6	≤0.1%	0.03–0.1%
Men's talcum (8)	1	1	≤0.1%	—
Preshave lotion (20)	—	—	—	0.01%
Shaving creams (135)	1	11	≤0.1%	0.08–0.2%
Other shaving preparations (64)	2	2	≤0.1%	0.03–0.04%
Skin care preparations				
Cleansing creams, lotions, liquids, and pads (1009)	58	195	≤0.1–5%	0.0006–0.54%
Depilatories (49)	—	—	—	0.15%
Face and neck skin care preparations (546)		157		0.09–0.4%
Body and hand skin care preparations (992)	104[a]	157	≤0.1–5%[a]	0.09–0.4%
Foot powders and sprays (43)	—	6	—	0.0004%
Moisturizers (1200)	91	278	≤0.1–1%	0.06–0.2%
Night skin care preparations (229)	33	65	≤0.1–1%	0.04–0.15%
Paste masks/mud packs (312)	11	73	≤0.1–1%	0.05–0.17%
Fresheners (212)	3	8	≤0.1–1%	0.06%
Other skin care preparations (915)	11	144	≤0.1–5%	0.0004–0.15%
Hormone skin care preparations[b]	1	—	≤0.1–1%	—
Skin lighteners[b]	2	—	≤0.1%	—
Wrinkle removers[b]	4	—	≤0.1%	—
Suntan preparations				
Suntan gels, creams, and liquids (138)	15	28	≤0.1–1%	0.03–0.4%
Indoor tanning preparations (74)	—	21	—	0.04–0.15%
Other suntan preparations (41)	4	13	≤0.1%	0.24%
Total Butylparaben uses/ranges	704	3001	≤0.1–5%	0.00004–0.54%

[a]In 1981, face and neck skin care preparations and body and hand skin care preparations were combined in one category.
[b]This category no longer exists.

Isobutylparaben

Industry reported to the FDA that Isobutylparaben was used in 642 products across a wide range of product categories (FDA 2006). This is an increase over the 86 products with Isobutylparaben reported to FDA in 1993 (Andersen 1995). No use concentration data were reported by Andersen (1995). A survey conducted by CTFA determined the current concentration of use of Isobutylparaben was between 0.000007% and 0.5%(CTFA 2004a).

Table 12 gives the available use data for Isobutylparaben.

Benzylparaben

Industry reported to the FDA that Benzylparaben was used in three underarm deodorants (FDA 2006). This is a decrease from

TABLE 12
Current and historical uses and concentrations of Isobutylparaben in cosmetic products.

Product category (number of product in category) (FDA 2006)	1993 uses (Andersen 1995)	2006 uses (FDA 2006)	1993 concentrations (Andersen 1995)	2003 concentrations (CTFA 2004a)
Baby products				
Baby shampoos (38)	—	1	—	—
Baby lotions, oils, powders, and creams (67)	—	5	—	—
Other baby products (64)	—	1	—	—
Bath preparations				
Oils, tablets, and salts (207)	—	1	—	0.01%
Soaps and detergents (594)	—	11	—	0.0001–0.1%
Bubble baths (256)	—	11	—	0.00002–0.04%
Capsules (5)	7	1	—	—
Other bath preparations (276)	—	16	—	0.02%
Eye makeup preparations				
Eyebrow pencil (124)	2	4	—	0.06%
Eyeliner (639)	1	9	—	0.02–0.1%
Eye shadow (1061)	—	3	—	0.05–0.4%
Eye lotion (32)	—	1	—	0.02%
Eye makeup remover (114)	—	6	—	0.02%
Mascara (308)	3	17	—	0.000007–0.1%
Other eye makeup preparations (229)	1	19	—	0.02–0.5%
Fragrance preparations				
Colognes and toilet waters (948)	—	3	—	0.01%
Powders (324)	—	2	—	0.04%
Other fragrance preparations (187)	—	2	—	0.02%
Noncoloring hair preparations				
Conditioners (715)	—	12	—	0.01–0.02%
Sprays/aerosol fixatives (294)	—	2	—	—
Shampoos (1022)	—	38	—	0.02–0.1%
Tonics, dressings, and other hair-grooming aids (623)	—	8	—	0.02–0.3%
Other hair preparations (464)	1	22	—	—
Hair-coloring preparations				
Other hair-coloring preparations (73)	—	1	—	—
Makeup preparations				
Blushers (459)	—	2	—	0.00001–0.04%
Face powders (447)	2	6	—	0.00001–0.04%
Foundations (530)	5	15	—	0.00001–0.06%
Leg and body paints (10)	—	—	—	0.02%
Lipstick (1681)	1	11	—	0.0001–0.4%
Makeup bases (273)	—	1	—	0.00003–0.02%
Fixatives (37)		1	—	—
Rouges (115)	—	1	—	—
Other makeup preparations (304)	1	17	—	0.03%
Nail care products				
Nail polish and enamel (398)	—	3	—	0.006%
Personal cleanliness products				
Underarm deodorants (281)	—	3	—	0.002%
Douches (8)	—	2	—	—
Other personal cleanliness products (390)	—	10	—	0.02%

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TABLE 12
Current and historical uses and concentrations of Isobutylparaben in cosmetic products. *(Continued)*

Product category (number of product in category) (FDA 2006)	1993 uses (Andersen 1995)	2006 uses (FDA 2006)	1993 concentrations (Andersen 1995)	2003 concentrations (CTFA 2004a)
Shaving preparations				
Aftershave lotions (260)	—	3	—	0.02–0.03%
Shaving cream (135)	—	1	—	—
Other shaving preparations (64)	1	1	—	0.02%
Skin care preparations				
Cleansing creams, lotions, liquids, and pads (1009)	7	67	—	0.003–0.1%
Face and neck skin care preparations (546)	12	75	—	0.02–0.09%
Body and hand skin care preparations (992)	4	52	—	0.02–0.4%
Foot powders and sprays (43)	—	2	—	0.4%
Moisturizers (1200)	12	67	—	0.0002–0.02%
Personal cleanliness product				
Night skin care preparations (229)	2	14	—	—
Paste masks/mud packs (312)	4	28	—	0.0004–0.4%
Fresheners (212)	1	5	—	0.02%
Other skin care preparations (915)	10	44	—	0.00002–0.02%
Suntan preparations				
Suntan gels, creams, and liquids (138)	5	9	—	0.02%
Indoor tanning preparations (74)	1	4	—	0.02–0.09%
Other suntan preparations (41)	3	2	—	0.2%
Total Isobutylparaben uses/ranges	86	642	—	0.000007–0.5%

the 45 uses reported in 1984 (Elder 1986). In 1984, industry reported broad concentration ranges in each product category—such uses were primarily in the ≤0.1% range, although one underarm deodorant was in the 0.1% to 1% range (Elder 1986). An industry survey conducted by the CTFA found no reported uses/use concentrations of Benzylparaben (CTFA 2004a).

Table 13 presents the available use data for Benzylparaben.

Product Analysis

According to Neidig and Burrell (1944), parabens formulate well because they have no perceptible odor or taste, are practically neutral in pH, do not produce discoloration, and do not cause hardening or muddying.

Rastogi et al. (1995) analyzed cosmetic products for the presence of Methyl-, Ethyl-, Propyl-, Butyl-, and Benzylparaben. Identification was based on HPLC retention times relative to the retention time of Isopropylparaben at a detection limit of 0.005%. Of the 57 rinse-off products analyzed, 77% contained parabens; and 99% of the 158 leave-on products contained parabens.

Table 14 presents the concentration (weight/weight) of each of the measured parabens in cosmetic products and the prevalence of each. With the exception of one suntan lotion at 0.87%, rinse-off products contained 0.01% to 0.5% and leave-on products contained 0.01% to 0.59% total parabens. The authors noted that the one product at 0.87% does not comply with the Danish and European Commission maximum concentration limit on total parabens of 0.8%, calculated as *p*-hydroxybenzoic acid. The limit for any one paraben is 0.4%, and none of the products exceeded that value for a single paraben (Rastogi et al. 1995).

Baby Products

Parabens are often used in combination in cosmetic products. Data provided in an industry survey (CTFA 2004b) found that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben are used to a total concentration of 0.3% in baby lotions, oils, powders, and creams. Likewise, Methylparaben, Propylparaben, and Butylparaben are used in this product category to a total concentration of 0.3%. Methylparaben and Propylparaben are used in this category to a total concentration of 0.5%.

This same survey (CTFA 2004b) found that Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Isobutylparaben are used to a total concentration of 0.08% in the other baby products category (cologne). Likewise, Methylparaben, Propylparaben, and Butylparaben are used in the other baby products category (cleansing cloths) to a total concentration of 0.33% to 0.53%. Methylparaben and Propylparaben are used

TABLE 13
Current and historical use and concentrations of Benzylparaben in cosmetic products.

Product category (number of products in category) (FDA 2002)	1984 uses (Elder 1986)	2005 uses (FDA 2006)	1984 concentrations (Elder 1986)	2003 concentrations (CTFA 2004a)
Fragrance preparations				
Sachets (28)	2	—	Unknown	—
Other fragrance preparations (173)	1	—	Unknown	—
Noncoloring hair preparations				
Conditioners (651)	5	—	≤0.1%	—
Shampoos (884)	3	—	≤0.1%	—
Personal cleanliness products				
Underarm deodorants (247)	13	1	≤0.1–1%	—
Other personal cleanliness products (308)	1	—	Unknown	—
Skin care preparations				
Cleansing creams, lotions, liquids, and pads (775)	4	—	≤0.1%	—
Face and neck skin care preparations (310)		—		—
Body and hand skin care preparations (840)	5[a]	—	≤0.1%[a]	—
Moisturizers (905)	3	—	≤0.1%	—
Night skin care preparations (200)	1	—	Unknown	—
Other skin care preparations (725)	1	—	Unknown	—
Skin lighteners[b]	4	—	Unknown	—
Suntan preparations				
Suntan gels, creams, and liquids (131)	2	—	Unknown	—
Total Benzylparaben uses/ranges	45	1	≤0.1–1%	—

[a]In 1981, face and neck skin care preparations and body and hand skin care preparations were combined in one category.
[b]This category no longer exists.

in the other baby products category (cleansing cloths) to a total concentration of 0.45%. Methylparaben was used alone at a total concentration of 0.05% in the other baby products category (cologne).

Noncosmetic

Food

The FDA has approved the use of parabens in foods as given in the specific Code of Federal Regulations (CFR) citations that follow. Methylparaben (21 CFR 184.1490) and Propylparaben (21 CFR 184.1670) are generally recognized as safe (GRAS) when used as chemical preservatives in foods, with use limits of 0.1% for each. Methylparaben and Propylparaben (indirect food additives) are permitted by prior sanction as antimycotics in food-packaging materials with no limits or restrictions (21 CFR 181.23), and Ethylparaben is similarly allowed as a component of adhesives intended for use in packaging, transporting, or holding food (21 CFR 175.105). Methylparaben and Propylparaben are specifically cited as preservatives, not to exceed 0.1%, acceptable for use in fruit jelly (21 CFR 150.141) and fruit preserves and jams (21 CFR 150.161). The FDA has established a tolerance of zero for residues of Methylparaben in milk from dairy animals (21 CFR 556.390).

TABLE 14
Concentration and prevalence of parabens in cosmetic products (Rastogi et al. 1995).

Paraben	Concentration (w/w)	% paraben-positive products in which found
Methyl-	0.01–0.32	98
Ethyl-	0.01–0.19	32
Propyl-	0.01–0.32	38
Butyl-	0.01–0.06	16
Benzyl-	0.01–0.07	16

The Joint WHO/FAO Expert Committee on Food Additives (JECFA) updated its specification for Methylparaben in 1998 and reiterated its 1973 finding that the group acceptible daily intake (ADI) for ethyl, methyl, and propyl *p*-hydroxybenzoic acid in foods is 0 to 10 mg/kg day^{-1} (JECFA 1998).

The European Food Safety Authority (EFSA) Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food adopted an opinion on the safety of paraben usage in food (EFSA 2004), which stated that the ADI of 0 to 10 mg/kg day^{-1} for the sum of Methylparaben and Ethylparaben is still valid. The opinion also stated, however, that Propylparaben should not be included in the ADI.

Pharmaceuticals

Sabalitschka (1930) reported that parabens were first used in drug products in 1924. Boehm and Maddox (1972) stated that combinations of Parabens are more active than individual esters. 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% (Neidig and Burrell 1944; Hassler 1954; Zacharias and Fisgus 1971; Boehm and Maddox 1972; Kassem et al. 1976).

FDA does not include preservatives in its over-the-counter (OTC) drug monographs. Preservatives are considered inactive ingredients and must meet the requirements specified in 21 CFR §330.1(e) that they be suitable ingredients that are safe and do not interfere with effectiveness.

FDA currently lists inactive ingredients used in approved drug products on-line (FDA 2004). Table 15 gives these data for parabens. According to FDA, this information can be used by industry as an aid in developing drug products. For example, if an inactive ingredient has been approved in a certain dosage form at a certain potency, a sponsor could consider it safe for use in a similar manner for a similar type of product.

In addition, FDA has specified 0.05% Propylparaben for standard preparation A and 0.10% Methylparaben for standard preparation B, the homosalate sunscreens used in SPF testing (21 CFR 352.70).

In 21 CFR 310.545(a)(22)(ii), FDA stated that the safety and effectiveness of Methylparaben as a topical antifungal for treating diaper rash is not demonstrated.

Other

Neidig and Burrell (1944) stated that parabens were historically used in textiles as antifungal agents, in gelatins and photographic emulsions, in bone glues, and in malt as antifermentation agents.

FDA listed Methylparaben and Propylparaben as components in its description of betamethasone acetate used as an animal drug (21 CFR 522.161).

GENERAL BIOLOGY

Absorption, Metabolism, and Excretion

Absorption

Whitworth and Jun (1973) studied the influence of surfactants on parabens absorption using frogs. Each of five frogs (*Rana pipiens*; 30 to 35 g) were immersed in 500 ml solutions of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben (concentration not given) for 2 h. The immersion liquid was sampled at 20-min intervals, assayed for parabens concentration, and returned to the beaker after analysis, over a 2-h period. Presence of parabens in frog skin was not determined, only the residual paraben concentration in the immersion fluid was measured.

Concentration of paraben in the immersion fluid decreased via linear kinetics as a function of time, with the greatest decrease (presumably the greatest uptake) reported for Butylparaben. The presumed uptake increased as the length of the ester carbon chain length increased.

When the experiment was repeated with polysorbate 20 at 0.5% or 1.0% added to the immersion fluid, the pattern of presumed uptake reversed. In this case, the greatest decrease (again, presumably the greatest uptake) was reported for Methylparaben. The presumed uptake decreased as the length of the ester carbon chain length increased in the presence of polysorbate 20.

When the experiment was repeated with sodium cholate (5×10^{-5} M) added to the immersion fluid, the pattern of presumed uptake was similar to parabens alone.

The authors suggested that these results confirm previous findings that the greater the lipid solubility of a chemical, the greater the rate of absorption in the frog (Whitworth and Jun 1973).

Fischmeister et al. (1975) applied parabens in an ointment vehicle (15% in Vaseline) to the skin of each of three healthy humans. Presence of residual parabens on the skin was determined at 1 and 8 h. One hour after application, parabens were identified; at 8 h, they were not detected.

Komatsu and Suzuki (1979) studied the percutaneous absorption of Butylparaben (0.015% to 0.1% 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. 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.

Hansen and Möllgaard (1990) reported that the permeability coefficient varied as a function of chain length for parabens using full thickness human skin in the following manner: methyl > ethyl > propyl > butyl > benzyl. No further details were provided.

Dal Pozzo and Pastori (1996) determined the percutaneous absorption through abdominal cadaver skin of a series of parabens from water, water with 50% propylene glycol, water with 20% PEG-400, liquid paraffin and three types of cosmetic formulation bases.

Type I formulation (oil/water) base consisted of 6.5 g squalane, 0.5 g stearic acid, 6 g octyl palmitate, 3 g isopropyl myristate, 2 g karitè butter, 0.35 g carbopol, 0.35 g triethanolamine, 4 g glyceryl stearate/PEG-100 stearate, 0.5 g sorbitan stearate, 3 g dimethicone, 3.2 g cyclomethicone, 0.7 g *p*-hydroxyalkyl benzoate, 0.02 g butylhydroxy anisol, 0.2 g allantoin, and 69.98 g water.

TABLE 15
Parabens in approved drug products (FDA 2004).

Route/dosage form	Maximum parabens concentration						
	Methyl-	Sodium Methyl-	Sodium Ethyl-	Propyl	Sodium Propyl-	Butyl-	Mixed Parabens
Infiltration/injection	0.1%	—	—	0.01%	—	—	—
Auricular (otic)/suspension	0.0014%	—	—	0.0006%	—	—	—
Caudal block/injection	0.1%	—	—	—	—	—	—
Epidural/injection	0.1%	—	—	—	—	—	—
IM–IV-SC/injection		—	—	20%	—	—	—
IM-IV/injection	0.75%	—	—	0.2%	—	—	—
IM-SC/injection	0.18%	—	—	0.02%	—	—	—
Inhalation/solution	0.07%	—	—	0.0375%	—	—	—
Intra-articular/injection	0.24%	—	—	0.16%	—	—	—
Intrabursal/injection	0.18%	—	—	0.02%	—	—	—
Intradermal/injection	0.1%	—	—	—	—	—	—
Intralesional/injection	0.15%	—	—	—	—	—	—
IM/injection	0.24%	—	—	—	—	—	—
IV/injection	0.75%	—	—	—	—	—	—
IV/powder, for injection solution	1.5%	—	—	—	—	—	—
Iontophoresis/solution	0.1%	—	—	—	—	—	—
Irrigation/solution	0.1%	—	—	—	—	—	—
IV-SC /injection	0.18%	—	—	0.02%	—	—	—
IV (infusion)/injection	0.44%	—	—	0.056%	—	—	—
IV (infusion)/powder, for injection solution	0.1%	—	—	—	—	—	—
Not applicable/liquid	0.12%	—	—	0.012%	—	—	—
Not applicable/not applicable	0.45 mg	—	—	0.06 mg	—	—	—
Nasal/solution	0.033%	—	—	0.017%	—	—	—
Nasal/metered spray	0.7%	—	—	0.3%	—	—	—
Nerve block/injection	0.1%	—	—	0.035%	—	—	—
Ophthalmic/ointment	0.05%	—	—	0.01%	—	—	—
Ophthalmic/solution	0.05%	—	—	0.015%	—	—	—
Ophthalmic/solution, drops	0.05%	—	—	0.015%	—	—	—
Ophthalmic/suspension	0.05%	—	—	0.01%	—	—	—
Ophthalmic/suspension, drops	0.05%	—	—	0.01%	—	—	—
Oral/capsule	1 mg	—	—	0.21 mg	—	0.002 mg	—
Oral/capsule (immediate/complete release), soft gelatin, perle	—	—	—	—	—	—	0.16 mg
Oral/soft gelatin coated capsule	0.156 mg	—	—	0.041 mg	—	—	—
Oral/soft gelatin capsule	0.48 mg	—	—	0.12 mg	0.35 mg	—	—
Oral/sustained action capsule	0.864 mg	—	—	0.216 mg	—	—	—
Oral/concentrate	0.2%	—	—	0.25%	—	—	—
Oral/drops	—	—	—	—	—	0.1%	—
Oral/granule	50 mg	—	—	—	—	—	—
Oral/powder for solution	0.1575%	—	—	0.01575%	—	—	—
Oral/powder for suspension	0.1%	0.1%	—	0.08%	0.1%	—	—
Oral/solution	13%	—	—	10%	—	0.5%	—
Oral/elixer solution	0.9%	—	—	0.1%	—	—	—
Oral/syrup solution	0.18%	—	—	0.02%	—	—	—
Oral/suspension	2.4%	0.65%	—	20%	0.1%	0.8%	—

(Continued on next page)

TABLE 15
Parabens in approved drug products (FDA 2004). *(Continued)*

Route/dosage form	Maximum parabens concentration						
	Methyl-	Sodium Methyl-	Sodium Ethyl-	Propyl	Sodium Propyl-	Butyl-	Mixed Parabens
Oral/liquid suspension	1%	—	—	0.3%	—	—	—
Oral/sustained action suspension	0.75%	—	—	0.15%	—	—	—
Oral/syrup	5%	—	—	20%	—	0.0075%	—
Oral/tablet	1.8 mg	0.1875 mg	—	0.2 mg	0.0625 mg	—	—
Oral/uncoated chewable tablet (immediate/complete release)	1.27 mg	—	—	0.142 mg	—	—	—
Oral/coated tablet	0.016 mg	—	—	0.002 mg	—	0.004 mg	—
Oral/film coated tablet	0.23 mg	—	—	0.04 mg	—	—	—
Oral/orally disintegrating tablet	—	0.3 mg	—	—	0.1 mg	—	—
Oral/repeat action tablet	—	—	—	—	—	0.006 mg	—
Oral/sustained action tablet	—	—	—	0.12 mg	—	0.04 mg	—
Oral/sustained action, multilayer, coated tablet	0.09 mg	—	—	—	—	—	—
Peridural/injection	0.1%	—	—	—	—	—	—
Rectal/metered aerosol	0.09%	—	—	0.009%	—	—	—
Rectal/enema	10.8%	—	—	—	—	—	—
Rectal/solution	13%	—	—	1.5%	—	0.5%	—
Rectal/suspension	2.4%	—	—	1.2%	—	—	—
Soft tissue/injection	0.15%	—	—	0.02%	—	—	—
Subcutaneous/injection	0.18%	—	—	0.02%	—	—	—
Topical/augmented cream	0.2%	—	—	0.032%	—	—	—
Topical/sustained release cream emulsion	0.2%	—	—	0.1%	—	—	—
Topical/aerosol foam emulsion	0.108%	—	—	0.011%	—	—	—
Topical/cream emulsion	18%	—	—	1%	—	0.4%	—
Topical/emulsion	—	—	—	0.06%	—	—	—
Topical/gel	0.3%	—	—	0.08%	—	—	—
Topical/gel, jelly	70%	—	—	30%	—	—	—
Topical/lotion	15%	—	—	10%	—	0.15%	—
Topical/metered aerosol	—	—	—	—	—	—	10%
Topical/ointment	0.2%	—	—	0.2%	—	—	—
Topical/shampoo	0.18%	—	—	0.03%	—	—	—
Topical/shampoo suspension	0.15%	—	—	—	—	—	—
Topical/solution	0.1%	—	—	0.033%	—	—	—
Topical/suspension	0.3%	—	—	—	—	—	—
Urethral/injection	0.18%	—	—	0.02%	—	—	—
Vaginal/cream emulsion	0.18%	—	—	0.1%	—	—	—
Vaginal/gel	0.08%	—	—	0.02%	—	—	—

Type II formulation (oil/water) base consisted of 23.72 g squalane, 0.5 g stearic acid, 0.5 g sorbitan stearate, 0.7 g *p*-hydroxyalkyl benzoate, and 70.58 g water.

Type III formulation (water/oil) base consisted of 4 g miglyol gel (triglycerides), 4 g squalane, 1.5 g PEG ethers, 1.5 g PEG-45 dodecylglycol copolymer, 1.5 g propylene glycol dipelargonate, 0.1 g versene, 4 g propylene glycol, 2 g glycerol, 1 g sorbitan stearate, 3 g PEG-7/hydrogenated castor oil, 5.5 g cyclomethicone/dimethicone, 4.5 g cyclomethicone, 0.7 g *p*-hydroxyalkyl benzoate, 0.15 g butylhydroxy anisol, and 66.05 g water.

The epidermis was isolated from abdominal cadaver skin and mounted in a diffusion cell. The receptor fluid was isotonic saline with 3% bovine serum albumin. Pure parabens in acetone (200 mg in 200 μl) were deposited on the stratum corneum and the solvent evaporated. For water, water with 50% propylene glycol, water with 20% PEG-400, or liquid paraffin, 2 ml was deposited. As a function of time after they were prepared, the three cosmetic

TABLE 16
Skin permeation of parabens as a function of vehicle (Dal Passo and Pastori 1996).

	Maximum Paraben flux ($\mu g/cm^2h$)					
Vehicle	Methyl	Ethyl	Propyl	Butyl	Hexyl	Octyl
Water	3.83	5.42	4.77	4.68	0.77	—[a]
Water/propylene glycol	6.5	3.5	2.6	3.6	3.0	—[a]
Water/PEG-400	4.01	7.17	2.51	5.92	2.32	—[a]
Liquid paraffin	0.42	0.74	1.00	2.65	2.29	0.96
Type I[b]	32.5	20.74	11.4	7.74	1.60	—[a]
Type II[b]	22.54	15.32	9.23	7.44	4.41	—[a]
Type III[b]	5.13	2.92	—[c]	1.60	—[c]	—[a]

[a]Not determined.
[b]Determined at 30 days after preparation of the cosmetic formulation.
[c]Emulsion unstable.

formulation bases were applied to the stratum corneum surface to a thickness of 1 cm. Appearance of parabens in the receptor fluid was determined by reverse phase HPLC at intervals from 1 to 8 h.

Table 16 presents the results of the maximum flux as a function of the vehicle for water, water with 50% propylene glycol, water with 20% PEG-400, and liquid paraffin, and as a function of the paraben side chain. Based on the maximum flux and the saturation concentration for each paraben in these vehicles, the authors further determined permeability constants and compared the log of the permeability constant with the log *P* of the various parabens. For each of the water-based vehicles, the permeability constant increased as a function of the log *P* of the paraben. The permeability constant decreased as a function of the addition of increasing proportion of glycols. For the lipophilic paraben vehicle (paraffin), the permeability constants actually decreased with increasing log *P*. The authors stated, however, that these simple models do not explain the behavior in complex cosmetic formulations.

As shown in the second part of Table 16, for the three cosmetic-type formulations, parabens fluxes decreased as a function of the paraben side chain. Using cosmetic-type formulations at 30 days post preparation, methy > ethyl > propyl > butyl > hexyl. As a function of time after preparation, the parbens fluxes also decreased, but maintained the same rank order of penetration. A steady-state flux was reached after 100 days and did not appreciably decrease further up to 200 days (last determination). The steady-state fluxes decreased as a function of the log *P* of the paraben used.

The authors concluded that the concentration of parabens in the aqueous phase of a cosmetic formulation (necessary for preservative effectiveness) can be increased by preferentially using higher-chain-length parabens. At the same time, they suggested that percutaneous absorption can be decreased by the same approach, coupled with the addition of solubilizers, such as glycols, to the formulation (Dal Passo and Pastori 1996).

Ishiwatari et al. (2005) conducted a study using human volunteers in which the levels of Methylparaben in the stratum corneum were measured. Cosmetic emulsions containing 0.15%, 0.25%, and 0.5% (*w*/*v*) Methylparaben were applied one time to the forearm (42 cm^2) of one male and one female subject. At 1, 2, 5, and 12 h after application, a small area was cleaned of emulsion using wet cotton and Methylparaben was extracted by application of a glass cylinder (3.1 cm^2) with 0.5 ml ethanol for 5 min. Methylparaben concentrations were determined in the ethanol solvent using HPLC (for the 1-, 2-, and 5-h durations) and gas chromatography–mass spectrometry (GC/MS) for other treatments.

Healthy Japanese adults (1 male, 11 female) applied a lotion only (6 subjects) or a lotion and an emulsion (6 subjects) containing Methylparaben (concentration not stated) twice a day for 1 month. Concentrations of Methylparaben in the stratum corneum were determined as above using (GC/MS) before the first application, at 1, 2, 3, and 4 weeks, and 2 days after stopping.

For the single application, Methylparaben reached its peak 1 to 2 h after application (peak was slightly higher for each higher use concentration) and returned to baseline after 12 h. Repeated applications resulted in an increase in Methylparaben concentration in the stratum corneum over time for both the lotion application and the lotion plus emulsion application. After 2 days, Methylparaben had returned to pretreatment levels.

These authors also determined the penetration of Methylparaben through Yucatan micropig skin with the fatty layers removed and the skin mounted in diffusion cells. An aqueous solution (10 μl) of Methylparaben (1%) was placed on each skin sample. At 15, 60, and 120 min, skin samples were removed from the diffusion cell and wiped. The stratum corneum was tape stripped 5× and the tape strips were extracted with methanol-water (50:50). The dermis was separated from the epidermis by heat treatment and each layer was weighed, homogenized, and extracted with methanol-water. Methylparaben in the methanol-water extracts was determined using HPLC. Methylparaben in the stratum corneum increased with time; increased in the epidermis from 15 to 60 min (no time 0 measurement), then decreased from 60 to 120 min; and increased in the dermis slightly from 15 to 60 min and remained essentially the same from 60 to 120 min (Ishiwatari et al. 2005).

Effects of Penetration Enhancers on Absorption. Kitagawa et al. (1997) measured the effect of penetration enhancers on the skin penetration of parabens using excised guinea pig skin mounted in a two-chamber diffusion cell. Penetration enhancers used were 15% ethanol, 15% ethanol plus 1% *l*-menthol, and a 0.025% suspension of *N*-dodecyl-2-pyrrolidone (NDP), which were added to the donor chamber with either Methyl-, Ethyl-, Propyl-, or Butylparaben.

TABLE 17
Skin permeability as a function of paraben type (Kitagawa et al. 1997).

Paraben	Log P	K_p ($\times 10^{-3}$ cm h^{-1})
Methyl-	1.66	6.51 ± 2.30
Ethyl-	2.19	32.67 ± 11.27
~Propyl-	2.71	66.26 ± 12.43
Butyl-	3.24	92.17 ± 27.18

In the absence of penetration enhancers, there was a direct relationship between the permeability coefficient for each paraben (K_p) and its octanol/water partition coefficient (log P) as shown in Table 17.

To confirm that penetration through the stratum corneum lipid layer was the rate limiting step, skin incubated with a chloroform-methanol mixture for 12 h was used. The permeability coefficient of each paraben was around 90 $\times 10^{-3}$ cm h^{-1}, eliminating the relationship with log P.

Addition of NDP stimulated the skin permeability of Methylparaben by a factor of 7 and Ethylparaben by a small amount, but had no effect on permeability of either Propyl- or Butylparaben. The mixture of 15% ethanol and 1% *l*-menthol increased the skin permeability of Methylparaben by a factor of 16, had no effect on Ethyl- or Propylparaben, and decreased the skin permeability of Butylparaben by a factor of 5. The same pattern, but to a lesser degree, was seen with 15% ethanol alone.

The authors concluded that the effect of NDP resulted partly as a result of NDP disruption of the stratum corneum lipid layer. They speculated that the decrease in Butylparaben with 15% ethanol and 15% ethanol plus 1% *l*-menthol related to a reduction in partitioning of Butylparaben between skin and vehicle because of an increase in solubility in the donor solution in the presence of alcohol (Kitagawa et al. 1997).

Effects of Occlusion and Vehicle on Absorption

Cross and Roberts (2000) examined the effect of occlusion on parabens skin penetration as a function of vehicle. Human female abdominal skin was used to prepare epidermal membranes, which were mounted in Franz-type diffusion cells. Methyl-, Ethyl-, Propyl-, and Butylparaben in a particular vehicle were added to the donor chamber and spread over the skin surface. The vehicles used were a commercial allergy test ointment, acetone, and ethanol. Occlusion was done using a piece of high-density polyurethane. Paraben concentration in the receptor fluid was determined by HPLC after 10 h. Occlusion resulted in a decrease in penetration of each paraben in ointment, but increased penetration of each paraben in acetone or ethanol. Table 18 shows the effects of vehicle and occlusion on each paraben.

Metabolism

In a study by Sabalitschka and Neufeld-Crzellitzer (1954), 2 g of Benzylparaben were consumed daily by each of two human volunteers for 5 days. Their urine was analyzed for metabolic

TABLE 18
Effect of occlusion on skin permeability of Methyl-, Ethyl-, Propyl-, and Butylparaben as a function of vehicle (Cross and Roberts 2000).

	Total penetration in 10 h (μg)	
Paraben/vehicle	Unoccluded	Occluded
Methylparaben		
Ointment	27.0 ± 1.3	11.9 ± 0.6
Acetone	86.4 ± 15.7	531.6 ± 68.6
Ethanol	90.3 ± 28.3	593.2 ± 43.0
Ethylparaben		
Ointment	87.1 ± 6.0	28.4 ± 3.1
Acetone	57.6 ± 12.3	976.7 ± 21.2
Ethanol	93.1 ± 30.5	894.7 ± 46.1
Propylparaben		
Ointment	78.0 ± 5.8	24.4 ± 3.1
Acetone	36.8 ± 9.5	494.6 ± 16.0
Ethanol	50.2 ± 19.0	450.1 ± 22.1
Butylparaben		
Ointment	75.7 ± 6.2	25.1 ± 2.8
Acetone	84.8 ± 22.7	650.1 ± 38.6
Ethanol	111.3 ± 49.0	684.3 ± 39.1

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.

Jones et al. (1956) studied the metabolism of parabens in dogs, rabbits, and in one human volunteer. Intravenous injections at 50 mg/kg Methylparaben, Ethylparaben, Propylparaben, or Butylparaben were administered to groups of three or more fasted dogs. Similarly, these compounds were administered orally at a dose of 1.0 g/kg. Blood and urine were analyzed at predetermined intervals.

Immediately following intravenous injection, very little ester remained in the blood. Metabolites were detectable in the blood up to 6 h post injection and 24 h post ingestion. Recovery of all esters but Butylparaben ranged from 58% to 94% of the administered dose. Absorption was essentially complete. Recovery of Butylparaben after oral administration was 40% and 48% after i.v. administration. The authors considered this finding a result of less effective hydrolysis of Butylparaben.

Dogs given 50 mg/kg were then killed and the distribution of esters and metabolites to organs was determined. 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 of the dog were extremely efficient in hydrolyzing Parabens—complete hydrolysis after 3 min for all Parabens except Butylparaben, which took 30 to 60 min.

No accumulation of Parabens was observed in the tissues of dogs given orally 1 g/kg/day Methylparaben or Propylparaben for 1 year. The rate of urinary excretion of esters and metabolites in these dogs increased to such an extent that after 24 h, 96 % of the dose was excreted in the urine. This is contrasted with dogs given a single dose of paraben in which the 96% excretion level was not attained until 48 h.

When 10% Methylparaben or Propylparaben in hydrophilic ointment was applied to the skin of a white rabbit for 48 h, esters and metabolites were not detected in the kidneys. The authors noted that there was no skin irritation at this dose.

These same authors gave 70 mg/kg Methylparaben orally to a fasted man. No ester was detected in his blood or urine. After 12 h, half of the dose was excreted in the urine as metabolites, with 11% as *p*-hydroxybenzoic acid (Jones et al. 1956).

Heim et al. (1957) reported that mouse liver perfused with Ethylparaben rapidly hydrolyzed it to the free acid within 60 min. 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.

No Ethylparaben was detected in the blood of six humans 4 h following oral administration of 10 to 20 mg/kg. High serum concentrations of *p*-hydroxybenzoic acid appeared rapidly. The authors stated that Ethylparaben, ingested in food by man, was probably completely hydrolyzed within 3 min after absorption (Heim et al. 1957).

Tsukamoto and Terada (1960, 1962) studied the metabolic fate of Methylparaben in rabbits. The compound was given by gastric intubation, and urine was analyzed by paper chromatography. Three major metabolites, *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% of the unchanged ester by 24 h. 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. At 24 h following paraben administration, 25% to 39% was recovered as *p*-hydroxybenzoic acid, 15% to 29% as the glycine conjugate, 5% to 8% as the ester glucuronide, 10% to 18% as the ether glucuronide, and 7% to 12% as the sulfate.

The metabolism of Methylparaben, Ethylparaben, and Propylparaben was studied in rats by Derache and Gourdon (1963). 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 min 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 min; its concentration then increased evenly during the next 4 h. The glucuronide and ethereal sulfate metabolites appeared only between 30 and 75 min post ingestion.

After 90 min, 67% to 75% of the total paraben dose was excreted as *p*-hydroxybenzoic acid, 10% to 12.5% as *p*-hydroxyhippuric acid, and 8% to 10% 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 h after ingestion.

The authors concluded that there were two stages of paraben detoxification: (1) absorption of paraben and excretion in urine of *p*-hydroxybenzoic acid, and (2) metabolic detoxification by glucuronic-, sulfo-, and glycino-conjugation (Derache and Gourdon 1963).

Tsukamoto and Terada (1964) dosed four male rabbits weighing between 2.25 and 3.50 kg with a 12% solution of 800 or 400 mg/kg of Isobutylparaben (as the sodium salt) 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% to 31% became *p*-hydroxybenzoic acid conjugated with glycine, and 7% to 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. 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.

Phillips et al. (1978) conducted a metabolic study 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 h; feces were collected at 72 h. At 72 h, total recovery was 96% for Ethylparaben and 95.6% for Propylparaben. Approximately 90% of the ^{14}C label was recovered in the urine at 24 h, whereas 6% and 3%, 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 h following oral administration.

Skin Metabolism

Hansen and Möllgaard (1990) reported an experiment using full thickness human skin and Methylparaben and Butylparaben. They noted that the parabens were converted to the alkyl alcohol and *p*-hydroxybenzoic acid, in a dose-dependent manner following Michaelis-Menton kinetics. They interpreted these findings as suggestive of enzymatic action in the skin. No further details were provided.

Lobemeier et al. (1996) examined the hydroylsis of parabens to hydroxybenzoic acid by extracts from different layers of the skin. They used the absorbance shift of the parabens (absorbance of parabens can be seen at 300 nm, whereas there is no absorbance of hydroxybenzoic acid at 300 nm) to determine if parabens were metabolized by extracts prepared from different skin layers. Because there is so much ultraviolet (UV) absorbing material in skin extracts, it was necessary to extract parabens and hydroxybenzoic acid from the reaction mixture. They tested recovery from standard reaction mixtures without incubation that had been spiked with free hydroxybenzoic acid and parabens. Recovery of 1 nmol of free hydroxybenzoic acid from reaction mixtures was stated to be 0.995 to 1.018 nmol. Virtually all of 4 nmol parabens with which reaction mixes were spiked was recovered.

Extracts were prepared from human abdominal skin. The cutis, subcutaneous fat, and stratum basale/stratum spinosum (skin keratinocytes) extract were each used. In addition, transformed keratinocytes in culture were used to prepare an extract and an extract of whole blood also was used. Skin keratinocytes did not produce reliable quantitative results. The subcutaneous fat extract produced the most hydrolysis for Methyl-, Ethyl-, Propyl-, and Butylparaben, although the activity decreased with increased chain length (e.g., Methylparaben $\gg$Butylparaben). The transformed keratinocyte extract had the opposite pattern, activity increased with increased chain length. The cutis extract was not significantly different across the parabens. Comparative results for the blood extract were not given.

The authors isolated the enzymatic activity in each extract using polyacrylamide gel electrophoresis and tested the material in each band against each of the parabens. They reported one B-type carboxylesterase in subcutaneous fat that was maximally active with Methylparaben and decreased in activity as the chain length increased to the Butylparaben. A second B-type carboxylesterase in subcutaneous fat preferred Butylparaben as a substrate. A third B-type carboxylesterase in keratinocytes also preferred Butylparaben as a substrate. A fourth carboxylesterase was present in human blood, but was not further characterized.

The authors concluded that the keratinocyte carboxylesterase was sufficient to completely hydrolyze the traces of parabens that may enter the skin from topically applied ointments. They speculated that the involvement of B-type carboxylesterases, which also catalyze acyl transfer reactions, may contribute to the hapten behavior of parabens in the skin and contribute to the contact allergy occasionally observed (Lobemeier et al. 1996).

Bando et al. (1997) studied the effects of skin metabolism on the percutaneous penetration of lipophilic drugs. Full-thickness rat abdominal skin (hair removed) was stripped of underlying adipose tissue, punched into a 3-cm-diameter disk, and mounted in a flow-through diffusion cell with Propyl- and Butylparaben added to the donor solution. Penetration of Propyl- and Butylparaben and hydroxybenzoic acid to the receptor cell was determined using HPLC. Determinations were made with and without an esterase inhibitor, diisopropyl fluorophosphate (DFP). In the absence of DFP, 96% of the total test material appeared in the receptor cell as hydroxybenzoic acid. In the presence of DFP, 30% of applied Propylparaben appeared in the receptor fluid unhydrolyzed and 100% of applied Butylparaben appeared unhydrolyzed.

Seko et al. (1999) performed a theoretical analysis of the effect of skin metabolism on penetration of Propylparaben and Butylparaben. These authors used a two-layer diffusion/metabolism model to describe data from an in vitro skin diffusion experiment using rat skin from which the fat layer had been removed. Diffusion was determined with and without pretreatment of the rat skin with 1 mM diisopropyl fluorophosphate, an esterase inhibitor.

When parabens were applied to untreated skin, both the parent paraben and *p*-hydroxybenzoic acid appeared in the receiver fluid. With diisopropyl fluorophosphate treatment, no *p*-hydroxybenzoic acid appeared in the receptor fluid and the appearance of the parent paraben was delayed. There was also a differential effect on the total penetration to the receptor fluid; Butylparaben penetration was decreased by 22%, compared to a 4% reduction for Propylparaben.

The authors concluded that the metabolism of parabens in the viable layer of the skin determines the lag time for skin penetration of intact parabens. They also noted that metabolism in the viable skin creates a steeper concentration gradient across the stratum corneum, increasing transport of these hydrophilic compounds (Seko et al. 1999).

Fasano (2004a) conducted a study of the in vitro dermal penetration and metabolism of Methylparaben and Butylparaben in rat and human skin. For each paraben, an oil in water emulsion with both radiolabeled (^{14}C in the carbon ring) and nonradiolabeled paraben was prepared to a target concentration (0.8% for Methylparaben and 0.4% for Butylparaben). Skin samples (10 replicates for rat skin and 13 replicates for human skin) were mounted in flow-through diffusion cells. Test emulsions were applied evenly at 10 $\mu l/cm^2$, one time, with no occlusion. Samples of the receptor fluid were mixed with acetonitrile, filtered, and analyzed for Methylparaben, Butylparaben, and hydroxybenzoic acid using liquid chromatography coupled with mass spectroscopy.

The majority of the radiolabeled Methylparaben that penetrated rat skin to the receptor fluid had been metabolized to hydroxybenzoic acid (54%), with around 24% as unmetabolized Methylparaben. For Butylparaben, 52.3% was metabolized to hydroxybenzoic acid, with only 5.5% as unmetabolized Butylparaben.

Metabolism was different in human skin with 35% of Methylparaben appearing as hydroxybenzoic acid and 60% remaining as unmetabolized Methylparaben. For Butylparaben, 32.8% appeared as hydroxybenzoic acid and 49.7% as unmetabolized Butylparaben.

Overall, based on the use of dermatomed skin, the availability of unmetabolized Methylparaben and Butylparaben from oil in

water emulsions was greater in the receptor fluid with human skin compared to rat skin.

Even though only Methylparaben and Butylparaben were applied, the authors noted that the receptor fluid in both species also contained detectable amounts (e.g., 200 dpm peak versus 750 dpm for hydroxybenzoic acid and 950 dpm for Methylparaben) of Ethylparaben (Fasano 2004a).

Fasano (2004b) also describe the penetration and metabolism of Butylparaben using viable, full-thickness human skin. Otherwise the study was conducted as described above. A total of 21% of the radiolabel penetrated to the receptor fluid after 24 h. In contrast with the above finding, the principle metabolite, hydroxybenzoic acid, was detected in the receptor fluid, with barely detectable levels of Butylparaben and no Ethylparaben, in this study of full-thickness skin. The author concluded that the first-pass metabolism of Butylparaben produced complete hydrolysis to hydroxybenzoic acid.

This work was repeated (Fasano 2005) to again examine the penetration and metabolism of Butylparaben (0.4%) in an oil/water emulsion applied to the same full thickness viable human skin described above. A finite dose (10 μl/cm^2) of the emulsion was applied to the skin surface and remained in contact over a 24-h period without occlusion. [^{14}C]Butylparaben (labeled in the carbon ring) was measured in the receptor fluid. A mean value of 14.9% (±3.73%) of the radioactive label penetrated the full thickness human skin after 24 h. The principle metabolite, hydroxybenzoic acid, was found in the receptor fluid (mean of 15.2% ± 5.23%) of all 10 replications (skin donated from two individuals), but barely detectable levels of the parent Butylparaben (mean of 0.225% ± 0.063%) were found only in 5 of 10 replications. The authors interpreted these results to confirm the near complete first-pass metabolism of Butylparaben to *p*-hydroxybenzoic acid in human skin.

Excretion

Kiwada et al. (1979) injected radiolabeled Ethylparaben (^{14}C in the carbon ring) 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 h. Little unmetabolized Ethylparaben was detected in samples of urine (0.03%) and bile (none detected). Radiolabeled metabolites recovered in the urine were 83.5% of the dose injected into the duodenum and 91.3% of that injected intravenously. Those recovered in the bile were 12.8% and 5.97%, respectively. The authors stated that the results suggested that hydrolysis of Ethylparaben to *p*-hydroxybenzoic acid and metabolism of the latter was rapid and complete.

Antimicrobial Effects

The antimicrobial activity of parabens has been extensively reported. This section highlights aspects of that activity.

Loos (1935) reported that Benzylparaben at 0.01% was effective in preventing the growth of the fungi *Epidermophyton interdigitale* and *Microsporum audouni*.

Neidig and Burrell (1944) reported that beyond pH 8, ester hydrolysis can occur, which reduces the preservative efficacy of parabens. Cavill and Vincent (1948) confirmed that the ester chain was necessary for antimicrobial activity and additionally reported that any branching (e.g., isobutyl versus butyl) reduced the effectiveness.

Murrell and Vincent (1950) reported that the activity of parabens increases as the length of the alkyl chain. Atkins (1950) stated that, although antimicrobial activity increases as the alkyl chain length increases, the water solubility decreases—because microbial replication generally occurs in the water phase of oil/water formulations, the amount of paraben in the water phase generally determines preservative effectiveness. Lang and Rye (1972, 1973) observed that the higher activity of the long-chain esters over the shorter-chain esters resulted from greater uptake of the former by bacterial cells. These authors suggested that because parabens are lipophilic, the action site was probably the cell membrane.

Gottfried (1962) stated that location of the phenolic hydroxy group on the benzene ring can increase or decrease the antimicrobial activity of parabens.

Bronswijk and Koekkoek (1971) tested the activity of Methylparaben against *Dermatophagoides pteronyssinus* (house dust mite). Methylparaben at 0%, 1%, 5%, or 7% was added to cultures, which were then incubated for 28 days. Growth of mites was suppressed by 1% Methylparaben; at 5% and 7% mite growth was completely inhibited.

Furr and Russell (1972a, 1972b, 1972c) explained the lack of preservative activity of Methylparaben and Ethylparaben against *Serratia marcescens* when they noted that Methylparaben and Ethylparaben were not taken up by whole cells and isolated cell walls of *S. marcescens*, whereas Propylparaben and Butylparaben were taken up and induced cell wall leakage.

According to Freese et al. (1973), parabens inhibit cellular oxidation by inhibiting compounds that 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 *Bacilus 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.

Allwood (1973) reported that nonionic surfactants at low concentrations may have a synergistic effect with parabens, whereas higher concentrations of the surfactant inhibit preservative activity.

Close and Neilson (1976) identified a Propylparaben-resistant strain of *Pseudomonas cepacia* with esterases able to hydrolyze Propylparaben and use the metabolites as a carbon source.

According to Shiralkar et al. (1976), growth inhibition occurs only after a minimum concentration of paraben is reached; once this value is exceeded, inhibition is rapid. Shiralkar et al. (1977) reported that Propylparaben was taken up by bacterial cells; 95% within 2 min after being added to cultures.

O'Neill and Mead (1982) studied the preservative capacity of parabens against *Aspergillus niger, Enterobacter hafnia, Enterobacter cloacae, Escherichia coli, Penicillium species, Pseudomonas aeruginosa, P. cepacia, Pseudomonas putida, Serratia liquifaciens, S. marcescens, and Serratia rubidaea.* Methylparaben in oil emulsions at 0.8% was effective against a very resistant *S. marcescens* isolate, ED-2. Equally effective was a mix of 0.4% of Methylparaben and 0.4% Ethylparaben. Methylparaben at 0.4% was not effective, nor was a mix of 0.4% Methylparaben and 0.4% Propylparaben. The authors concluded that Methylparaben is the most effective preservative among the parabens and recommended that it should be used at the highest practical concentration and supplemented with Ethylparaben only when there is some limitation on the concentration of Methylparaben.

Nes and Eklund (1983) reported the effect of Methyl-, Propyl-, and Butylparaben on DNA, RNA, and protein synthesis in *Esherichia coli* and *Bacillus subtilis*. Cell cultures were made permeable by toluene treatment (0.075 ml in 7.5 ml resuspension of washed cells) and stored frozen (liquid nitrogen) in buffer at a cell concentration between 5 and 10×10^9 cells/ml. DNA synthesis was measured by adding standard mixtures of buffer, ATP, unlabeled DNA precursors, and ^{3}H-labeled dTTP to toluenized cells. RNA synthesis was performed in a similar fashion, except that RNA precursors were used with ^{3}H-labeled dUTP. Protein synthesis was done using a poly(U) substrate with buffer, ATP, GTP, and ^{14}C-phenylalanine with each of the other 19 amino acids unlabeled, mixed with phosphoenolpyruvate, phosphoenolpyruvate kinase, and the S30 fraction from either *E. coli* or *B. subtilis*.

No significant differences were seen between the two bacterial strains in DNA and RNA synthesis. Inhibition of DNA synthesis was greatest with Butylparaben and least with Methylparaben. For all parabens, DNA synthesis inhibition increased as a function of the paraben concentration. The same pattern was seen for RNA synthesis. Although protein synthesis was inhibited by parabens in the same order described above, the effect was much less in *B. subtilis* compared to *E. coli*. The authors speculated that DNA, RNA, and protein synthesis could be targets affected by parabens (Nes and Eklund 1983).

Protein Binding

Tzortzatou and Hayhoe (1974) reported that Methylparaben and Propylparaben increased 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.

Albumin

Patel (1968) reported that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben bind to bovine serum albumin (BSA). Binding increased with increasing ester chain length. The binding process is endothermic and hydrophilic in nature. Additionally, protein-bound paraben is devoid of its antifungal activity.

Jun et al. (1971) used a fluorescent probe to determine that the paraben sidechain is the primary binding site to BSA. Brodersen (1974) and Echeverria et al. (1975) observed that Methylparaben and Propylparaben bilirubin to serum albumin at concentrations of 400 μg/ml.

Rasmussen et al. (1976) observed that, whereas 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. (1976) observed that Methylparaben interacts with components of icteric newborn sera, increasing the availability of free, unconjugated bilirubin.

Otagiri and Perrin (1977) reported that the serum albumin-binding constant increases significantly from Propylparaben to Butylparaben.

Cytotoxicity

Ansel and Cadwallader (1964) examined the effects of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben on human and rabbit erythrocytes in vitro. Butylparaben, at 0.02%, induced hemolysis in 12% of the rabbit and 6% of the human erythrocytes. Concentrations of 0.25% Methylparaben, 0.17% Ethylparaben, and 0.05% Propylparaben induced no hemolysis.

Krauze and Fitak (1971) tested Methylparaben, Ethylparaben, and Propylparaben in cultures of embryonic mouse fibroblasts. They reported 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.

Sheu et al. (1975) determined that the doses of Methylparaben, Ethylparaben, and Propylparaben that produced 50% cell inhibition (IC_{50}) in HeLa cells were 1.3, 0.6, and 0.22 mM, respectively. These were similar to IC_{50} values in *B. subtilis* and *E. 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. The authors stated that 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.

Brown et al. (1978b) reported that contact lenses treated with 0.02% Propylparaben were cytotoxic to the L929 strain of mouse fibroblasts and S3 HeLa cells.

Ishiwatari et al. (2005) studied the effect of Methylparaben on human keratinocytes in culture. EpiLife-KG-2 medium was used to grow the cells to confluence and then they were subcultured using EpiLife-KG-2 medium containing 0.001% or 0.003% Methylparaben. Control cultures received no Methylparaben after subculturing. Cells were counted and the number of population doublings determined, along with the number of apoptotic cells.

Until day 20, control and Methylparaben-treated cultures grew at the same rate. After day 20 (at just over 8 doublings), the rate of growth for cultures treated with Methylparaben slowed. At 70 days, the controls had leveled off at around 19 doublings and the treated cells leveled off at around 16 doublings. There was no difference between the two Methylparaben concentrations.

Apoptotic cells were determined at time 0, 16 days, and 32 days. There was no difference between control and treated cultures at 16 days, but at 32 days apoptotic cells had increased to around 9% in the 0.003% Methylparaben culture and 5% in the 0.001% Methylparaben culture. Necrotic cells exhibited the same pattern, except that at 32 days there were almost 60% necrotic cells in the 0.003% Methylparaben culture compared to an almost indistinguishable difference between the control and 0.001% Methylparaben groups. The authors also noted morphological changes in the keratinocytes treated with Methylparaben—they became enlarged and flattened. The authors speculated that Methylparaben exposure might influence the aging and differentiation of keratinocytes and might induce dermatological disorders (Ishiwatari et al. 2005).

Phototoxicity

Handa et al. (2005) exposed human keratinocytes in culture to UV radiation, with and without Methylparaben pretreatment; and at various Methylparaben concentrations without UV radiation. Cell viability was determined at 6 and 24 h for Methylparaben concentrations of 0.003%, 0.03%, and 0.3%. At 6 h, the highest Methylparaben concentration that did not cause a reduction in cell viability compared to controls was 0.03%; at 24 h, that concentration was 0.003%. A concentration of 0.03% was chosen for the UV experiments.

Cells were cultured with 0.03% Methylparaben to confluence, the medium was removed and replaced with phospate-buffered saline. Cultures were exposed to fluorescent sunlamps (30% UVA, 54% UVB, 0.2% UVC) to levels of UVB of 15 or 30 mJ/cm^2. There was no indication that UVA and UVC radiation were filtered out. After exposure, cells were again incubated in culture medium (without Methylparaben). The number of necrotic or apoptotic cells was determined by staining.

No apoptotic cells were found in any of the control or treatment cultures. Cultures receiving no UV exposure and no Methylparaben had 2.27% (± 0.11%) necrotic cells. The complete results of the various combinations of exposures to UV and Methylparaben are given in Table 19.

TABLE 19
Human keratinocyte cell death associated with UV radiation and/or Methylparaben exposure (Handa et al. 2005).

	Necrotic cells (%)		
Methylparaben	No UV radiation	15 mJ/cm^2 UVB	30 mJ/cm^2 UVB
None	2.27 ± 0.11	3.00 ± 0.45	6.02 ± 1.21
0.003%	2.54 ± 1.06	10.61 ± 2.73	19.25 ± 3.39

The authors concluded that Methylparaben itself appeared to have no effect on the number of necrotic cells, but it did increase the number of necrotic cells produced as a result of UV radiation exposure (Handa et al. 2005).

In a commentary on this study, Shiseido Co., Ltd. (2005) noted that the absorption maximum for Methylparaben is 256 nm in the UVC region and that at 300 nm, the absorption is not significant. Because the light source used by Handa et al. (2005) contained 0.2% UVC, not found in sunlight at the earth's surface, it was suggested that the phenomenon is not relevant to normal solar exposures.

Tissue Effects

Pomerat and Leake (1954) studied Methylparaben for toxicity to tissue cultures of embryonic chicken spleen and adult human skin. In splenic tissue, concentrations of 520 to 1040 μg/ml inhibited growth, whereas concentrations of 30 to 60 μg/ml induced detectable injury. In cultures of skin, concentrations required for least growth inhibition and detectable injury were 175 to 350 μg/ml and 140 to 175 μg/ml, respectively.

White (1967) studied the effects of Methylparaben and Propylparaben on cultured embryonic chicken femoral bones in vitro. At concentrations 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 concentrations. When mixtures of the two were tested, growth inhibition occurred, even at the lowest concentration tested (0.025 g/ml of each). The authors suggested that the parabens' effect may be due to their ability to stabilize lysosomes.

Mostow et al. (1979) studied the effects of Methylparaben and Propylparaben on the ciliary activity of epithelial cells in cultures of ferret tracheal rings. 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.

Nerve Tissue

Nathan and Sears (1961) reported the effects of 0.1% and 0.2% Methylparaben on vagus and sympathetic nerves and

spinal nerve roots, in vivo, in cats. When applied directly, Methylparaben blocked nerve impluse conduction in myelinated and unmyelinated nerves. Conduction block was reversible and anesthetic-like. The authors suggested that injection of Methylparaben may cause degeneration in a number of the surrounding nerves.

Kitamura (1979) 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.

Muscle

Karasek and Slavicek (1967) studied the effect of Methylparaben on the sensitivity of the isolated frog rectus abdominus muscle to acetylcholine (ACh). 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.

The effect of Methylparaben and Propylparaben on smooth muscle of isolated guinea pig trachea was studied by Geddes and Lefcoe (1973). Both compounds induced dose-dependent, rapid, reversible relaxation of tracheal smooth muscle. In addition, these ingredients potentiated isoproterenol and dibutyryl cyclic adenosine monophosphate (AMP) at concentrations 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. (1975) 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.

The authors concluded that 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. The authors noted that the direct action of Methylparaben could have clinical implications, because 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 (Jones et al. 1975).

Physiological Effects

Bubnoff et al. (1957) 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. They reported spasmolytic action in cerebral vessels of cats after intravertebral injection of 5 mg/kg of Benzylparaben. The authors concluded that a relationship may exist between the effects of parabens as vasodilators and anticonvulsants.

Adler-Hradecky and Kelentey (1960) tested Methylparaben, Ethylparaben, Propylparaben, and Butylparaben for surface analgesia in rats, infiltration analgesia in guinea pigs, and conduction anesthesia in frogs. Surface analgesia was studied by applying parabens (0.01%) 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% 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 significant effects. In the conduction anesthesia study, isolated frog muscle-nerve preparations were treated with 1% parabens and then electrically stimulated. Conduction was measured by the electric potential required to stimulate muscle contraction. Only Butylparaben and Propylparaben significantly (but slightly) inhibited contraction when compared to controls.

Goodwin et al. (1979) identified Methylparaben 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 min, 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 suggested that Methylparaben is a sex pheromone of the dog.

Person (1985) noted that Methylparaben has been identified as the main volatile component of vaginal secretions of female Beagle dogs during estrus. Application of pure Methylparaben to the vulva and in the vagina of anestrus females reportedly resulted in sexual arousal of males, with mounting as if the females were in estrus. The author opines that avoiding Methylparaben-containing creams "could possibly relieve the mounting tensions of dog owners throughout the world."

Hamilton et al. (1990) used an in vitro system to study the direct cerebrovascular effect of pure succinylcholine, 1.8 mg/ml Methylparaben, 0.2 mg/ml Propylparaben, 1.8 mg/ml Methylparaben, 0.2 mg/ml Propylparaben combined, a multidose prescription Rx form of succinylcholine (20 mg/ml) containing 1.8 mg/ml Methylparaben and 0.2 mg/ml Propylparaben, a multidose Rx form of succinylcholine (20 mg/ml) containing 1.0 mg/ml Methylparaben or a single dose Rx form without parabens. Basilar artery preparations from dogs and guinea pigs were treated with Methylparaben or Propylparaben, pure succinylcholine, or the three prescription forms of succinylcholine. Measurements were taken of the basilar artery precontracted with KCl. Pure succinylcholine or the single dose form had no statistically significant effect, but the multidose Rx forms did cause relaxation of the artery. Vasodilation was seen in direct proportion to the amounts of parabens present.

To examine the possible role of the arterial endothelium, measurements were taken of arteries in which the endothelium had been rubbed off. No difference was seen compared to arteries with an intact endothelium. The authors speculated that the site of action of the parabens, therefore, was most likely directly on the arterial smooth muscle (Hamilton et al. 1990).

Pompy et al. (1991) examined the effect of paraben preservatives on intracranial pressure in vivo using cats. Succinylcholine, with and without paraben preservatives, and paraben preservatives alone were injected into each of six anesthetized and instrumented cats in specific sequences. Contrary to the in vitro finding discussed above, preservative-free succinylcholine did produce an increase in intracranial pressure that was not statistically different from succinylcholine with 1.8 mg/ml Methylparaben and 0.2 mg/ml Propylparaben (the prescription version); both were statistically significantly increased over controls. Injection of 1.8 mg/ml Methylparaben and 0.2 mg/ml Propylparaben produced an increase in one animal, but not in the other five, and the overall effect was of a nonsignificant increase in intracranial pressure compared to controls. These authors reported that there was a small, transitory decrease in arterial pressure, consistent with in vitro findings, suggesting that parabens have some systemic vasodilatory effect.

Gelb et al. (1992) further examined the effects of parabens on cerebral vasodilation and intracranial pressure in healthy humans. Cerebral blood flow was determined with inhaled ^{133}Xenon in eight volunteers and cerebral blood flow velocity was determined using transcranial Doppler ultrasound in a different group of eight volunteers. Methylparaben (9 mg) and Propylparaben (1 mg) were given together intravenously to mimic the preservative that would be given in a 100 mg dose of a commercially available multidose vial of succinylcholine. No adverse hemodynamic or neurological effects resulted from the paraben injection. Although the authors stated that they could not discount the possibility that parabens could have an effect in the presence of cerebral dysfunction and impaired autoregulation, they concluded that these findings and those in cats described above, suggest no adverse effects of parabens on intracranial pressure.

Noting that Methylparaben has been reported to have pharmacological effects, Harvey et al. (1992) attempted to examine the effect of Methylparaben on cyclic nucleotides (cAMP and cGMP) and cyclic nucleotide phosphodiesterase isozymes using male Wistar rats (200 to 250 g). The authors described the characteristics of the various phosphodiesterase isozymes as follows: form I has affinity for both cAMP and cGMP; form II was stimulated by micromolar concentrations of cGMP, but binds both cAMP and cGMP; and form IV is insensitive to cGMP and sensitive to cAMP. Methylparaben (0.4%) in feed was provided to five groups of four rats over a period of 3 weeks. An additional five groups of four rats served as control animals. At the end of the exposure period, the animals were killed, cortices were dissected and halved. For each group, two right and two left halves were processed for cyclic nucleotide determinations and the other halves were processed for phosphodiesterase separation and activity.

There was a statistically significant drop in cAMP levels and a small, but statistically significant, increase in cGMP levels in the Methylparaben group. Three separate phosphodiesterase isozymes (I, II, and IV) were identified and assayed. No significant effect of Methylparaben was seen on two of the isozymes (forms I and II), but phosphodiesterase IV activity was increased. Given the small increase in cGMP levels the authors expressed surprise that phosphodiesterase forms I and II were not increased. The authors did not comment on the increase in phosphodiesterase IV, given the decrease in cAMP levels. The authors concluded that these results provide support for a Methylparaben effect on cell membranes (Harvey et al. 1992).

Toxic Effects Mechanisms

Nakagawa and Moldéus (1998) used isolated rat hepatocytes and mitochondria to examine the mechanism of toxic effects of parabens. Incubation of rat hepatocytes with concentrations of Propylparaben of 0, 0.5, 1.0, and 2.0 mM produced cell death that increased with both concentration and time of incubation with the control group exhibiting minimal cell death over the 3-h incubation time. The authors postulated that diazinon, an esterase inhibitor, would reduce the toxic effect if *p*-hydroxybenzoic acid is responsible for the damage. Addition of 100 μM diazinon to 1.0 mM Propylparaben increased the cytotoxicity of Propylparaben over the 3-h incubation time, suggesting that *p*-hydroxybenzoic acid is not the active agent.

The effect of different parabens (at 2.0 mM) was determined by measuring cell death, ATP, adenine nucleotide pools, and mitochondrial membrane potential during a 1-h incubation. Methylparaben produced the least toxic effects and Isobutylparaben produced the most. The authors concluded there was no difference in toxicity between isomers (Propylparaben/Isopropylparaben and Butylparaben/ Isobutylparaben). Table 20 presents those results.

TABLE 20
Toxic effects of parabens (2.0 mM, 1 h) in isolated rat hepatocytes in culture (Nakagawa and Moldéus 1998).

Paraben	% cell death	Cellular ATP	Cellular adenine nucleotide pool	Mitochondrial membrane potential (% of control)
None (control)	21 ± 4	15.1 ± 0.9	20.7 ± 3.5	100
p-Hydrozybenzoic acid	23 ± 8	14.1 ± 1.5	19.9 ± 3.1	96.3
Methylparaben	29 ± 5	11.0 ± 2.6*	19.3 ± 1.9	93.3
Ethylparaben	32 ± 6	9.7 ± 2.0*	15.7 ± 2.8*	91.5
Propylparaben	50 ± 4*	2.1 ± 0.3*	15.7 ± 3.0*	48.5
Isopropylparaben	47 ± 7*	3.3 ± 0.6*	16.6 ± 2.4*	55.1
Butylparaben	88 ± 4*	0.3 ± 0.2*	7.1 ± 1.8*	39.3
Isobutylparaben	98 ± 2*	0.2 ± 0.1*	7.1 ± 0.7*	37.1

*Significantly different from control.

The authors also determined the effect of parabens on respiration in isolated hepatocyte mitochondria (in the presence of adenosine triphosphate [ATP]; state 3). The authors concluded that the decrease in oxygen uptake in state 3 was greater with the longer-chain parabens compared to the shorter ones, and no difference between chain isomers.

Overall, the authors concluded that the effects of parabens on isolated rat hepatocytes was mediated by reduced mitochondrial function, the consequent reduction in ATP, and limitation of all energy-requiring functions, eventually leading to cell death (Nakagawa and Moldéus 1998).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Methylparaben

Litton Bionetics (1974) performed a series of acute oral toxicity studies using rats. Methylparaben in 0.85% 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 h. Necropsy findings included reddened gastric mucosa and congested lungs. No animals died at 100 and 500 mg/kg. The acute oral LD_{50} was determined to be 2100 mg/kg.

These authors repeated the study using Methylparaben as a 21.8% saline suspension 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.

Methylparaben at 37% to 79% 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 authors concluded that the rat acute oral LD_{50} for 21.8% to 79% Methylparaben was >5600 mg/kg (Litton Bionetics 1974).

CTFA (1976a) reported a study in which Methylparaben was administered by gastric intubation to five female rats at a dose of 15,000 mg/kg. All animals appeared normal throughout the study, and there were no gross lesions at necropsy on the seventh day.

Products containing 0.2% or 0.8% Methylparaben administered by gastric intubation to rats at doses up to 15,000 mg/kg caused no deaths (CTFA 1979a, 1979b, 1981a; Leberco Laboratories 1978a, 1979a).

Ethylparaben

Moriyama et al. (1975) administered Ethylparaben 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 weights increased normally. No macroscopic abnormalities were found at necropsy.

CTFA (1980a) reported that Ethylparaben was tested for acute oral toxicity as a 20% dilution in propylene glycol. Doses of 4.64 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.

Products containing 0.2% Ethylparaben produced no deaths when administered to groups of five rats at a dose of 15 g/kg (CTFA 1981b, 1981c).

Propylparaben

Products containing 0.2% or 0.3% Propylparaben caused no deaths when administered to rats at doses of 15 g/kg (CTFA 1977a; Leberco Laboratories 1978b).

Butylparaben

Products containing 0.2% or 0.3% Butylparaben produced no deaths when administered orally to rats at doses of 5 and 25 g/kg, respectively (CTFA 1976b, 1980b).

Benzylparaben

Loos (1935) stated that 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.

Sabalitschka and Neufeld-Crzellitzer (1954) fed two guinea pigs 2 g of Benzylparaben per day; no injurious effects to the animals were noted. The duration of dosing was unspecified.

CTFA (1985) reported a study in which 5 g/kg of Benzylparaben given to groups of Charles River CD rats produced no deaths.

Comparing Parabens

Schuebel (1930) 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 and 6 g/kg. Toxicity decreased as the alkyl chain length increased.

Matthews et al. (1956) determined the acute oral toxicity of parabens and their sodium salts in an unspecified number of mice. Test compounds were suspended in 3% starch, propylene glycol, or olive oil. Animals were observed for 1 week post treatment.

The reported acute oral LD_{50} values 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 alkyl chain length increased, toxicity increased due to longer hydrolysis times (Matthews et al. 1956).

Multiple Parabens

Applied Research Laboratories (1939) administered a 60:40 mixture of the sodium salts of Propylparaben and Ethylparaben, respectively, 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% mortality). Animals were observed for 10 days post treatment. The minimum lethal dose was determined to be 5.0 g/kg.

Sado (1973) studied the acute oral toxicity of Ethylparaben, Propylparaben, Butylparaben, and paraben combinations in dd-strain mice. The acute oral LD_{50} values for Ethyl-, Propyl-, and Butylparabens were 6008, 6332, and 13,200 mg/kg, respectively. Additional tests revealed that the toxicity of mixtures did not exceed theoretical values, indicating that these compounds do not exhibit synergistic toxicity.

Products containing both Methylparaben at 0.2% and Propylparaben at 0.1% resulted in oral LD_{50} values in rats greater than 98.9 g/kg in one study (Stillmeadow 1978a) and greater than 5 g/kg in another (CTFA 1979c).

A product containing both 0.2% Propylparaben and 0.1% Butylparaben produced no deaths when administered orally at 5 ml/kg to 10 rats (CTFA 1980c).

Acute Dermal Toxicity

Methylparaben

A hairdressing product containing 0.2% 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 h. No toxic effects were observed for 14 days post treatment (CTFA 1981d).

Multiple Parabens

The acute dermal toxicity of eye makeup formulations containing 0.2% Butylparaben or 0.2% Methylparaben and 0.1% Propylparaben was studied using rats. The LD_{50} values were greater than 2 g/kg (CTFA 1979c, 1980b).

Acute Subcutaneous Toxicity

Methylparaben

Bijlsma (1928) administered Methylparaben subcutaneously to mice at 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 reported to be greater than 333 mg/kg, since no animals died from this dose.

Homburger (1968) gave groups of eight C57BL/6 mice 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.

Mason et al. (1971) administered Methylparaben subcutaneously to five groups of 20 Fischer rats at doses up to 500 mg/kg (10 males/10 females per group). No animals died and the acute LD_{50} was reported to be >500 mg/kg.

Isobutylparaben

According to an entry in the RTECS (1993), the subcutaneous LD_{50} of Isobutylparaben in mice was reported to be 2.6 g/kg.

Multiple Parabens

Adler-Hradecky and Kelentey (1960) administered the sodium salts of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben subcutaneously to groups of five mice. The reported acute LD_{50} values were 1.20, 1.65, 1.65, and 2.5 g/kg, respectively.

Acute Intravenous Toxicity

Methylparaben

Simonelli and Marri (1939) administered Methylparaben to three rabbits at intravenous 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.

Homburger (1968) reported on a study in which 6 A/Jax mice were each given 2.5 mg Methylparaben intravenously. Gasping respiration and shock were observed immediately. Animals returned to normal within 90 min.

Benzylparaben

Kohn (1933) stated that intravenous injection of Benzylparaben (dose not given) to dogs and cats caused no variation in blood sugar concentration of the animals. Ghirardi (1940) reported that intravenous injection of dogs with 0.7 g/kg Benzylparaben produced no ill effects.

Comparing Parabens

Matthews et al. (1956) injected Methylparaben or Propylparaben intravenously in dogs in increasing doses (1 to 1400 mg/kg), and the effects on the cardiovascular and autonomic nervous system were monitored. Parabens had no effect on the nervous system. Death was associated with the hypotensive action including a sharp but brief fall in blood pressure and a corresponding rise in the jugular venous pressure. The rate of injection and the cardiovascular effect were correlated. These authors reported that the acute intravenous LD_{50} values in mice of the sodium salts of Methylparaben and Propylparaben were 170 and 180 mg/kg, respectively.

Acute Intraperitoneal Toxicity

Comparing Parabens

Matthews et al. (1956) reported the following acute intraperitoneal LD_{50} values in mice for various parabens and their salts: Methylparaben, 960 mg/kg; Methylparaben (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 which the authors attributed to local irritation.

Acute Subarachnoid Toxicity

Methylparaben

Adams et al. (1977) studied the effect of 0.1%, 0.3%, and 1% Methylparaben (in saline) on the spinal cords and spinal nerve roots of rabbits following subarachnoid injection. Vehicle 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.

Acute Inhalation Toxicity

Methylparaben

Jian and Po (1993) reported that Methylparaben is mildly ciliotoxic to male Wistar rats at an inhaled concentration of 1.18 mM (4-h exposure).

Subchronic Oral Toxicity

Methylparaben

Bijlsma (1928) 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

Moriyama et al. (1975) administered Ethylparaben orally to groups of 10 rats (5 males/5 females per group) at concentrations of 2.0%, 1.0%, and 0.2% 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.

From weeks 22 to 25, significant increases in mean body weight were observed in males at the 0.2% level. Significant decreases in mean body weights were observed in males at the 1.0% and 2.0% 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.

These authors also administered Ethylparaben 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. Over the period of the study, body weights increased. No animals died and no abnormalities were observed upon necropsy (Moriyama et al. 1975).

Benzylparaben

Ishizeki et al. (1955) reported that guinea pigs fed 1 g of Benzylparaben per day for 19 days had no signs of toxicity.

Comparing Parabens

Inai et al. (1985) administered 0.6%, 1.25%, 2.5%, 5%, and 10% Isobutylparaben or Butylparaben in the feed of groups of 10 male and 10 female ICR/Jcl mice for 6 weeks. A group of 20 males and 20 females served as a control.

All mice of the 5% and 10% dose groups died during the first 2 weeks of the study. Body weight gain percentages for mice of the 1.25% and 2.5% 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% or higher. 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 or Butylparaben or in the control animals (Inai et al. 1985).

Multiple Parabens

CTFA (1980d) reported a study in which a product formulation containing 0.2% Methylparaben and 0.2% 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% and 10% 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 (CTFA 1980d).

CTFA (1980e) reported a study in which a product formulation containing 0.2% Propylparaben and 0.1% 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 (CTFA 1980e).

Subchronic Dermal Toxicity

Methylparaben

CTFA (1980f) reported results of a 3-month dermal toxicity study conducted to test the effects of daily dermal exposure to a product formulation containing 0.2% Methylparaben. A treatment group of five male and five female albino rabbits received daily topical doses of 5.5 mg/cm^2 over 8.4% of the 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 (CTFA 1980f).

CTFA (1980g) reported a 3-month dermal toxicity study similar to that described above on another product formulation containing 0.2% Methylparaben. The formulation was administered to groups of five male and five female rabbits at doses of 6.6 mg/cm^2 and 11 mg/cm^2 over 8.4% of the 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 (CTFA 1980g).

Comparing Parabens

CTFA (1981f) reported the results of a 13-week dermal toxicity study in rats conducted on a medicated cream containing 0.7% Methylparaben or a medicated lotion containing 0.3% Propylparaben. Groups of 10 rats received daily topical doses of the cream at 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% 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 (CTFA 1981f).

Multiple Parabens

CTFA (1981e) reported a 3-month dermal toxicity study on a product formulation containing 0.2% Methylparaben and 0.2% 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 and 6 mg/cm^2 over 10% of the body surface area.

After dosing, rabbits in one control group and one group treated with 6 mg/cm^2 of the product were exposed daily to one-half the minimal erythema dose of UV radiation (4 min at 6 inches from Westinghouse FS-20 lamps producing UV in the range of 280 to 400 nm).

The product alone caused persistent moderate erythema, slight edema, and mild desquamation. Epidermal fissures with bleeding and papuloerythema were observed occasionally. The high dose was slightly more irritating than the low dose. UV 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 chemistry, 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 (CTFA 1981e).

Chronic Oral Toxicity

Comparing Parabens

Matthews et al. (1956) reported a chronic oral toxicity study in which Methylparaben or Propylparaben were incorporated into the diets at 2% or 8% and the diets fed to groups of 24 rats for 96 weeks. Ethylparaben or Butylparaben were fed to the same numbers of rats at concentrations of 2% or 8% in the diet for 12 weeks. Negative controls were included in the study. Rats, especially the males, fed the 8% Methylparaben or Propylparaben diets had decreased weight gain in the early part of the study. At 8% dietary concentration, Ethylparaben reduced growth rate, decreased motor activity, and, in some cases, caused death within the first week. All males fed 8% Butylparaben died before the 12th week. Females fed this diet exhibited signs of toxicity. At 2% of the diet, Parabens exerted no toxic effect. Rats killed at the conclusion of the feeding test had no treatment related abnormalities.

These authors also dosed weanling dogs 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 (Matthews et al. 1956).

Inai et al. (1985) administered 0.15%, 0.3%, and 0.6% Butylparaben or Isobutylparaben in the feed of groups of 50 male and 50 female 8-week-old ICR/Jcl mice for 102 weeks. A group of 50 males and 50 females served as a control and were fed a basal diet. In a range-finding subacute toxicity test, mice were fed concentrations of both parabens of 0.6%, 1.25%, 2.5%, 5%, and 10%. All mice of both sexes in the two highest concentration groups died. Significant reductions in weight gain were seen in the 1.25% and 2.5% groups. The 0.6% level in feed was determined to be the maximum tolerated dose.

In the chronic toxicity phase of the study, 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 were killed and necropsied. There was no significant difference between groups in the amount of feed consumed.

Data were compiled from animals surviving the study for 78 weeks or more. Although tumors were observed in treated and control animals, there were no significant differences in the incidence of tumors or the time to tumor development between the treated mice and the controls or between groups given different doses of Isobutylparaben or Butylparaben.

Tumors in Butylparaben-treated mice included thymic lymphoma, nonthymic lymphoid leukemia, and myeloid leukemia; with adenomas and adenocarcinomas of the lung and soft tissue myosarcomas and osteosarcomas in several dose groups.

Among Isobutylparaben-treated mice, a high incidence of thymic lymphoma and nonthymic lymphoid leukemia was noted in the 0.6% group; with soft tissue myosarcomas and osteosarcomas also high. In male mice treated with Isobutylparaben, 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. Systemic amyloidosis was noted in 58% of dosed males and 33% of dosed females compared with 25% of control males and 10% of control females.

The authors calculated that the maximum ingested dose of Butylparaben that was considered nontumorigenic was ~40 mg/mouse; equivalent to a daily human intake of 65.8 g. Comparing this nontumorigenic level with permitted food additive levels of 0.25 g/L of Butylparaben, they noted that this nontumorigenic level is much higher than the average daily intake of Butylparaben by humans (Inai et al. 1985).

Although no information is available concerning the incidence of amyloidosis in historical controls in this laboratory, it has been reported 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).

Multiple Parabens

Applied Research Laboratories (1942) fed a 60:40 mixture of the sodium salts of Propylparaben and Ethylparaben, respectively, 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.

Chronic Subcutaneous Toxicity

Methylparaben

Mason et al. (1971) administered Methylparaben via subcutaneous injection at doses of 3.5, 2.0, 1.1, and 0.6 mg/kg to groups of 80, 60, 40, and 20 Fischer rats, respectively, twice weekly, for 52 weeks. At 52 weeks, 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.

Dermal Irritation

Methylparaben

CTFA (1976c) reported that 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 h. The resultant primary irritation index (PII) was 0.67 (maximum score 4.0), a value indicative of mild skin irritation according to these authors.

Ethylparaben

CTFA (1980 h) reported that the Draize skin irritation technique was used to test Ethylparaben at100% and at 10% in water on groups of nine rabbits. The undiluted and diluted ingredient produced no signs of irritation.

Benzylparaben

According to CTFA (1985), the PII of 500 mg of Benzylparaben 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). 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.

Comparing Parabens

Sokol (1952) stated that pastes containing hydrophilic ointment and either 10% Methylparaben or Propylparaben were applied to the shaved backs of albino rabbits for 48 hours produced no irritation. Neither Methylparaben, Propylparaben, nor their degradation products were detected when the animals were then killed and their kidneys removed for analysis.

Product Dermal Irritation Tests

Methylparaben

Several Draize rabbit skin irritation tests have been conducted on product formulations containing parabens (CTFA 1979d, 1979e, 1981g; Leberco Laboratories 1978c, 1978d, 1979b). Product formulations containing 0.2 to 0.8% Methylparaben 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.

CTFA (1981o) reported that a hairdressing product formulation containing 0.2% 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. The authors considered this degree of irritation to be typical for this type of product.

Ethylparaben

Products containing 0.2% Ethylparaben produced minimal to mild irritation in studies using rabbits, with PIIs of 0.17 to 0.56 (CTFA 1981h and i).

Propylparaben

CTFA (1977b) reported a study in which a product formulation containing 0.3% Propylparaben was applied daily to the shaved skin of nine albino rabbits for 4 consecutive days. The product produced minimal irritation with a PII of 0.5 (maximum score 4.0).

Butylparaben

CTFA (1976d) reported that a product containing 0.3% Butylparaben was similary tested on the backs of six rabbits for 3 consecutive days. Almost all rabbits showed mild irritation.

Multiple Parabens

CTFA (1980c) reported a test in which a product containing 0.2% Propylparaben produced minimal irritation in studies using rabbits, with a PII of 0.5. A product containing 0.2% Butylparaben was reported to be nonirritating, but the PII of 2.75 indicated moderate irritation. There were no signs of irritation with a product formulation containing 0.2% Propylparaben and 0.1% Butylparaben.

CTFA (1979c) reported that a product containing both 0.2% Methylparaben and 0.1% Propylparaben was minimally irritating in studies using rabbits, with a PII of 0.5.

Dermal Sensitization

Methylparaben

Aldrete and Klug (1970) injected Methylparaben (0.1%) intradermally into the shaved dorsal skin of four guinea pigs 5 days per week for 8 weeks. Sites were scored 24 h 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.

Maurer et al. (1980) injected Methylparaben (0.1%) intracutaneously every other day for 3 weeks (10 injections) into the dorsal skin of each of 20 guinea pigs. Sites were scored 24 h postinjection. During the second and third weeks of induction, Methylparaben was incorporated at 0.1% 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 h and compared to induction reactions. Ten days later, a 5% Methylparaben challenge patch was applied to the skin site, which was scored for irritation 24 h 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.

CTFA (1981q) also reported that a product formulation containing 0.2% 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 h. Applications were made three times per week for a total of nine applications. 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.

Butylparaben

Brulos et al. (1977) gave 20 albino guinea pigs intradermal injections of Freund's complete adjuvant on days 0 and 9, at which time 5% Butylparaben was applied under 48-h 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 h to a previously untested site. One, 7, 24, and 48 h 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% 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.

Multiple Parabens

Sokol (1952) reported that Methylparaben, Ethylparaben, Propylparaben, and Butylparaben (0.1% in saline), was injected intracutaneously into an unspecified number of guinea pigs, three times weekly for 3 weeks (10 injections). No reaction was observed 24 h after the first injection. Two weeks following the last induction injection, a challenge injection was administered into an adjacent site and observed for 48 h. No allergic response was induced by any of the parabens.

Matthews et al. (1956) reported that the same four parabens (at 0.1%) 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 h later. There were no reactions in the animals to any of the parabens. It was observed that these ingredients are nonsensitizing.

In a procedure described by Marzulli et al. (1968), 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% 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% 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% intradermally at induction, and 1% intradermally or 10% topically at challenge (Marzulli et al. 1968).

CTFA (1981p) reported that a Magnusson-Kligman guinea pig maximization test was used to determine the sensitization potentials of Methylparaben and Ethylparaben. The procedure calls for a protocol of induction with Methylparaben or Ethylparaben at 1% and 5% in 50% Freund's complete adjuvant, booster of 10% sodium lauryl sulfate followed by 50% of the relevant paraben in petrolatum 24 h later, and challenge with Methylparaben at 5% and 10% and Ethylparaben at 1% and 2% in petrolatum. A total of 80 female guinea pigs were used. Phenylacetaldehyde (concentration not given) served as a positive control, with 7/8 and 8/8 animals in two groups having a reaction. No animals in any of the Methylparaben or Ethylparaben groups showed a reaction.

Ocular Irritation

Methylparaben

Simonelli and Marri (1939) reported a study in which Methylparaben, at concentrations up to 0.2% was instilled into the eyes of rabbits. At the highest concentration tested, Methylparaben induced slight, transient conjunctival hyperemia.

Soehring et al. (1959) reported that, in an investigation concerning the irritancy of various ophthalmic drug ingredients, 0.1% to 0.2% Methylparaben in isotonic solution did not induce ocular irritation when instilled in the eyes of rabbits and guinea pigs.

CTFA (1976e) reported on a study in which Methylparaben at 100% 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.

Ethylparaben

CTFA (1980i) reported that Ethylparaben at 100% instilled into the eyes of two groups of six albino rabbits was slightly irritating, with a maximum eye irritation score of 2/110 on day 1. Ethylparaben at 10% in water produced no signs of irritation.

Benzylparaben

CTFA (1985) reported no adverse ocular responses in three New Zealand rabbits at 1, 24, 48, or 72 h after the instillation of 0.1 g of Benzylparaben into the conjunctival sac.

Multiple Parabens

Weinreb et al. (1986) reported intercellular vacuolization and thickening of the endothelial layer in rabbit corneal endothelium 1 day following subconjunctival administration of solutions containing Methylparaben and Propylparaben.

Product Ocular Irritation Studies

A number of rabbit eye irritation studies have been conducted on products containing Methylparaben, Ethylparaben, Propylparaben, and/or Butylparaben at concentrations of 0.1% to 0.8%. Most products produced no signs of eye irritation (CTFA 1979c, 1979f, 1979g, 1980h, 1981j, 1981k; Leberco Laboratories 1978e, 1978f, 1979c). Other products produced slight or minimal eye irritation, with scores of 1.0 to 3.3/110 (CTFA 1980c, 1981l, 1981m, 1981n; Stillmeadow 1978b).

Mucous Membrane Irritation

Multiple Parabens

CTFA (1980c) reported a study in which a product formulation containing 0.2% Propylparaben and 0.1% Butylparaben was applied to the genital mucosa of six female albino rabbits. The single 0.1-ml application of the undiluted product produced no evidence of mucosal irritation during the 7-day observation period.

Phototoxicity

Multiple Parabens

As noted earlier, CTFA (1981e) reported a 3-month dermal toxicity study of a product formulation containing 0.2% Methylparaben and 0.2% Propylparaben using rabbits. The formulation was administered at doses of 2 mg/cm^2/10% body surface area and 6 mg/cm^2/10% body surface area. After dosing, rabbits in one control group and one group treated with 6 mg/cm^2 of the product were exposed daily to one-half the minimal erythema dose of ultraviolet light (4 min 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 slightly more irritating than the low dose. Ultraviolet light exposure had no apparent effect on the severity of the irritation in either treatment group.

GENOTOXICITY

Methylparaben

Litton Bionetics (1974) reported the result of 3 different assays to evaluate the genotoxicity of Methylparaben: a host-mediated assay, a cytogenic assay, and a dominant lethal assay.

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 in vivo host-mediated assay, 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 h later, and peritoneal fluid was extracted, bacterial counts were made, and the number of mutants was recorded. In the subchronic in vivo host-mediated assay, each of 10 mice received orally 0 to 3500 mg/kg Methylparaben daily for 5 consecutive days. Within 30 min after the last treatment, animals were inoculated with indicator organisms and treated as above. In the in vitro host-mediated assay, 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 *Salmonella typhimurium* or *Saccharomyces cerevisiae* in these in vitro or in vivo host-mediated assays.

The cytogenic assay also consisted of acute and subchronic in vivo tests and an in vitro study. In the acute cytogenic assay, 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 mitosis. Five animals at each dose level were killed at 6, 24, and 48 h. Bone marrow was removed and the chromosomes of cells evaluated for abnormalities. Positive and negative controls were used. In the subchronic cytogenic assay, groups of five mice received 0 to 5000 mg/kg Methylparaben daily for 5 consecutive days. Animals were killed 6 h following the last dosing, and tissue was taken for evaluation as above. In the in vitro cytogenic assay, 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.

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 (Litton Bionetics 1974).

Matsuoka et al. (1979) 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 homogenates (S9 mix). Chromosome preparations were then made and aberrations were scored. When assayed without S9 mix, induction of chromosomal aberrations was negative (1%). In the presence of S9 mix, however, aberration incidence increased to 13.0% and was judged to be significant. Gaps, breaks, exchanges, and

rings were observed. The significance of these effects was not assessed.

Propylparaben

McCann et al. (1975) reported the use of the *Salmonella* /microsome test 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 (S9). When tested at doses of 10 to 2000 μg/plate, Propylparaben was nonmutagenic both with and without metabolic activation.

Litton Bionetics (1975) also used the Ames test to evaluate the mutagenic potential of Propylparaben in *S. cerevisiae* strain 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% Propylparaben was added to cultures. In suspension tests, 0.025% to 0.15% Propylparaben was used. Propylparaben was nonmutagenic with and without metabolic activation in all assays.

Odashima (1976) reported that Propylparaben was evaluated 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 h later. Bone marrow cell chromosomes were examined for aberrations. Mutagenic activity was evaluated in *S. typhimurium* strains TA1535, TA1536, TA1537, and TA1538, and repair testing was performed with *E. coli* strains H-17, M-45, and WP-2. In all assays except the repair test, Propylparaben was nongenotoxic.

Sugimura et al. (1976) used a modified Ames test in which 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 without metabolic activation and in strains TA98 and D-2 with metabolic activation, but was mutagenic in strain TA100 with metabolic activation.

Isopropylparaben

Ishidate and Odashima (1977) reported that, at a concentration of 1 mg/plate in DMSO, Isopropylparaben was negative in Ames tests using *S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98, with and without metabolic activation.

Butylparaben

Ishizaki et al. (1978) reported that when Butylparaben (1%) 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.

Isobutylparaben

Ishidate and Odashima (1977) reported that at a concentration of 1 mg/plate in DMSO Isobutylparaben was negative in Ames tests using *S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98. These authors also performed a chromosomal abberation assay 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.

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).

Comparing Parabens

Ishidate et al. (1978) studied the ability of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben 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 h later and aberrations scored. The maximum tolerated concentrations 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% increases in polyploid 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% increases, respectively). Aberrations observed included chromatid breaks, chromatid gaps, chromosomal exchanges, and ring formations.

Ishidate et al. (1984) summarized the results of mutagenicity screening of food additives, including Ethylparaben, Isopropylparaben, and Isobutylparaben. Results of reverse mutation assays using *S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98 (Ames test) were considered negative (<4.9% mutation frequency) for all three parabens. In chromosomal aberration assays using a Chinese hamster fibroblast cell line, after 48 h, cells treated with 0.25 mg/ml Ethylparaben, 0.125 mg/ml Isopropylparaben, or 0.6 mg/ml Isobutylparaben in ethanol had 1%, 2.0%, and 3.0% polyploid cells and a 11%, 1%, and 1% incidence of structural chromosomal aberrations, respectively. The authors stated that the control incidence of aberrations was usually less than 3% and that any result less than 4% was considered negative. A result more than 10% was positive.

CARCINOGENESIS

Cell Proliferation

Methylparaben

Homburger (1968) reported on a study in which 100 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 8th week. By the 10th 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 stated that Methylparaben was not carcinogenic under these test conditions.

In a second 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 CF-1 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.

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, because the positive-control compound (croton oil) had no effect, the author stated that the test was inconclusive (Homburger 1968).

Mason et al. (1971) conducted a study in which 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% incidence for Methylparaben; 1% for negative control). The incidence of injection site tumors, pituitary adenomas, uterine polyps, and leukemias did not differ significantly from controls.

Rodrigues et al. (1986) fed Methylparaben to weanling Fischer 344 rats (eight animals). Analyses of the rat stomach were performed as in the study by Hirose et al. (1986). The authors reported no increase in the labeling index in the prefundic region. Because this finding was different from earlier results demonstrating an increase in the labeling index with Propylparaben treatment, these authors conducted a further study comparing *p*-hydroxybenzoic acid, Methylparaben, Ethylparaben, Propylparaben, and Butylparaben as described under "Comparing Parabens" in this section.

Propylparaben

Odashima (1976) stated that 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. The author stated that, in both studies, Propylparaben was noncarcinogenic.

In a study primarily examining the pathological and proliferative effects of butylated hydroxyanisole (BHA), Nera et al. (1984) reported the short-term effects of Propylparaben in the forestomach of Fischer 344 rats. Finely ground Propylparaben at 1.0% and 4.0% (five rats each group) was incorporated in powdered rat diet of weanling rats and given for 9 days. Untreated diet was the negative control. One hour before killing with CO_2, each rat was injected intraperioneally (i.p.) with 0.25 μCi/g [methyl-^{3}H] thymidine. Each rat was necropsied and the stomach removed and processed for autoradiography using a longitudinal bisection of the entire stomach, and different parts of the stomach were taken for histological examination.

Results for Propylparaben were presented by comparison with the effects of BHA. At 1% Propylparaben, a 1.5-fold increase in the labeling index was found in the prefundic region, with hyperplasia seen histologically. At 4% Propylparaben, there was a 2.5- fold increase in the labeling index in the prefundic region, with rete pegs and papillae, slight acanthosis, and minimal hyperkeratosis seen with intercellular edema. These findings were comparable to 0.5% BHA in the powdered diet (Nera et al. 1984).

Hirose et al. (1986) compared the effects of 13 phenolic compounds, including Propylparaben, using Syrian golden hamsters. Each of 15 7-week old hamsters received 3% Propylparaben in feed for 20 weeks. A control group received only basal feed. At the end of the exposure, animals were killed and their liver, kidneys, cheek pouch, stomach, esophagus, lungs, pancreas, and urinary bladder were removed.

Three animals received an intraperitoneal injection of [methyl-^{3}H]thymidine 1 h before killing. Tissue for histological and autoradiographic examination was taken from the anterior and posterior walls of the forestomach, glandular stomach, and urinary bladder. Mild hyperplasia in the forestomach was seen in five animals, but no moderate or severe hyperplasia or papillomatous lesions were found. Radiolabel indicies were not different compared to controls.

Shibata et al. (1990) reported on the early proliferative responses of forestomach and glandular stomach of rats treated with five different phenolic antioxidants, including Propylparaben. Five 6-week-old rats were given 3% Propylparaben in

feed for 8 weeks. A control group received basal feed. At week 8, rats were injected intraperitoneally with bromodeoxyuridine (BrdU) and killed 1 h later. Stomachs were removed and fixed. Samples for histological examination and BrdU immunohistochemical staining were taken from the forestomach and the glandular stomach. Propylparaben had no hyperplastic effect and there was no increase in the labeling index in the forestomach. Likewise, Propylparaben had no effect on the glandular stomach.

Comparing Parabens

As described in "Chronic Toxicity" earlier, Inai et al. (1985) examined the tumorigenicity of Butylparaben or Isobutylparaben administered orally to mice. There were no statistically significant differences in the tumor incidence between control and treated mice, or between groups of treated mice. The incidence and time to death with neoplasms in different organs also was not different between control and treated mice. The authors reported a higher incidence of amyloidosis in treated animals, with effects in the spleen, liver, kidney, and/or adrenal gland, compared to controls.

Rodrigues et al. (1986) conducted a study using *p*-hydroxybenzoic acid, Methylparaben, Ethylparaben, Propylparaben, and Butylparaben using the methods of Hirose et al. (1986). Treatment chemicals were given to Fischer 344 male rats at 4% for 9 days in the dry diet. BHA at 2% was the positive control.

No effect was seen in the prefundic region in control animals or in animals fed 4% *p*-hydroxybenzoic acid or Methylparaben. Around a 2-fold increase in labeling index was seen for 4% Ethylparaben, an 8-fold increase for Propylparaben, and almost a 14-fold increase for 4% Butylparaben. BHA at 2% produced an increase in labeling index similar to 4% Butylparaben.

The authors stated their view that the finding of labeling index related to the chain length of the paraben used in the study probably reflects the inability of esterases present in the forestomach epithelium to hydrolyze parabens with higher chain lengths (Rodrigues et al. 1986).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Teratogenesis

Methylparaben

The Food and Drug Research Labs (1972) studied the teratogenic effects of Methylparaben in rats, mice, and hamsters. Groups of 21 to 25 pregnant animals were given Methylparaben orally at doses of 5.0 to 550 mg/kg (rats, mice) or 3.0 to 300 mg/kg (hamsters) 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 weights were 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 control groups.

A similar study (Food and Drug Research Labs 1973) 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 control groups.

Ethylparaben

Moriyama et al. (1975) added Ethylparaben to the feed of groups of 12 pregnant rats at concentrations of 0.1%, 1%, or 10% between gestation days 8 and 15. On day 21 of pregnancy, rats were killed, and the number of fetal implantations, status of maternal visceral organs, fetal body weights, and numbers of skeletal, visceral, and external defects in fetuses were recorded.

At the 10% level, cerebral hemorrhages, abnormal enlargement in the ventricles of the brain, and, in some, hydronephrosis and hypo-osteogenesis were observed in fetuses. Some fetuses at 1% Ethylparaben had no blood in the cardiac ventricle; some had intraperitoneal hemorrhages. Fetuses of rats of the 0.1% group had no significant visceral or skeletal defects. The authors considered the incidence of visceral and skeletal abnormalities in the 363 test fetuses evaluated to be insignificant when compared to control animals.

In addition, two groups of six pregnant rats each were given 0.1% or 10% Ethylparaben administered in their feed for 1 week during gestation days 8 to 15. Neonates from these dams were nursed for 1 month and growth, body weights, and abnormalities were recorded. These neonates grew normally. None had malformations or abnormal behavior. The authors concluded that at concentrations up to 10%, Ethylparaben was not teratogenic (Moriyama et al. 1975).

Butylparaben

Daston (2004) conducted a feeding study to determine the developmental toxicity of Butylparaben in rats. Singly housed Sprague-Dawley rats were divided into three treatment groups and one control group—each group consisted of 25 presumed pregnant rats. Dose levels in the three treatment groups were 10, 100, and 1000 mg/kg day^{-1} daily on gestational days (GDs) 6 to 19. Based on range-finding studies, the 10 and 100 mg/kg day^{-1} groups were expected to be the same as the control group.

Body weights and clinical signs were determined daily, but feed consumption was recorded only on GDs 0, 6, 9, 12, 15, 18

and at sacrifice (by CO_2 inhalation) on GD 20. Fetuses were obtained by caesarean section. Uteri were stained and examined for implantation sites. The number and distribution of corpora lutea, implantation sites, live and dead fetuses, and early (embryonic structures not evident) and late resorptions were recorded. Fetal observations included sex, external abnormalities, and body weights. Live fetuses were sacrificed by i.p. injection of a commercial euthanasia solution. Around half of the fetuses were examined for soft tissue abnormalities and the other half examined for skeletal abnormalities.

In the high-dose group, maternal body weight gains were reduced compared to controls, reaching statistical significance at GDs 18 to 20. Maternal feed consumption values were significantly reduced in the high-dose group on GDs 12 to 15 and 18 to 20 compared to controls. Even with some maternal toxicity, the author concluded that none of the measures of developmental toxicity were affected by any of the Butylparaben doses.

The author noted that this study protocol measures parameters that are influenced by a large number of developmental mechanisms that may be sensitive to toxicants, including estrogens. Although these parameters are not the most sensitive indicators of estrogenic activity, the author indicated that this study design is responsive to such agents. The author concluded that Butylparaben at these dose levels does not have strong estrogenic potential during development, consistent with other observations of weak in vitro estrogenicity and limited response in the in vivo uterotrophic assay. The findings are not consistent, the author stated, with the findings of Oishi (2001, 2002a). Given that Butylparaben is rapidly and completely hydrolyzed to *p*-hydroxybenzoic acid by esterases throughout the body and that *p*-hydroxybenzoic acid has no estrogenic activity (Routledge et al. 1998), the author suggested that this is a plausible explanation for the absence of Butylparaben developmental toxicity (Daston 2004).

Harvey (2005) provided commentary on the above study. This author noted that the Lemini et al. (1997) study of *p*-hydroxybenzoic acid injected subcutaneously did demonstrate a uterotrophic effect and further argued that parabens may be absorbed intact. This author's suggestion is for a complete evaluation of the reproductive toxicity of parabens using accepted protocols.

Daston (2005) responded to this by reiterating that by relevant exposure routes, Butylparaben does not appear to have significant estrogenic effect when administered orally or applied to the skin. Noting that oral administration data in reports by Oishi (2001, 2002a) are the exception to this statement, this author suggests the need to replicate this study.

Male Reproductive Effects

Methylparaben

Charles River Discovery and Development Services (2005a) conducted a study using Crl:(WI) BR male rats. Four exposure groups (16 rats each) received Butylparaben at concentrations of 0, 100, 1000, and 10000 ppm in the diet for a minimum of 56 days. Diet was prepared by combining Butylparaben in acetone with the meal form of CE-2 diet. The authors reported that the acetone evaporated. Mean values for actual consumed doses of Butylparaben were estimated to be 0, 11.2, 110.0, and 1141.1 mg/kg day^{-1}.

All rats were 21 days of age at the start of the study. Body weights, clinical observations, and feed consumption were recorded. At 21 days after the start of exposure (42 days of age), blood samples were collected (biweekly after the initial collection) and analyzed for luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. Animals were observed for viability at least 2× daily and examined for clinical signs and general appearance at least 1× daily. At the end of the study, all surviving rats were killed and a final blood sample taken and analyzed. Sperm evaluations (concentration, motility, and morphology) were made. The left testis from each animal was collected for evaluation of daily sperm production (DSP).

A gross necropsy was performed and reproductive organs, livers, adrenal glands, thyroid glands, and pituitary glands were weighed and prepared for histological evaluation. Histological examination of the reproductive organs was performed on animals from the control and high-dose groups, including 25 cross-sections of seminiferous tubules from each animal grouped into each of six stages of spermatid development.

Urine-stained abdominal fur was observed in three rats in the highest dose group. No other dose-related effects of Methylparaben consumption on body weights, weight gain, feed consumption, organ weights, daily sperm production, or sperm morphology were found in any dose group. One rat in the highest dose group was found dead on day 31, but no cause of death could be determined. This death was not considered related to treatment because the rat was normal in all aspects until being found dead. Histopathology of the testes using the semiquantitative staging identified no cell or stage related changes in either control or treated animals (Charles River Discovery and Development Services 2005a).

Propylparaben

Oishi (2002b) reported the effects of Propylparaben on the male reproductive system in rats. Crj:Wistar rats, 19 to 21 days old (52.5 ± 2.17 g) were placed into four groups of eight animals each. Propylparaben was given in the diet at 0%, 0.01%, 0.10%, and 1.0%. At the end of 4 weeks, rats were killed by decapitation, reproductive organs were examined, sperm counts performed in the testis, sperm reserves determined in the cauda epididymides, and testosterone levels were measured. Intake of the test material calculated from the amount of food consumed resulted in values of 12.4 ± 3.04, 125 ± 30.0, and 1290 ± 283 mg/kg day^{-1} for the 0.01%, 0.10%, and 1.0% groups, respectively.

Food intake was not different in any group, nor were there any significant body weight differences. A significant reduction in cauda epididymal sperm reserves and sperm concentrations was seen at 0.1% and 1.0% (compared to controls), but it was

not determined if the two levels were different from each other. Sperm counts in the testis of rats exposed to Propylparaben were 37.5 ± 5.32, 26.2 ± 2.34, 27.0 ± 9.07, and 25.9 ± 3.90 (DSP × 10^6 ± *SD*) for the 0%, 0.01%, 0.1%, and 1.0% groups, respectively. The sperm counts at all treatment levels were statistically different from controls. Serum testosterone was said to be reduced in a dose-dependent manner, but only the 1.0% level was significantly reduced compared to controls (Oishi 2002b).

Ashby et al. (2003) reviewed available data on control sperm counts from their own and several other studies using Wistar rats. Control sperm counts in the testis from different reported studies were given as 12.5 ± 1.2, 18.8 ± 2.7, 25.5 ± 4.4, 27.2 ± 3.4, 27.5 ± 3.2, and 34.4 ± 4.3 (DSP × 10^6 ± *SD*). These values encompass the values reported by Oishi (2002b).

Butylparaben

Fisher et al. (1999) studied the effect of subcutaneous injections of estrogen and other compounds on the development of the efferent ducts of the rat testis through puberty to adulthood. The efferent ducts join the testis to the initial segment of the epididymis and are comprised of a ciliated and nonciliated epithelium that express estrogen receptors α and β. Although the primary focus of the study was on diethylstilbestrol (DES) compared to ethinyl estradiol and tamoxifen, they also examined Butylparaben (at ~2 mg/kg day^{-1} in corn oil on postnatal days 2 to 18). Animals exposed to Butylparaben were killed on postnatal day 18, so that time became a point of comparison across all treatments. At postnatal day 19, DES produced dose-related changes in all parameters measured, but Butylparaben produced only minor effects on one parameter, epithelial cell height. No effect of Butylparaben on the expression of the water channel protein aquaphorin-1, efferent duct distension, or rete testis morphology was seen.

Oishi (2001) reported on the effects of Butylparaben on the male reproductive system in rats. Butylparaben was given in feed at doses of 0.01%, 0.10%, and 1.0% (8 rats per dose) to 3-week-old male Wistar rats for 8 weeks. A control group received basal diet only. The average Butylparaben intake from calculated food consumption was 10.4 ± 3.07, 103 ± 31.2, and 1026 ± 310 mg/kg day^{-1} for the 0.01%, 0.10%, and 1.0% dose groups, respectively. Animals were killed and reproductive organs dissected and weighed. Sperm counts were performed. Serum testosterone levels were measured after diethyl ether extraction using an enzyme immunoassay kit.

Body weights were not affected by Butylparaben, but there was a decrease in epididymis and seminal vesicles weights at the 1.0% dose level, and a decrease in the relative weights of the epididymis at both the 0.1% and 1.0% dose levels. There were no effects on the testes, ventral prostates, or preputial glands. Sperm counts at all dose levels were significantly decreased compared to controls, with a dose-dependent decreasing trend. Serum testosterone was significantly decreased at the 0.1% and 1.0% dose levels, but it was not determined if the effect at the two dose levels were different from each other. The author concluded that Butylparaben can adversely affect the secretion of testosterone and alter the functions of the male reproductive system. Recalling that sperm counts were reduced at 0.01% (~10 mg/kg) Butylparaben, the author went on to compare this with the acceptable daily intake (ADI) levels set by the European Commission (10 mg/kg), Japan (10 mg/kg), and the average daily intake in the United States (1 to 16 mg/kg for infants and 4 to 6 mg/kg after 2 years of age) and suggest that an adverse effect of Butylparaben is possible at doses well below the ADI or average daily intake (Oishi 2001).

Kang et al. (2002) reported decreased sperm number and motile activity in F1 offspring of rats maternally exposed to Butylparaben. Female Sprague-Dawley rats (22 9-week-old animals) were mated with male Sprague-Dawley rats. After mating, animals were randomly assigned to treatment or control groups (six to eight animals per group). Treatment groups received 100 or 200 mg/kg day^{-1} Butylparaben (in DMSO) by subcutaneous injection. The total treatment period was from gestation day 6 to postnatal day 20, with a 2-day interruption at parturition. Dams were killed when their litters were weaned, and body and organ weights measured. Implantation sites were determined. Live pups were counted, weighed, examined, and anatomical measurements made. Pups were killed and examined at postnatal days 21, 49, 70, and 90. Body and organ weights were measured and gross morphology of internal and external genitalia was examined. Histopathology was performed on the testes, prostate glands, seminal vesicles, uteri, and ovaries. Sperm in the caudal epididymides were counted and sperm motility determined. Spermatogenesis in the seminiferous tubules was examined. RNA was extracted from the testes of three male offspring of each group to determine expression of estrogen receptor mRNA.

There were no signs of toxicity in treated dams. Implantation sites, total pups, and pup sex ratio were not affected by treatment. The proportion of live pups, however, was decreased at both Butylparaben doses and the proportion of pups surviving to weaning was decreased in the high-dose group. Effects in male F1 offspring varied as a function of postnatal time and dose level, but no apparent pattern emerged; e.g., decreased body weights on postnatal day 49 in the low-dose group, but not in the high-dose group. No abnormalities were reported from the histopathological examination of the reproductive organs of male F1 animals.

The number of sperm in the caudal epididymis was 50% of control levels, sperm motility was reduced, and the numbers of round and elongated spermatid cells was decreased at both doses. The pattern of expression of estrogen receptor mRNA appeared to be affected, but at both doses only on postnatal days 21 (decrease) and 70 (increase). At postnatal day 49, the expression was decreased only at the low dose, and at postnatal day 90, the expression was increased only at the high dose. The authors suggested that maternal exposure to these high doses of Butylparaben delayed late stage of spermatogenesis by affecting the hormonal regulation process (Kang et al. 2002).

Oishi (2002a) reported the effects of Butylparaben on the male reproductive system in mice. Using the same protocol described by this author above for rats, 4-week-old Crj:CD-1 mice were treated in groups of eight to Butylparaben in the diet for 10 weeks. Butylparaben doses calculated from food consumption were 14.4 ± 3.60, 146 ± 35.9, and 1504 ± 357 mg/kg day^{-1}. There were no treatment-related effects on the liver, ventral prostates, seminal vesicles, and preputial glands. Both the absolute and relative epididymis weights were significantly higher in the high-dose group, compared to controls. A dose-dependent decrease in round and elongated spermatid counts was found, although the numbers of spermatogonia and spermatocytes did not differ from controls. Serum testosterone was significantly decreased only at the high dose, but a dose-dependent trend was noted. Comparing the doses in the study to the ADI in Japan, the author noted that Butylparaben can have adverse effects on the male reproductive system at doses below the ADI.

Charles River Discovery and Development Services (2005b) conducted a study using Crl:(WI) BR male rats. Four exposure groups (16 rats each) received Butylparaben at concentrations of 0, 100, 1000, and 10000 ppm in the diet for a minimum of 56 days. Diet was prepared by combining Butylparaben in acetone with the meal form of CE-2 diet. The authors reported that the acetone evaporates and is not a permanent part of the diet mixture. The authors noted the difficulty in preparing Butylparaben for the 10000 ppm group because of solubility problems. These were overcome by adding small increments of Butylparaben and small incremental additions of acetone, etc., until the desired solution concentration was reached. Actual consumed doses of Butylparaben were estimated to be 0, 10.9, 109.3, and 1087.6 mg/kg day^{-1}.

All rats were 21 days of age at the start of the study. Body weights, clinical observations, and feed consumption were recorded. At 21 days after the start of exposure (42 days of age), blood samples were collected (bi-weekly after the initial collection) and analyzed for LH, FSH, and testosterone.

At the end of the study, all surviving rats were killed and a final blood sample taken and analyzed. Sperm evaluations (concentration, motility, and morphology) were made. One testis was collected for evaluation of DSP.

A gross necropsy was performed and reproductive organs, livers, adrenal glands, thyroid glands, and pituitary glands were weighed and prepared for histological evaluation. The authors noted that the histological examination of the testes was done in such a manner to identify treatment related effects such as missing germ cell layers or types, retained spermatids, multinucleate or apoptotic germ cells, and sloughing of spermatogenic cells into the lumen. Cross-sections (25) of seminiferous tubules were evaluated from each animal and grouped into one of six staging groups. In this way, the authors stated, cell or stage specificity of testicular findings could be noted.

No effects of Butylparaben consumption on body weights, weight gain, feed consumption, or organ weights were found. Two rats (one control and one in the 100 ppm group) were killed on days 32 and 44, respectively, because of lesions of the eye from retro-orbital bleeding. In those two animals, no other clinical observations were noted during the study or at necropsy. No other control or treatment animals had adverse clinical observations during the study or at necropsy.

Histopathology of the testes using the semiquantitative staging described above identified no cell or stage related changes in either control or treated animals, except that one rat given 10,000 ppm had a single cross-section (25 cross-sections obtained) of a seminiferous tubule with a loss of germinal epithelium. The authors interpreted the small area affected and the failure to find any equivalent findings in the testes of any other animal to suggest this effect was not treatment related. Histopathological evaluation of the adrenal, pituitary, or thyroid glands or the liver uncovered no treatment related effects.

DSP was unaffected by Butylparaben consumption. Likewise, no effect on sperm motility, count, or morphology was found. No consistent differences in LH, FSH, or testosterone levels were reported in the treatment groups compared to controls. In the 1000 and 10,000 ppm groups at the second blood sample interval (3 weeks), there was a significant reduction in testosterone. At 9 weeks, the 10,000 ppm group had an increased testosterone level. LH levels were reduced in the 100 and 10,000 ppm groups at 5 weeks, but not at other doses. At 9 weeks, LH levels were increased in the 10,000 ppm group. None of these findings were considered dose-related. These authors concluded that 10,000 ppm was a no observed effect level (NOEL) for general toxicity, including specific male reproductive toxicity as determined by hormone level determinations and sperm analysis (Charles River Discovery and Development Services 2005b).

Comparing Parabens

Song et al. (1989) reported the effects of Methyl-, Ethyl-, Propyl-, and Butylparaben on human spermatozoa in a study designed to screen for potential spermicidal agents. Semen was obtained from healthy donors and accepted only if the sperm density was not less than 50 × 10^6/ml and at least 40% to 50% fully motile with rapid forward motion. Semen (0.2 ml) was mixed with 1.0 ml of parabens by shaking for 10 s at the concentrations shown in Table 21. If immediate viewing showed signs of sperm viability, the sample was incubated for 30 to 60 min and reexamined.

TABLE 21
Concentrations of parabens tested for spermicidal activity (Song et al. 1989).

Paraben	Concentration (mg/ml)				
Methyl	10.0	9.0	8.0	7.0	6.0
Ethyl	8.0	7.0	6.0	5.0	4.0
Propyl	5.0	4.0	3.0	2.0	1.0
Butyl	2.0	1.5	1.0	0.5	0.25

The lowest concentration at which none of the spermatazoa showed any signs of viability was reported for each paraben as follows: Methylparaben, 6.0 mg/ml; Ethylparaben, 8 mg/ml; Propylparaben, 3 mg/ml; and Butylparaben, 1.0 mg/ml. Because 6.0 mg/ml was the lowest concentration of Methylparaben tested, the authors suggested that it may be that total inactivation could occur at a lower concentration (Song et al. 1989).

Oishi (2004) reported an absence of spermatotoxic effects in male Crj:Wistar rats fed 0.1 and 1.0% Methylparaben or Ethylparaben. Test compounds were administered in the diet of 25-to 27-day-old rats (75.9 ± 2.87 g). Animals were divided into five groups of eight each. One group served as controls, and the other four were given one of the two test parabens at one of the two concentrations. At the end of 8 weeks, the rats were weighed and then decapitated. The weights of the testes, epididymides, prostates, seminal vesicles, and preputial glands were measured. Sperm counts in the testes and epididymides were made. Sperm cell stages were determined in a sectional analysis of each rat testis. The concentrations of testosterone, LH, and FSH in serum were measured.

The paraben intakes for the 0.1% and 1.0% diet groups were approximately 100 and 1000 mg/kg day^{-1}, respectively. Compared to controls, no statistically significant differences were found in any of the organ weights or the total body weights, sperm counts were not different, the distribution pattern of sperm developmental stages was not altered, and the serum levels of the hormones tested were not different. The author contrasted these findings with the adverse effects on sperm seen with orally administered Butylparaben and Propylparaben (Oishi 2004).

Uterotrophic Assays

p-Hydroxybenzoic Acid

Lemini et al. (1997) reported on the estrogenic effects of *p*-hydroxybenzoic acid in immature CD-1 mice and in ovariectomized CD-1 female mice. Subcutaneous injection of *p*-hydroxybenzoic acid was made at 5, 50, 500, and 5000 μg/kg for 3 consecutive days for the ovariectomized animals and the three highest doses for the immature animals. Estradiol (10 μg/kg) was used as a positive control and the corn oil vehicle was the negative control.

The authors found an apparent dose-dependent increase in the vaginal cornification (relative abundance of cornified cells) in both groups of animals, although only the 5000 μg/kg level was statistically significant in the immature females and only the 500 and 5000 μg/kg levels in the ovariectomized females. Uterine weights were statistically significantly increased at the 5000 μg/kg level in both groups. The authors concluded that sc administration of *p*-hydroxybenzoic acid produces an estrogen-like effect in CD-1 mice and that the effect is dose-dependent (Lemini et al. 1997).

The Central Toxicology Laboratory (CTL) performed a dose-setting study (Twomey 2000a) followed by a uterotrophic assay (Twomey 2000b) using immature CD-1 female mice.

In the dose-setting study, two female mice were administered 0.5 ml *p*-hydroxybenzoic acid per 100 g body weight by sc injection for 3 consecutive days. The stated dose levels in each of four treatment groups was 0.5, 5.0, 50.0, and 100.0 mg/kg day^{-1}. Clinical observations and animal body weights were recorded daily. No effects on body weights were noted and no clinical signs were observed. The authors concluded that these dose levels were suitable for a subsequent uterotrophic assay (Twomey 2000a).

In the uterotrophic assay (Twomey 2000b), immature female Alpk:AP$_f$CD-1 mice (20-21 days of age) received a single sc injection of *p*-hydroxybenzoic acid at each of the above dose levels for three consecutive days. Each treatment group consisted of 10 animals. A vehicle control (arachis oil) group and a positive control group (diethylstilbesterol at 0.01 mg/kg day^{-1}) were also included. Body weights were determined daily, along with clinical observations. At approximately 24 h after the last dose was administered, all animals were killed. Each uterus was removed and its blotted weight recorded.

As in the dose-setting study, no adverse clinical effect was noted and there was no effect on body weights or weight gain. Blotted uterus weights in animals administered diethylstilbesterol were significantly increased compared to controls, as expected. Uterus weights in animals administered *p*-hydroxybenzoic acid were significantly decreased compared to controls, although no dose-response was reported (Twomey 2000b).

Isobutylparaben

Darbre et al. (2002) reported that Isobutylparaben showed significant estrogenic activity in the mouse uterotrophic assay (1.2 or 12 mg per mouse, injected subcutaneously, once daily for 3 days (72 and 720 mg/kg day^{-1} equivalent)). The estrogenic potency, however, was low compared to estradiol. The authors concluded that branching of the alkyl chain (Isobutylparaben vs. Butylparaben) increases estrogenic activity.

Koda et al. (2005), in a study of the uterotophic effects of benzophenones and Isobutylparaben used in ultraviolet sunscreens, reported that Isobutylparaben increased rat uterine weights. Isobutylparaben was given by subcutaneous injection to ovariectomized female Crj:CD (SD) rats for 3 successive days at doses of 100, 250, and 625 mg/kg day^{-1}. On day 4, the rats were killed and the uteri removed. Wet weights were determined, followed by mincing, blotting, and dry weight determination. Both wet and dry uterine weights were increased, indicating estrogenic activity. Compared to 17β-estradiol, however, Isobutylparaben was 4,000,000 times less potent.

Benzylparaben

Darbre et al. (2003) reported on the estrogenic activity of Benzylparaben in the mouse uterine weight bioassay. When applied topically at 33 mg per mouse (2500 mg/kg day^{-1} equivalent), once daily for 3 days, estrogenic activity was reported, but not when applied daily at a 100-mg level. The estrogenic potency,

however, was low compared to estradiol and there was no dose response. The authors compared these findings with results reported by Byford et al. (2002) and concluded that estrogenic activity of parabens increases with the addition of an aryl group (Benzylparaben vs. Methylparaben).

Comparing Parabens

Routledge et al. (1998) used an in vivo (rat) uterotrophic assay and in vitro assays to examine the estrogenic effects of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. The uteri of immature rats were used as the source of cytosolic estrogen receptors in a competitive binding assay. A yeast system, in which activation of the human estrogen receptor was measured in terms of reporter gene (β-galactocidase) activity, was used to measure the presence of estrogens (positive findings were confirmed using an estrogen antagonist). Immature female A1pk:AP rats (21 to 22 days old, 38 to 55 g) and ovariectomized (at 6 to 8 weeks of age) rats of the same strain were used in a uterotrophic assay. Positive controls for comparison in the competitive binding assay were DES and 4-nonylphenol. Butylparaben did compete with [^{3}H]estradiol for binding to the rat estrogen receptor, but with an affinity 5 orders of magnitude lower than DES and between 10 and 100 times lower than 4-nonylphenol.

In the yeast system, 17β-estradiol and 4-nonylphenol were positive controls. Parabens were positive in this assay, but only at levels far higher than 17β-estradiol. The level at which induction of β-galactosidase began to increase was at molar concentrations of around 10^{-11} for 17β-estradiol, 5×10^{-7} for Butylparaben, 10^{-6} for Propylparaben and 4-nonylphenol, 10^{-5} for Ethylparaben, and 10^{-4} for Methylparaben. A negative control, p-hydroxybenzoic acid, did not induce β-galactocidase.

Methylparaben and Butylparaben were tested in the uterotrophic assay in vivo at a range of doses. Butylparaben was given either orally or by subcutaneous injection at doses of 4, 40, 200, 400, 600, 800, 1000, or 1200 mg/kg day^{-1} and Methylparaben at 40, 80, 400, or 800 mg/kg day^{-1} on each of 3 successive days. Estradiol was used as the positive control (at 0.4 mg/kg day^{-1} for oral gavage and 0.04 mg/kg day^{-1} for subcutaneous injection) and arachis oil as the negative control.

Methylparaben did not increase uterine weights at any dose level via any route of administration. Butylparaben given orally produced a small, not statistically significant, increase in wet and dry uterine weights at 800 and 1200 mg/kg day^{-1}. Subcutaneous doses of Butylparaben increased uterine wet weights at doses between 400 and 800 mg/kg day^{-1}, depending on the group studied. All 800 mg/kg day^{-1} groups had increased wet and dry uterine weights. The lowest level of Butylparaben that produced an effect (dry uterine weight increase) in any group was 200 mg/kg day^{-1} via subcutaneous injection (Routledge et al. 1998).

Hossaini et al. (2000) investigated the estrogenic activity of p-hydroxybenzoic acid, Methyl-, Ethyl-, Propyl-, and Butylparaben in the mouse (B6D2F$_1$ strain) and rat (Wistar strain) uterotrophic assays. Test compounds were dissolved in ethanol and then diluted with peanut oil to give a final ethanol concentration of 10%. In the mouse studies, oral doses of Methylparaben ranged from 1 to 1000 mg/kg day^{-1}; Propylparaben from 1 to 100 mg/kg day^{-1}; and a combination of Methyl-, Ethyl-, and Propylparaben at 100 mg/kg day^{-1}were given; and subcutaneous (s.c.) injection doses of Methyl-, Ethyl-, Propyl-, and Butylparaben and a combination of Methyl-, Ethyl-, and Propylparaben were given at 100 mg/kg day^{-1}. In mice, s.c. doses of p-hydroxybenzoic acid were given of 5 and 100 mg/kg day^{-1}. In the rat study, only Butylparaben was given, sc at doses of 100, 400, and 600 mg/kg day^{-1}.

These authors reported an increase in wet and dry uterine weights, but only at the 600 mg/kg day^{-1} Butylparaben dose, confirming the results of Routledge et al. (1998) of a weak estrogenic effect. In the mouse studies, no uterotrophic effect was reported for any of the parabens alone or in combination, either by oral or subcutaneous injection at levels up to 100 mg/kg day^{-1}, thus failing to confirm the positive findings of Lemini et al. (1997) reportedly at a 20× lower dose. The authors concluded that the parabens are not potent estrogens in vivo (Hossaini et al. 2000).

Lemini et al. (2003) reported the estrogenic activity of parabens in the uterotrophic assay using mice and rats. Immature CD1 female mice, ovariectomized adult CD1 female mice, and immature Wistar (IW) female rats were randomly assigned to different treatment groups. Treatment groups were administered (one subcutaneous injection per day for 3 days) one of the parabens in propylene glycol at doses equivalent to 0.36, 3.62, 36.2, 108, 362, or 1086 μmol/kg for the mice and 3.62, 36.2, 108, 362, or 1086 μmol/kg for the rats. Positive controls were administered estradiol at 10 μg/kg and vehicle controls received the vehicle alone. These authors calculated the relative uterotrophic effect (RUE), compared to estradiol at 100 and relative uterotrophic potency (RUP), compared to estradiol on a dose basis of each significant increase in uterotrophic weights.

Methylparaben produced a significant increase in uterine weights in immature CD1 mice at doses of 108 μmol/kg (RUE = 34; RUP = 0.096), 362 μmol/kg (RUE = 47; RUP = 0.021), and 1086 μmol/kg (RUE = 54; RUP = 0.006), but not at 0.36, 3.62, or 36.2 μmol/kg.

Ethylparaben produced a significant increase in uterine weights in immature CD1 mice at doses of 36.2 μmol/kg (RUE = 35; RUP = 0.289), 362 μmol/kg (RUE = 36; RUP = 0.027), and 1086 μmol/kg (RUE = 64; RUP = 0.005), but not at 0.36, 3.62, or 108 μmol/kg.

Propylparaben produced a significant increase in uterine weights in immature CD1 mice at doses of 108 μmol/kg (RUE = 54; RUP = 0.062), 362 μmol/kg (RUE = 51; RUP = 0.020), and 1086 μmol/kg (RUE = 66; RUP = 0.005), but not at 0.36, 3.62, or 36.2 μmol/kg.

Butylparaben produced a significant increase in uterine weights in immature CD1 mice at doses of 36.2 μmol/kg (RUE = 44; RUP = 0.229), 108 μmol/kg (RUE = 58; RUP = 0.057), 362 μmol/kg (RUE = 62; RUP = 0.016), and 1086 μmol/kg (RUE = 91; RUP = 0.003), but not at 0.36 or 3.62 μmol/kg.

Methylparaben produced a significant increase in uterine weights in ovariectomized CD1 mice only at a dose of 1086 μmol/kg (RUE = 46; RUP = 0.006).

Ethylparaben produced a significant increase in uterine weights in ovariectomized CD1 mice only at 108 μmol/kg (RUE = 60; RUP = 0.056), 362 μmol/kg (RUE = 68; RUP = 0.014), and 1086 μmol/kg (RUE = 85; RUP = 0.004).

Propylparaben produced a significant increase in uterine weights in ovariectomized CD1 mice only at doses of 108 μmol/kg (RUE = 61; RUP = 0.054), 362 μmol/kg (RUE = 55; RUP = 0.018), and 1086 μmol/kg (RUE = 91; RUP = 0.004).

Butylparaben produced a significant increase in uterine weights in ovariectomized CD1 mice only at 108 μmol/kg (RUE = 69; RUP = 0.081), 362 μmol/kg (RUE = 32; RUP = 0.032), and 1086 μmol/kg (RUE = 55; RUP = 0.006).

Methylparaben produced a significant increase in uterine weights in immature Wistar rats only at doses of 362 μmol/kg (RUE = 33; RUP = 0.029), and 1086 μmol/kg (RUE = 59; RUP = 0.006).

Ethylparaben produced a significant increase in uterine weights in immature Wistar rats only at 1086 μmol/kg (RUE = 62; RUP = 0.008).

Propylparaben produced a significant increase in uterine weights in immature Wistar rats only at doses of 362 μmol/kg (RUE = 58; RUP = 0.019) and 1086 μmol/kg (RUE = 65; RUP = 0.008).

Butylparaben produced a significant increase in uterine weights in immature Wistar rats only at 1086 μmol/kg (RUE = 67; RUP = 0.006).

The authors interpreted these data as supporting a no observed effect level (NOEL) that varied as a function of both the species and the chain length of the paraben, as shown in Table 22. Overall, the authors concluded that these data confirm the estrogenic effect of parabens (Lemini et al. 2003).

In another approach, Lemini et al. (2004) conducted an analysis of the physical measurement parameters of uteri from mice treated with parabens, vehicle alone, or estradiol. Groups of adult ovariectomized CD1 mice were administered Methylparaben (55 and 165 mg/kg), Ethylparaben (60 and 180 mg/kg), Propylparaben (65 and 195 mg/kg), and Butylparaben (70 and 210 mg/kg) s.c. on each of 3 consecutive days. The positive control, estradiol (10 μg/kg), and vehicle control (10 ml/kg polypropylene glycol) were given to other groups in the same fashion.

A day after the last exposure, animals were killed and uteri dissected, blotted, weighed, and fixed for morphological analysis. The uteri were cut into 7-μm transverse sections. Luminal epithelium heights, glandular epithelium heights, and myometrium widths were determined.

Morphometric results were given in bar graphs with numerical expressions of percent increase over the vehicle control and have been converted to a tabular format as shown in Table 23.

Overall, the authors concluded that this approach allows a determination if the utertrophic effect involves both the endometrium and the myometrium. They stated that the most relevant responses to Propylparaben and Butylparaben were seen in the endometrium height and myometrium width (Lemini et al. 2004).

TABLE 22
NOEL as a function of animal used in uterotrophic assay and of paraben chain length (Lemini et al. 2003).

	NOEL (mg/kg)		
Paraben	Immature CD1 mice	Ovariectomized CD1 mice	Immature Wistar rats
Methylparaben	5.5	5.5	16.5
Ethylparaben	0.6	6.5	60
Propylparaben	6.5	7.0	20
Butylparaben	0.7	6.0	21

Table 24 summarizes the results of the uterotrophic assays described above. Golden et al. (2005) provided the potency comparisons, except for Lemini et al. (2003, 2004); those values (the normative value of 100 for estradiol divided by the RUP) were taken from the studies themselves.

In Vivo Fish Assay

Comparing Parabens

Pedersen et al. (2000) used an in vivo fish assay to determine the estrogenic effects of Ethyl-, Propyl-, and Butylparaben. In this assay system, induction of the yolk precursor protein, vitellogenin, is the measure of a positive estrogenic effect. Juvenile rainbow trout (80 to 120 g) were given intraperitoneal injections of test compounds, always using a 1 ml/kg injection volume.

TABLE 23
Morphometric changes in mouse uteri exposed to parabens (Lemini et al. 2004).

		Morphometric parameter (% higher than control)		
Treatment	s.c. dose	GEH[a]	LEH[a]	MW[a]
Estradiol	10 μg/kg	60	153	88
Methylparaben	55 mg/kg	10	33	15
Methylparaben	165 mg/kg	20	87	38
Ethylparaben	60 mg/kg	30	80	48
Ethylparaben	180 mg/kg	30	106	43
Propylparaben	65 mg/kg	20	87	39
Propylparaben	195 mg/kg	10	110	43
Butylparaben	70 mg/kg	30	87	26
ButylParaben	210 mg/kg	40	113	35

[a]Glandular epithelium heights (GEH), luminal epithelium heights (LEH), and myometrium widths (MW).

TABLE 24
Summary of results of uterotrophic assays.

Chemical and study	Significant response in rats (route and dose)[a]	Significant response in mice (route and dose)[a]	Estradiol/chemical potency ratio[a]
p-Hydroxybenzoic Acid			
Lemini et al. 1997		Yes (s.c.; 5 mg/kg day^{-1})	1000
Hossaini et al. 2000	No (s.c.; up to 5 mg/kg day^{-1})	No (s.c.; up to 100 mg/kg day^{-1})	
Twomey 2000		No (s.c.; up to 100 mg/kg day^{-1})	
Methylparaben			
Routeledge et al. 1998	No (oral; up to 800 mg/kg day^{-1})		
	No (s.c.; up to 80 mg/kg day^{-1})		
Hossaini et al. 2000		No (oral; up to 1000 mg/kg day^{-1})	
		No (s.c.; up to 100 mg/kg day^{-1})	
Lemini et al. 2003	Yes (s.c.; 16.5 mg/kg day^{-1})		1041
		Yes (s.c.; 55 mg/kg day^{-1})	3448
Lemini et al. 2004		Yes (s.c.; 55 mg/kg day^{-1})	5000
		Yes (s.c.; 165 mg/kg day^{-1})	20000
Ethylparaben			
Hossaini et al. 2000		No (oral; up to 1000 mg/kg day^{-1})	
		No (s.c.; up to 100 mg/kg day^{-1})	
Lemini et al. 2003	Yes (s.c.; 6 mg/kg day^{-1})		346
		Yes (s.c.; 180 mg/kg day^{-1})	12,500
Lemini et al. 2004		Yes (s.c.; 60 mg/kg day^{-1})	3333
		Yes (s.c.; 180 mg/kg day^{-1})	25,000
Propylparaben			
Hossaini et al. 2000		No (oral; up to 100 mg/kg day^{-1})	
		No (s.c.; up to 100 mg/kg day^{-1})	
Lemini et al. 2003	Yes (s.c.; 20 mg/kg day^{-1})		1612–1851
		Yes (s.c.; 65 mg/kg day^{-1})	5263
Lemini et al. 2004		Yes (s.c.; 65 mg/kg day^{-1})	3333
		Yes (s.c.; 195 mg/kg day^{-1})	20,000
Butylparaben			
Routeledge et al. 1998	No (oral; up to 1200 mg/kg day^{-1})		
	Yes (s.c.; 600 mg/kg day^{-1})		15,000
Hossaini et al. 2000	Yes (s.c.; 600 mg/kg day^{-1})	No (s.c.; up to 100 mg/kg day^{-1})	6000
Lemini et al. 2003	Yes (s.c.; 7 mg/kg day^{-1})		436
		Yes (s.c.; 210 mg/kg day^{-1})	16,666
Lemini et al. 2004		Yes (s.c.; 70 mg/kg day^{-1})	5000
		Yes (s.c.; 210 mg/kg day^{-1})	11,111
Isobutylparaben			
Darbre et al. 2002		Yes (s.c.; 72 and 720 mg/kg day^{-1})	240,000–2,400,000
Koda et al. 2005	Yes (s.c; 100, 250, 625 mg/kg day^{-1})		4,000,000
Benzylparaben			
Darbre et al. 2003		Yes (topical; 2500 mg/kg day^{-1})	330,000–3,300,000

[a] All values from Golden et al. 2005, except for Twomey 2000, Lemini et al. 2003, 2004, and Koda et al. 2005.

Groups of 6 or 10 fish were injected at day 0 and 6 with one of the parabens, with 17β-estradiol as the positive control and *p*-hydroxybenzoic acid as the negative control. Butylparaben was dissolved in 48% ethanol and given at doses of 50, 150, and 200 mg/kg. All others were dissolved in DMSO. Propylparaben, Ethylparaben, and *p*-hydroxybenzoic acid were given at 100 and 300 mg/kg and 17β-estradiol at 1 mg/kg. Vitellogenin was determined in plasma using an enzyme-linked immunosorbent assay (ELISA).

At day 12, there was a statistically significant increase in vitellogenin in fish given 100 or 300 mg/kg Butylparaben (700× that seen at day 0) when compared to controls; the authors did

not report if the two dose levels were different from each other. Similar results were reported for Propylparaben (1000× that seen at day 0), but Ethylparaben only increased vitellogenin at the high dose level (60× that seen at day 0). The 17β-estradiol dose did increase the levels of vitellogenin by a factor of 150 and *p*-hydroxybenzoic acid had no effect at either dose (Pedersen et al. 2000).

Estrogen Receptor Binding

Isobutylparaben

Darbre et al. (2002) reported on the estrogenic activity of Isobutylparaben. The assays were similar to the study from the same laboratory (Byford et al. 2002) described later, absent the molecular modeling. Isobutylparaben was able to displace [^{3}H]estradiol from the estrogen receptor α (Erα) of MCF-7 cell cytosol (beginning at around 10^4 molar excess). In addition, these authors reported an increase expression of estrogen-regulated genes (at 10^{-6} to 10^{-5} M concentration) and an increase in the growth of two estrogen-dependent human breast cancer cell lines (also at 10^{-6} to 10^{-5} M concentration). The authors compared these results with the Byford et al. (2002) study and concluded that branching of the alkyl chain (Isobutylparaben versus Butylparaben) increases estrogenic activity.

Benzylparaben

Darbre et al. (2003) reported on the estrogenic activity of Benzylparaben. The assays were similar to the study from the same laboratory (Byford et al. 2002) described later, absent the molecular modeling. Benzylparaben was shown to displace [^{3}H]estradiol from the ERα of MCF-7 cell cytosol beginning at around 10^3 molar excess. In addition, these authors reported that Benzylparaben increased expression of estrogen-regulated genes (at 10^{-5} to 10^{-4} M concentration) and increased the growth of two estrogen-dependent human breast cancer cell lines (also at 10^{-6} to 10^{-5} M concentration). The authors compared these findings with previous results (Byford et al. 2002) and concluded that the estrogenic activity increases with the addition of an aryl group (Benzylparaben > Methylparaben).

Comparing Parabens

Satoh et al. (2000) determined the competitive binding of Butylparaben, Ethylparaben, Isobutylparaben, Isopropylparaben, Methylparaben, or Propylparaben to human estrogen receptor α (ERα) and ERβ. DES and bisphenol A were the positive controls. The relative binding affinity (RBA) was calculated as a ratio of the IC_{50} values of the test compound to DES and the values are shown in Table 25.

The IC_{50} values for DES were 1.6×10^{-8} M and 1.7×10^{-8} M, respectively, for ERα and ERβ and an arbitrary value of 100 was set for the RBA for DES. The authors concluded that parabens may be endocrine disrupters (Satoh et al. 2000).

Okubo et al. (2001) assessed the estrogen receptor–dependent estrogenic activity of Butylparaben, Ethylparaben, Isobutylparaben, Isopropylparaben, and Propylparaben in vitro using proliferation of human breast cancer (MCF-7) cells and expression of ERα and progesterone receptor (PR).

TABLE 25
Parabens relative binding affinities for human ERα and ERβ (Satoh et al. 2000).

	Relative binding affinity	
Compound tested	ERα	ERβ
Methylparaben	No binding detected	No binding detected
Ethylparaben	0.009 ± 0.002	0.009 ± 0.002
Propylparaben	0.029 ± 0.003	0.040 ± 0.004
Isopropylparaben	0.043 ± 0.004	0.044 ± 0.004
Butylparaben	0.068 ± 0.005	0.072 ± 0.006
Isobutylparaben	0.267 ± 0.027	0.340 ± 0.031
Bisphenol A	0.205 ± 0.025	0.155 ± 0.020
DES	100	100

Positive controls were DES and 17β-estradiol. Estrogen-sensitive MCF-7 cells were inoculated into 96-well culture plates, allowed to attach, decanted, and covered with phenol red–free Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% charcoal-dextran–treated human serum. Test compounds were dissolved in ethanol, diluted in phenol red–free DMEM, added to the wells, and the cells incubated for 6 days. Cell numbers were estimated using a fluorescence assay, which parallels the amount of nucleic acid. Total cellular RNA was isolated from treated cells and subjected to a reverse transcriptase–polymerase chain reaction (RT-PCR). A Western blot analysis was done using treated cells disrupted by sonication. Binding of parabens were determined using a commercial ERα and ERβ system in the presence of excess 17β-estradiol. Inhibition was calculated from absorbance values with and without 10^{-6} M DES.

Maximum MCF-7 cell proliferation with 17β-estradiol was seen at 3×10^{-11}M, and with DES around 10^{-9}M.

For Ethylparaben, Propylparaben, and Butylparaben, the peak was at 2×10^{-5}M, for Methylparaben, 2×10^{-4}M, and for Isopropylparaben and Isobutylparaben, 5×10^{-6} M.

RT-PCR amplification of RNA was performed only on cells treated with Butylparaben and Isobutylparaben, with 17β-estradiol as the positive control.

A decrease in ERα expression and a large increase in PR expression (25×) was seen with 17β-estradiol. Butylparaben and Isobutylparaben did not cause any ERα expression decreases up to 24 h, but did at 48 h; PR expression increases were 4× and 5× at 24 and 48 h, respectively. The Western blot assay identified that 17β-estradiol had the expected effect of a large decrease in ERα compared to the control and Butylparaben and Isobutylparaben had a small decrease.

The RBAs of parabens calculated in this study are given in Table 26.

TABLE 26
Relative binding affinity of parabens to human ERα and ERβ (Okubo et al. 2001).

	Relative binding affinity	
Compound tested	ERα	ERβ
Ethylparaben	0.011	0.011
Propylparaben	0.033	0.044
Isopropylparaben	0.040	0.054
Butylparaben	0.053	0.123
Isobutylparaben	0.110	0.093
DES	100	100

The authors stated that all parabens examined stimulated MCF-7 cell growth, affected expression of ERα and PR, and bound to ERα and ERβ. Parabens with longer and branched alkyl chains were more potent than those with short and straight chains. Because ERβ was not detected in the RT-PCR or Western blot assay, the authors suggested that ERα was primarily expressed in MCF-7 cells.

The authors speculated that parabens could have an endocrine disruption function (Okubo et al. 2001).

Byford et al. (2002) reported results of a study of the estrogenic effects of parabens in MCF-7 cells. A series of assays were performed: (1) competitive ERα binding between [2,4,6,7-^{3}H]estradiol and 1× to 10^6× molar excess of Methyl-, Ethyl-, Propyl-, or Butylparaben; (2) up regulation of an estrogen-responsive reporter gene transfected into MCF-7 cells; (3) regulation of expression of the *pS2* gene (also estrogen-regulated); and (4) cell proliferation. With some minor differences, preparation and treatment of MCF-7 cells was the same as described in the above study.

Cell proliferation was weakly stimulated by 10^{-5}M Methylparaben, with stronger stimulation at 5×10^{-5}M and higher. Ethylparaben was a weak stimulant at 5×10^{-6}M and 10^{-5}M, but stronger at 5×10^{-5}M and higher; at 10^{-4}M, Ethylparaben had the same stimulation as 17β-estradiol at 3×10^{-11}M. Propylparaben was a weak stimulant at 10^{-6}M, but stronger at 5×10^{-6}M and higher; Propylparaben at 5×10^{-5}M was indistinguishable from 17β-estradiol at 3×10^{-11}M. Butylparaben had a stimulant effect at 10^{-6}M, but was stronger at 5×10^{-6}M and higher; Butylparaben at 10^{-5}M was indistinguishable from 17β-estradiol at 3×10^{-11}M. Inclusion of the antiestrogen ICI 182,780 reversed the effect of 17β-estradiol and each of the parabens, suggesting that the parabens stimulation of MCF-7 cell proliferation is an estrogenic phenomenon.

The results of the other assays were consistent with these findings. 17β-Estradiol eliminated [2,4,6,7-^{3}H]estradiol binding to ERα in MCF-7 lysates at a molar excess of 10:1. Tamoxifen had the same effect at a molar excess of 1000:1. As a negative control, dexamethasone had no effect up to a molar excess of 10^5:1. Propyl- and Butylparaben began to reduce the binding of labeled estradiol at a molar excess of 10^4:1, a pattern that continued with addition of more paraben; at a molar excess of 10^6:1, however, there was still on the order of 20% binding of the radiolabeled estradiol. Methyl- and Ethylparaben did not affect binding until a molar excess of 10^5:1 was reached.

Up-regulation of an estrogen-responsive reporter gene transfected into MCF-7 cells appeared to be less consistent a measure of estrogenic potential of parabens. Over 24 h, Methylparaben up to 10^{-4}M had no effect, but 10^{-4}M did up regulate over 7 days. Over 24 h, Ethylparaben up regulated at 10^{-5} and 10^{-4}M, but only at 10^{-4}M over 7 days. Over 24 h, Propylparaben up regulated at 10^{-5} and 10^{-4}M, but only at 10^{-5}M over 7 days—the 10^{-4}M concentration appeared to down-regulate expression. Over 24 h, Butylparaben up-regulated at 10^{-5}M, but not at 10^{-4}M, and over 7 days, Butylparaben up-regulated at 10^{-5}M, but again not at 10^{-4}M. Each of the parabens also had the effect of up-regulating *pS2* as determined by Northern blotting in which *pS2* mRNA levels were increased, but this assay did not yield quantitative results.

These authors also used molecular modeling of the ERα ligand-binding domain (LBD) using the crystal structure from the Brookhaven Protein Database. Of the three dimers in the asymmetric structure, the one with the A and B subunits was selected and modified to remove duplicate residues remote from the LBD and complexed 17β-estradiol, but to retain all water molecules and add nonpolar hydrogens. Paraben ligands were placed within the LBD site with their phenolic hydroxyl group taking the position formerly occupied by the phenolic hydroxy group of the 17β-estradiol. Energy minimization were carried out on both protein and ligand with a nonbonded interaction energy cut-off of 15 Å. A dielectric constant of 1.0 was used. The interaction energies of the protein ligand complexes were calculated as the energy of the complex minus the individual energies of the protein and the paraben. Parabens with alkyl chain lengths up to 10 were used in the model, as were combinations of two ligand molecules. The resulting interaction energies were all negative, implying to the authors that there was no steric hindrance in accommodating these ligands in the LBD (Byford et al. 2002).

Lemini et al. (2003), in their uterotrophic assay in mice and rats, also measured the relative binding affinities of parabens to estradiol. These data are given in Table 27. The authors stated that these results are in agreement with those of Byford et al. (2002) noted above.

Pugazhendhi et al. (2005) extended the work of Okubo et al. (2001) and Byford et al. (2002) by examining the activity of *p*-hydroxybenzoic acid compared to Methylparaben using a competitive binding assay in MCF-7 (human breast cancer) cell lysates, regulation of expression of an estrogen-responsive reporter gene (ERE-CAT) in MCF-7 cells, and proliferation of estrogen-dependent MCF-7 and ZR-75-1 cells (another human breast cancer cell line).

TABLE 27
Relative binding affinities (RBAs) of parabens in competing for estradiol receptor sites (Lemini et al. 2003).

	RBA
Estradiol	100
Methylparaben	Did not compete with estradiol
Ethylparaben	1.2×10^{-3}
Propylparaben	3.5×10^{-3}
Butylparaben	1.5×10^{-3}

In a competitive binding assay, a MCF-7 cell lysate was incubated with 16×10^{-10} M 17β-estradiol. Increasing concentrations of Methylparaben demonstrated that a 43.3% inhibition of estradiol binding could be obtained with a 2.5×10^6-fold molar excess of Methylparaben. A 66.7% inhibition required a 5.0×10^6-fold molar excess of Methylparaben and a 71.5% inhibition required a 1.0×10^7-fold molar excess. Increasing concentrations of *p*-hydroxybenzoic acid demonstrated that a 53.9% inhibition of estradiol binding could be obtained with a 5.0×10^6-fold molar excess of Methylparaben. A 98.7% inhibition required a 1.0×10^7-fold molar excess of *p*-hydroxybenzoic acid.

CAT gene expression was stimulated 2-fold by 17β-estradiol at 10^{-8} M and higher concentrations. CAT gene expression was stimulated 1.5-fold by Methylparaben at 10^{-3} M (highest concentration tested) and 1.25-fold by *p*-hydroxybenzoic acid at 5×10^{-4} M (highest concentration tested).

In an MCF-7 cell proliferation assay in which the culture medium was changed every 24 h, *p*-hydroxybenzoic acid resulted in a small (less than a doubling) but statistically significant increase in proliferation at 10^{-6}, 10^{-5}, and 10^{-4} M, but not at 10^{-7} or 10^{-3} M. More than a 10-fold increase in cell proliferation was seen with 17β-estradiol at 10^{-8} M. The authors noted that the results using MCF-7 cells were not reproducible when the culture medium was changed every 3 to 4 days. Using ZR-75-1 cells, *p*-hydroxybenzoic acid was tested at the same concentrations, with medium changes every 24 h, but only 10^{-5} M *p*-hydroxybenzoic acid caused a small increase in cell proliferation, compared to a 10-fold increase in cell proliferation seen with 17β-estradiol at 10^{-8} M.

The authors interpreted the findings as indicative of estrogenic activity of *p*-hydroxybenzoic activity in these assays (Pugazhendhi et al. 2005).

Androgen Receptor Binding

Fang et al. (2003) described a recombinant androgen receptor (AR) competitive binding assay and used the results to survey 202 chemicals, including Methylparaben and Propylparaben. A radiolabeled competitor molecule was combined with radiolabeled 17α-methyl-[^{3}H]methyltrienolone and the androgen receptor protein in a test tube on ice. Incubation for 18 to 20 h was followed by adding a hydroxylapatite (HAP) slurry (60% HAP and 40% Tris buffer). The HAP pellet was alcohol extracted and counted. The AR binding affinity was expressed as the relative binding affinity (RBA), which was the inhibitory concentration for 17α-methyl-methyltrienolone divided by that for the test chemical, expressed as a percent. The RBA of 17α-methyl-methyltrienolone was, by definition, 100%. The RBA for Propylparaben was 0.0010%. No RBA was reported for Methylparaben because it was found to be a nonbinder.

The authors interpreted these findings in terms of the structure/activity relationship between the androgen receptor and each class of chemicals. For the phenols, they stated that the RBA for the androgen receptor correlated positively with the octanol/water partition coefficient. The authors noted that some of the phenols that are typical ER receptor ligands are also active in AR binding (Fang et al. 2002).

Reporter Cell Lines

Comparing Parabens

Gomez et al. (2005) used three reporter cells lines: HELN cells, which are ER negative; HELNERα cells, which express ERα; and HELNERβ cells, which express ERβ, to determine the binding of various parabens. These cell lines were derived from HeLa cells by transfection with the appropriate plasmid. Nonspecific binding was measured by interference with luciferase production.

At concentrations up to 10^{-5} M, *p*-hydroxybenzoic acid, Methylparaben, Ethylparaben, and Propylparaben had no effect on nonspecific binding. Butylparaben, however, beginning at 3×10^{-6} M did exhibit nonspecific binding as measured by a decreased luciferase production.

Specific binding to ERα in HELNERα cells and ERβ in HELNERβ cells was determined. At concentrations up to 10^{-5} M, *p*-hydroxybenzoic acid and Methylparaben had no effect. At 10^{-5} M, the ranking of effect was Butylparaben > Propylparaben > Ethylparaben. There was no difference between binding to ERα or ERβ (Gomez et al. 2005).

Gene Expression Profiling

Comparing Parabens

Terasaka et al. (2006) used expression of estrogen-responsive genes to examine the estrogenic activity of parabens and other phenols. Based on a determination of up-regulation or down-regulation by estrogen, a DNA microarray assay system (Info-Genes, Tsukuba, Japan) was prepared containing 172 estrogen-responsive genes (108 up-regulated and 64 down-regulated) and 31 calibration/expression markers.

MCF-7 cells (human breast carcinoma cell line) were cultured for 3 days and then treated with estrogen at 10 nM concentration or 10 μM of Methylparaben, Ethylparaben, Propylparaben, or Butylparaben for 3 days. mRNA was extracted and purified. A 2-μg aliquot from the control and each test sample was labeled using Cy3 and Cy5 dyes, respectively. Both Cy3 and Cy5 labeled probes were mixed and denatured and hybridized with the

prepared DNA microarray. Image analysis was used to determine the ratio of Cy3 and Cy5 signal from each spot in the microarray. Data from spots with poor hybridization were removed from the data processing.

Although the set of 172 estrogen-responsive genes had been devised, these authors further narrowed the selection to 120 genes that more reliably (in the view of the authors, based on reproducibility as influenced by mRNA stability, background level, cross-hybridization) responded to the phenol group of chemicals while showing differences between them.

Using the binding of mRNA from treated and control MCF-7 cells to DNA from these 120 optimal genes, the authors prepared scattergrams that depicted the response of each phenol and estrogen. A correlation coefficient (R) for each test compound was calculated on the basis of linear regression and the statistical significance of the correlation. For Methylparaben the R value was negative (0.21); and positive for Ethylparaben, 0.19, Propylparaben, 0.74, and Butylparaben, 0.60. Of these results, significant correlations were observed for Propylparaben and Ethylparaben only. The authors further noted that the profiles of Propylparaben and Ethylparaben were closer to each other than were the profiles of either compared to estrogen and suggested that the expression of genes specific to parabens is contributing to the profiles.

Endocrine Disruption

Overview

Harvey and Johnson (2002) suggested approaches to the assessment to toxicity data with end points related to endocrine disruption. They noted that studies of endocrine disruption have proliferated, with most relating to estrogenic effects. The authors suggested that all glands, tissues, receptors, transporter proteins, and enzymes that comprise the endocrine system should be considered as targets and toxicity evaluated using a weight-of-evidence approach considering all available data. Although structure-activity relationships and in vitro/in vivo screens provide useful data, the authors asserted that repeated-dose studies with defined end points will provide the most powerful tools for hazard assessment. The authors noted that the Scientific Committee on Toxicity, Ecotoxicity, and the Environment of the European Commission has stated its view that toxicity of the endocrine system should be considered in the same way as other target organs and that *endocrine disruption*, per se, is not a toxic end point in itself, but is a mechanism by which toxic effects may occur. Harvey and Johnson (2002) argued that knowledge of the toxic effect, the no observed effect level, and reversibility are as important as identifying the inherent hormone-like property.

Carcinogenesis or reproductive/developmental toxicity are end points, according to these authors, that are of importance, regardless of whether caused by an endocrine disrupter or not, but that endocrine effects may help identify mechanism of action. They also noted that endocrine effects are usually reversible upon removal of treatment/stimulation.

These authors further suggested that consideration be given to exposure issues, including the possibility that the effects of very low potency estrogens may be irrelevant at low exposures. They noted that endocrine disruption may not always be the critical or most sensitive end point for a given chemical; e.g., again for low potency estrogens, if endocrine effects occur only at extreme doses, then it may be that other system toxicity may be the critical toxicity in hazard evaluation (Harvey and Johnson 2002).

Foster (2004) presented an overview of endocrine active chemicals in which he discussed the major hypothesis proposed for the effects of endocrine active agents on human reproduction and development. He also commented on the use of animal surrogates to help test the hypothesis and establish biological plausibility and the strengths and weaknesses of current and proposed testing methods and potential improvements.

Endocrine active chemicals may be

- estrogens or estrogen mimics, in which case the safety concern relates to acceleration in puberty, fertility, pregnancy/birth, and female reproductive target tissues (ovary, uterus, and breast);
- (anti-)androgen-like, with concerns regarding birth defects in males, delays in puberty, fertility, and male reproductive target tissues (prostate and testis);
- anti-thyroid-like, with concerns about retarded growth, central nervous system (CNS) effects, and hearing defects.

The safety concerns are significant because of increases in prevalence of human male reproductive disorders, including decreases in sperm parameters, increases in testicular maldescent or other genitalia problems, and increased incidence of testicular cancer (germ cell derived). These end points may be linked to a critical period in utero and the perinatal period as a result of fetal hormone action. During pregnancy weeks 7 to 8, the sexually indifferent fetus experiences a window of hormone susceptibility. Development as a female is largely hormone-independent, but development as a male is entirely hormone-dependent. For example, the male-determining gene, SRY, controls differentiation of Sertoli cells, which in turn influence Leydig cell proliferation and production of testosterone. Testosterone influences testicular descent and masculinization of the internal and external genitalia, etc. Sertoli cells also influence sperm production in adult life and regression of Mullerian (female) ducts.

Pharmaceuticals, such as diethylstilbesterol and progesterone analogues, are understood to disturb reproduction and development in humans, but there is esentially no evidence that environmental endocrine active chemicals can cause similar responses. Possible explanations for the absence of findings include low concentrations that never reach the threshold needed to trigger adverse responses and the low power that epidemiology studies have to detect and link prenatal exposure with an outcome that may only manifest in the adult.

As a consequence, animal surrogates have been used. In the case of Dibutyl Phthalate, for example, end points that suggest adverse effects include anogenital distance reduction (feminization); nipple development (areolae retention), immature testis and epididymis, hypospadias, and testicular lesions. Dibutyl Phthalate, however, does not interact with the androgen receptor. Considering all end points, the lowest no observed adverse effect level (NOAEL) was stated to be 50 mg/kg day^{-1} (from a gavage study). Comparing this to a maximal level of exposure to Dibutyl Phthalate from all sources of women of childbearing age of 113 μg/kg day^{-1} results in a safety factor of almost 500.[*Note:* The Cosmetic Ingredient Review (CIR) Expert Panel, in its re-review of Dibutyl Phthalate, considered the same 50 mg/kg day^{-1} NOAEL, and combined it with a Dibutyl Phthalate exposure of 9.13 μg/kg day^{-1} from use of cosmetics—with a margin of safety of around 5000 (Andersen 2005).]

It was noted that other phthalate esters have effects similar to Dibutyl Phthalate, including Diethylhexyl Phthalate and Butylbenzyl Phthalate, but that other phthalate esters have no endocrine activity at all, including Diethyl Phthalate and Dimethyl Phthalate.

Linuron, a herbicide structurally related to flutamide, was presented as another endocrine active chemical. Animal testing has identified increased resorption sites (at maternally toxic levels), but no birth defects. In a three-generation reproduction study, decreased weight gain in F_0 males, females, and offspring were seen, along with reduced pup survival. In a two-generation reproduction study, no effects on fertility were seen, but tubular atrophy in the testis and epididymal inflammation were found. Overall, Linuron was considered a weak, competitive, androgen receptor antagonist, with effects seen in multigenerational studies where all offspring were examined.

Reiterating the absence of human data that can identify adverse effects associated with endocrine active chemicals, it was stated that animal studies are necessary. It is critical that such studies, themselves, be designed to maximize the likelihood that adverse effects will be detected (Foster 2004).

Government Programs

The Environment Directorate General of the European Commission organized a European workshop on endocrine disrupters in June 2001. The workshop highlighted the potential effects of endocrine disrupting chemicals on human health and wildlife as an issue of increasing concern to all sectors of society. It was stated that a significant number of questions still need to be answered and the challenge facing regulators, industry, and academia is how these can be resolved in a rapid cost-effective manner whilst still providing scientifically robust outputs. The workshop recommended information exchange and international coordination, identified research and development needs, suggested needed test methods development and testing strategies, and establishment of monitoring programs, but no short-term action regarding specific chemicals was suggested (Report of the European Workshop on Endocrine Disrupters 2001).

The U.S. Environmental Protection Agency (EPA) has established an Endocrine Disrupter Methods Validation Subcommittee of the National Advisory Council for Environmental Policy and Technology to address the development and refinement of assays to reduce animal use, refine procedures involving animals to make them less stressful, and replace animals where scientifically appropriate (EPA 2006a). When complete, EPA will use these validated methods or assays to identify and characterize the endocrine activity of pesticides, commercial chemicals, and environmental contaminants, specifically in relation to estrogen, androgen, and thyroid hormones. In addition, EPA is working with the Organization for Economic Cooperation and Development's Endocrine Testing and Assessment Task Force to validate and harmonize endocrine screening tests of international interest. EPA's Web site lists a table of the current status of the development and validation of endocrine disrupter assays (EPA 2006b).

FDA's National Center for Toxicological Research has established an Endocrine Disrupter Knowledge Base (EKDB). A computer model has been validated for predicting estrogen receptor binding for estrogenic or estrogen-like compounds and data are available for 791 chemicals, but do not include the parabens (FDA 2008).

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

Comparing Parabens

In a review article, Sokol (1952) described a study in which Methylparaben, Ethylparaben, Propylparaben, and Butylparaben were each applied to the backs of 50 humans at concentrations of 5%, 7%, 10%, 12%, and 15% 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%; Ethylparaben, 7%; Propylparaben, 12%; and Butylparaben, 5%. Higher concentrations produced some evidence of irritation. In a repeated-insult patch test (RIPT), each paraben at the "no effect" concentration above was applied to the skin of 50 subjects (25 males/25 females) for 4 to 8 h every other day for 3 weeks (10 applications). Following a 3-week rest, the materials were reapplied at induction concentrations for 24 to 48 h. No sensitization was reported.

Hjorth and Trolle-Lassen (1963) 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. In further testing in 32 patients, using

TABLE 28
Cross-sensitivity between paraben esters (Hjorth and Trolle-Lassen 1963).

			Methylparaben		Ethylparaben		Propylparaben	
			Positive	Negative	Positive	Negative	Positive	Negative
		Cases[a]	21	11	27	5	22	9
Ethylparaben	Positive	27	18	9	—	—	—	—
	Negative	5	3	2	—	—	—	—
Propylparaben	Positive	22	15	7	20	2	—	—
	Negative	9	5	4	7	2	—	—
Benzylparaben	Positive	14	10	4	12	2	13	1
	Negative	17	16	7	15	2	9	8

[a] 32 cases tested with 5% paraben esters in petrolatum or in equal parts Eucerin and water; one case was not tested with Propylparaben or Benzylparaben.

parabens at 5% in petrolatum or in Eucerin and water to determine cross-reactions, about two thirds of the patients sensitive to one of the paraben esters also reacted to one or several other esters. These data are shown in Table 28.

Wuepper (1967) also reported cross-reactivity to parabens. Four patients with known paraben sensitivity were patch-tested with Methylparaben, Ethylparaben, Propylparaben, and Butylparaben (5% in petrolatum). In addition, three of these patients were patch-tested with 0.1% and 1% of each paraben and 0.1%, 1%, and 5% *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%; only one patient reacted at 0.1%. One patient had positive reactions to intradermal and topical *p*-hydroxybenzoic acid.

Marzulli et al. (1968) reported results of an RIPT 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 h; 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% sodium lauryl sulfate (SLS) under occlusion for 24 h, followed by application of parabens for 48 h. Five such cycles were used for induction. Following a 2-week rest, the test mixtures were reapplied under 72-h challenge patches. On one skin site in all subjects, 10% SLS was applied for 1 h before challenge application. At another site, no SLS was used.

Results are summarized in Table 29. The authors concluded that sensitization to parabens is not a problem in the United States where these compounds are used at 0.1% to 0.3% in topical medicaments (Marzulli et al. 1968).

Evans (1970) observed that, in most cases, individuals who are sensitive to parabens have chronic dermatoses that may be in continual contact with these ingredients. Fisher (1971) stated that the incidence of paraben contact sensitization in healthy Americans is low, considering the extensive use of these materials, and concluded that topically applied parabens do not pose any significant hazard to the public. Marzulli and Maibach (1973) reaffirmed this conclusion.

Pevny and Glassl (1971) reported on a new test method for sensitization in the oral mucosa. Methylparaben and Ethylparaben, in increasing concentrations, were studied for their effect on the oral mucous membrane of 39 subjects. They described toxic limit concentrations for Methylparaben and Ethylparaben of 5% and 10%, respectively. One subject had a reaction of the oral mucous membrane to Methylparaben.

TABLE 29
Methylparaben and Propylparaben mixture sensitization results (Marzulli et al. 1968).

	Number sensitized to challenge	
Concentration of mixture in petrolatum at induction	Without SLS at challenge	With SLS at challenge
0.2% Methylparaben + 0.05% Propylparaben	0/102	0/102
1.0% Methylparaben + 0.25% Propylparaben	0/101	0/101
5.0% Methylparaben + 1.25% Propylparaben	1/98	1/98
10% Methylparaben + 10% Propylparaben	0/74	0/74
10% Methylparaben + 10% Propylparaben + 5% SLS pretreatment	0/22	—

Thune and Granholt (1975) administered 37 patients with recurrent urticaria a tablet orally 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. Larson (1977) stated 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.

Fisher (1979) coined the term "paraben paradox" in which paraben-sensitive patients who present 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, including the eyelid. He concluded that 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.

Hegyi (1979) noted a tendency toward increased incidence in paraben contact allergy in Europe over time. From 1968 to 1972, a 0.3% incidence of paraben sensitization was reported. From 1973 to 1977, the incidence increased to 1.5%.

Table 30 summarizes results of patch tests of parabens on patients with and without skin problems reported between 1962 and 1982. The studies by Cramer and Unrein (1963) and Maucher (1974) in Table 30 involved patients with high sensitivity toward "*para*-agents," a group of compounds in which parabens are considered a member.

Adams and Maibach (1985) reported a five-year study of cosmetic reactions. Of the 713 patients with cosmetic related reactions, 554 were reported to have no chronic skin disease, 115 had atopic dermatitis or a history of same, and 36 patients had a history of other chronic dermatitis. Patch testing identified 19 individuals with positive reactions to unspecified parabens.

Menne and Hjorth (1988) reported on results of routine patch testing with paraben esters. In 8020 patients tested consecutively with a paraben mixture in a standard series, 76 individuals reacted positively. Testing with the individual parabens was used to confirm the result, to reduce the chance that the result was due to excited skin syndrome. The authors stated that the parabens are weak sensitizers, and around 1% sensitization should be expected.

Menne et al. (1991) published a multicenter study of reactions to Methylchloroisothiazolinone/Methylisothiazolinone (MCI/MI) preservative in which they presented data on 4713 patients seen in 22 European clinics and patch-tested with MCI/MI, nickel, formaldehyde, and parabens. There was a variation from 0% positive reactions to parabens to 4.7%. Overall there were 51 positive reactions to parabens, for an overall percentage of 1.08%.

Goh and Yuen (1994) reported patch test results in 274 metal workers (180 male, 94 female) with dermatitis. One female worker had a positive patch test to paraben mix (3% each of Benzylparaben, Butylparaben, Ethylparaben, Methylparaben, and Propylparaben).

On the supposition that individuals sensitized to parabens in topical medications and cosmetics may experience flares of dermatitis from parabens in food and systemic medications, Veien et al. (1996) reported on oral challenge with parabens in paraben-sensitive patients. A placebo-controlled oral challenge with a mixture of 100 mg of Methylparaben and 100 mg of Propylparaben was performed in 14 patients with a positive patch test to paraben-mix. Two of the 14 patients had flares of their dermatitis after challenge with oral parabens, but not the placebo. One patient had a flare at a paraben patch test site on the back. The other 11 patients had no reaction to the oral challenge. The two patients with flares of their dermatitis were instructed regarding foods to avoid. At follow-up after attempts to avoid these foods for 1 to 2 months, no improvement in their dermatitis was seen.

Schnuch et al. (1998) presented the results of a 5-year multicenter study (24 allergy departments) of patch testing with preservatives, antimicrobials, and industrial biocides. Patch testing procedures were not identical across the range of facilities.

Patch test data from 22,602 patients tested with paraben-mix (15% in petrolatum) in the standard series indicated 364 positives (1.6%). In women, the figure was 1.5%, and in men, 1.6%. No difference in reactions was seen up to 60 years of age, but a jump in percentage of positive reactions was seen in the 60- to 80-year-old group and in the >80 age group (Schnuch et al. 1998).

Lestringant et al. (1999) reported on allergic contact dermatitis in the United Arab Emirates. From 1989 to 1996, 373 patients (143 men and 230 women) presented with possible contact allergies and were patch-tested at a single dermatology clinic. Positive patch test results to paraben-mix were seen in 4 men (2.8%) and 15 women (6.5%) for an overall frequency of 5.1%.

Wilkinson et al. (2002) reported a 10-year overview of results of preservatives patch testing. The percentage of positive reactions was relatively flat over this time period, varying between 0.5% and 1%. This may be compared with the frequency of reactions to Methyldibromo Glutaronitrile, which steadily increased in Europe from just under 0.5% in 1991 to 3.5% in 2000.

In a review of hypersensitivity to preservatives, Sasseville (2004) stated that the rate of sensitization to parabens has remained remarkably constant over the years.

North American Contact Dermatitis Group Patch Test Results

Storrs et al. (1989) reported the prevalence of allergic reactions in patients with suspected allergic contact dermatitis who were tested with 19 vehicle and preservative allergens. Of the 661 patients tested from 1984 to 1985 with paraben mix (12% in petrolatum—3% each of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben), there were seven allergic reactions, three doubtful allergic reactions, and three irritant reactions.

Marks et al. (1995) updated the North American Contact Dermatitis Group standard tray patch test results. Parabens mix (15% in petrolatum—3% each of Methylparaben, Ethylparaben,

TABLE 30
Results of paraben patch tests 1962–1982.

Ingredient	Concentration	Number of subjects	Previous sensitivity or dermatitis	Procedure	Positive reactions	Percentage positives	Reference
Paraben-mix[a]	14%	5799	Yes	Patch test	—	1.13%	Hjorth and Trolle-Lassen 1962
Ethylparaben	5%					1.15%	
Ethylparaben	1%	210	Yes	Standard epicutaneous test	43	20.5%	Cramer and Unrein 1963
Paraben-mix[a]	1%	160	No		0	0%	
Paraben-mix[a]	5% in petrolatum	30	No	Patch test	0	0%	Schorr and Mohajerin 1966
Methylparaben, Ethylparaben, Propylparaben	15% in kaolin (5% each)	91	Yes	Patch test	4	4.4%	Wuepper 1967
Paraben-mix[a]	5% in petrolatum	273	Yes	Patch test	2	0.8%	Schorr 1968
		260	No		0	0%	
Methylparaben, Ethylparaben, Propylparaben	15% in petrolatum (5% each)	100	Yes	Patch test	3	3%	Fisher 1971
Paraben-mix[a]	12% in petrolatum (3% each)	2000	Yes	24-h patch	—	1.3% males	Bandman et al. 1972
		2000	Yes	48-h patch	—	2.3% females	
Paraben-mix	15% in paraffin (3% each)	1000 (477 males, 523 females)	Yes	Patch test	4 males, 6 females	0.84% males, 1.15% females	Cronin 1972
Paraben-mix	15% in petrolatum	2061	Yes	Patch test	44	2.1%	North American Contact Dermatitis Group (NACDG) 1972
Methylparaben, Ethylparaben, Propylparaben	15% in petrolatum (5% each)	1200	Yes	48-h patch	38	3%	NACDG 1972
Methylparaben, Ethylparaben, Propylparaben[a]	2% in lanolin	148	Yes	Patch test	45	30.4%	Maucher 1974
Paraben-mix[a]	15% in petrolatum	4097	Yes	24-h chamber	14	0.3%	Hannuksela et al. 1976

TABLE 30
Results of paraben patch tests 1962–1982. *(Continued)*

Ingredient	Concentration	Number of subjects	Previous sensitivity or dermatitis	Procedure	Positive reactions	Percentage positives	Reference
Methylparaben, Ethylparaben, Propylparaben	30% in petrolatum (10% each)	4825	Yes	24-h patch	91	1.9%	Marzulli and Maibach 1976
Paraben-mix[a]	15% in paraffin	1312 (603 males, 709 females)	Yes	48-h patch	18 males, 13 females	3% males, 1.86% females	Husain 1977
Paraben-mix[a]	15%	192	Yes	48-h chamber	7	3.6%	Fraki et al. 1979
Methylparaben, Propylparaben	1%	60 (14 males, 46 females)	Yes	Patch test	7	11.7%	Jenni and Zala 1980
Paraben-mix[b]	15% in petrolatum	1862 (716 males, 1146 females)	Yes	Patch test	40	2.1%	NACDG 1980
Paraben-mix[a]	15% in petrolatum	4600	Yes	Patch test	57	1.24%	Romaguera and Grimalt 1980
Paraben-mix[a]	15% in petrolatum	465	Yes	Patch test	9	1.5	Meynadier et al. 1982

[a]Paraben-mix not described.
[b]3% each of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben.

Propylparaben, Butylparaben, and Benzylparaben) had 2.3% positive test results in 3476 patients tested from 1992 to 1994. This was compared to a positive reaction response of 1.3% in patients tested from 1989 to 1990.

In 3086 patients tested from 1994 to 1996 (Marks et al. 1998), there were 1.8% positive allergic reactions to parabens mix (15%–3% each of Methylparaben, Ethylparaben, Propylparaben, Butylparaben, and Benzylparaben).

In 4096 patients tested from 1996 to 1998 (Marks et al. 2000), there were 1.7% positive allergic reactions to parabens mix (12%–3% each of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben).

In 5803 patients patch-tested between 1998 and 2000 (Marks et al. 2003), there were 1.0% positive reactions to parabens mix (12%–3% each of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben).

In 4898 patients patch-tested from 2001 to 2002 (Pratt et al. 2004), there were 0.6% positive allergic reactions to parabens mix (12%–3% each of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben).

Product Irritation and Sensitization Testing

A number of product formulations containing parabens at concentrations of 0.1% to 0.8% have been tested for human skin irritation. These studies are summarized in Table 31.

Several product formulations containing parabens have been tested for skin sensitization. Tests include: Schwartz-Peck prophetic patch tests on product formulations containing 0.2% Methylparaben and 0.1% Propylparaben, or 0.2% Butylparaben; Draize-Shelanski repeated-insult patch tests on product formulations containing 0.1% to 0.8% Methyl-, Propyl-, Butyl-, and/or Ethylparaben; and Kligman maximization tests on product formulations containing 0.2% Methylparaben and 0.1% Propylparaben. The results and other details of these studies are summarized in Table 32.

Photocontact Sensitization

Multiple Parabens

Each of four products containing 0.2% Methylparaben and/or 0.2% Propylparaben were tested for evidence of photo-induced contact sensitization in 27 to 30 subjects (Food and Drug Research Labs 1978a, 1978b, 1979, 1980). 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. About 0.2 ml of the test material was applied under an occlusive patch for 24 h.

The irradiated test site was subjected to nonerythrogenic ultraviolet radiation for 15 min 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 UVA 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 (Food and Drug Research Labs 1978a, 1978b, 1979, 1980).

Six of the Draize-Shelanski repeated-insult patch tests summarized in Table 25 used supplemental ultraviolet light exposure after the 1st, 4th, 7th, 10th, and challenge patches. 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%. Of the 607 subjects thus treated, none had reactions indicative of photosensitization.

Phototoxicity

Multiple Parabens

Four product formulations, each containing 0.2% Methylparaben and/or 0.2% Propylparaben, were tested for human phototoxicity (Food and Drug Research Labs 1978a, 1978b, 1979, 1980). 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 h. The test site on one forearm was subjected to nonerythemogenic ultraviolet light for 15 min at a distance of 10 to 12 cm from the source, receiving a UVA 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 UVA 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.

The Schwartz-Peck prophetic patch tests summarized in Table 32 used a single supplemental UV light exposure after the second patch. Test sites were irradiated for 1 min 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% Butylparaben or both 0.2% Methylparaben and 0.1% Propylparaben. Of the 1034 subjects thus tested, only 3 had mild skin reactions (CTFA 1978c; Research Testing Laboratories 1978; CTFA 1980k).

Ocular Irritation

Methylparaben

Aqueous solutions of 0.10% to 0.30% Methylparaben instilled in the eyes of humans produced moderate hyperemia, slight lacrimation, and slight burning. All symptoms disappeared within 1 min. These results were confirmed when instillation of these solutions several times daily into the eyes of more than 100 subjects produced no irritation (Simonelli and Marri 1939).

TABLE 31
Clinical skin irritation tests with product formulations containing parabens.

Test method	Material tested	Paraben concentration	Number of Subjects	Results	Reference
24-h single insult occlusive patch	Unspecified product	0.8% Methylparaben	20	No irritation	CTFA 1978a
	Unspecified product	0.8% Methylparaben	20	No irritation	CTFA 1978b
	Unspecified product	0.3% Propylparaben	20	PII of 0.10 (out of 4.0 max.); minimal irritation in 2 subjects	CTFA 1977c
5-day cumulative irritancy (daily occlusive patch)	Hairdressing formulation	0.2% Methylparaben	50	No cumulative irritation	CTFA 1981g,r
20-day cumulative irritancy (23 h occlusive patch, 5 days/week)	Facial mask	0.3% Propylparaben	13	Slightly irritating; total composite score of 50 (out of 520 max.)	CTFA 1977d
21-day cumulative irritancy (23-h occlusive patch for 21 consecutive days)	White cream	0.2% Methylparaben	12	Essentially nonirritating; total score of 0.83 (out of 630 max.)	Hill Top Research 1979a
	White cream	0.2% Methylparaben	13	Essentially nonirritating; total score of 31 (out of 630 max.)	Hill Top Research 1981
	White cream	0.2% Methylparaben, 0.2% Propylparaben	11	Slightly irritating; total score of 72 (out of 630 max.)	Hill Top Research 1978b
	Orange cream	0.2% Methylparaben, 0.2% Propylparaben	9	Essentially nonirritating; total score of 0 (out of 630 max.)	Hill Top Research 1979b
	Lotion	0.2% Methylparaben, 0.1% Propylparaben	13	Slightly irritating; total score of 141 (out of 630 max.)	Hill Top Research 1978a
	Red wax	0.2% Propylparaben, 0.1% Butylparaben	9	Essentially nonirritating; total score of 2.2 (out of 630 max.)	Hill Top Research 1980
Controlled use test for 4 weeks	Eye makeup	0.2% Methylparaben, 0.1% Propylparaben	57	No irritation	CTFA 1979h
		0.2% Butylparaben	56	No irritation	CTFA 1980j

Case Reports

Methylparaben

Saiki et al. (1972) 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.

Kaminer et al. (1982) reported a delayed hypersensitivity reaction to orally administered Methylparaben. A male patient given haloperidol syrup developed an urticarial maculopapular rash over his entire body, except his face. All medications were discontinued and blood drawn for a macrophage migration inhibition test. There was no response in the test to haloperidol tablets, but there was to haloperidol in solution and to Methylparaben. The authors suggest that immunologic tests may help

TABLE 32

Clinical skin sensitization tests with product formulations containing parabens.

Test method	Material tested	Paraben concentration	Number of Subjects	Results	Reference
Kligman maximization test	Unspecified product	0.2% Methylparaben, 0.1% Propylparaben	25	No sensitization	Ivy Research Laboratories 1978
	Unspecified product	0.2% Methylparaben, 0.1% Propylparaben	25	No sensitization	
Schwartz-Peck prophetic patch test	Eye makeup	0.2% Methylparaben, 0.1% Propylparaben	202	No irritation or sensitization; supplemental UV exposure after 2nd insult produced mild reactions in 2 subjects	CTFA 1978c
	Lotion	0.2% Methylparaben, 0.1% Propylparaben	104	Mild irritation with closed patch in 6 subjects at 1st exposure and in 2 subjects at 2nd exposure; no sensitization; supplemental UV exposure after 2nd insult produced mild reactions in 1 subject	Research Testing Laboratories 1978
	Lotion	0.2% Methylparaben, 0.1% Propylparaben	104	Mild irritation with closed patch in 2 subjects at 2nd exposure; no sensitization; supplemental UV exposure after 2nd insult produced no reactions	
	Eye makeup	0.2% Butylparaben	728	Mild irritation with closed patch in 2 subjects at 1st exposure and in 4 subjects at 2nd exposure; no sensitization; supplemental UV exposure after 2nd insult produced no reactions	CTFA 1980k
Draize-Shelanski repeated insult patch test	Eyeshadow	0.8% Methylparaben	87	Isolated transient irritation in 2 subjects; no sensitization	CTFA 1980k
	Foundation	0.8% Methylparaben	103*	Isolated transient irritation in 11 subjects; no confirmed sensitization	Research Testing Laboratories 1979a
	Blush	0.8% Methylparaben	198	Mild to moderate irritation in 10 subjects; no confirmed sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 produced no reactions	Research Testing Laboratories 1979b
	Foundation	0.8% Methylparaben	198	Mild to moderate irritation in 8 subjects; no confirmed sensitization; supplemental UV exposure in half the subjects produced no reactions	
	Hand lotion	0.2% Methylparaben	103	Isolated transient irritation in 3 subjects; no sensitization	Testkit Laboratories 1978
	Body scrub	0.2% Methylparaben	91	Doubtful reactions in 2 subjects during induction; no other evidence of irritation or sensitization	Testkit Laboratories 1979
	Hand cream	0.2% Methylparaben	205	Isolated transient irritation in 3 subjects; no sensitization	CTFA 1979i
	Unspecified product	0.2% Methylparaben	108	No irritation; no sensitization	CTFA 1979j

(Continued on next page)

TABLE 32
Clinical skin sensitization tests with product formulations containing parabens. *(Continued)*

	Unspecified product	0.2% Methylparaben	108	Isolated transient irritation during induction in 1 subject; mild irritation at challenge on original site, no reaction at virgin site	CTFA 1979k
	Suntan lotion	0.2% Methylparaben	56	No irritation; no sensitization	Food and Drug Research Labs 1984
	Unspecified product	0.2% Methylparaben, 0.2% Propylparaben	57	Mild reactions in 1 subject at induction patch 10 and at challenge on original site; no reaction at virgin site	Food and Drug Research Labs 1978a
	Orange paste	0.2% Methylparaben, 0.2% Propylparaben	27	Mild to marked irritation in 2 subjects; no sensitization	Food and Drug Research Labs 1979
	Eye makeup	0.2% Methylparaben, 0.1% Propylparaben	102	No irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 produced no reactions	CTFA 1978c
Draize-Shelanski repeated insult patch test	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 produced no reactions	Research Testing Laboratories 1978
	Lotion	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 produced no reactions	
	Moisturizing facial mask	0.3% Propylparaben	99	Minimal to mild irritation in most subjects; no sensitization	CTFA 1976f
	Orange jelly	0.3% Propylparaben	108	No irritation; no sensitization	Hill Top Research 1976
	Mascara	0.3% Propylparaben	94	Slight irritation; no sensitization	Hill Top Research 1977
	Protective face cream	0.2% Propylparaben	56	Isolated transient irritation in 1 subject; no sensitization	Food and Drug Research Labs 1978b
	Unspecified product	0.2% Propylparaben, 0.1% Butylparaben	205	Mild to moderate irritation in 10 subjects; no sensitization	CTFA 1980l
	Unspecified product	0.2% Propylparaben, 0.1% Butylparaben	205	Mild irritation in 1 subject during induction; mild, transient reactions at challenge in 2 subjects at original site and 1 subject at virgin site—investigators concluded there was no significant evidence of sensitization	CTFA 1980m
	Eyeliner	0.3% Butylparaben	180	No irritation; no sensitization	CTFA 1976g
	Eye makeup	0.2% Butylparaben	353	Mild to moderate irritation in few subjects; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 produced no reactions	CTFA 1980k
	Moisture milk lotion	0.2% Ethylparaben	111	Mild irritation in 3 subjects; 1 mild reaction 48 hours after challenge in subject who had not previously reacted—investigators concluded there was no significant evidence of sensitization	Techni-Med Consultants 1980
	Night cream	0.2% Ethylparaben	111	Mild irritation in 3 subjects; no reactions indicative of sensitization	

the clinician isolate the specific cause of an antigenic reaction and allow therapy to continue with a different formulation.

Kojima (1992) reported a case of facial contact urticaria in response to Methylparaben in a cosmetic product.

Propylparaben

Lindner et al. (1989) reported a case of allergic contact dermatitis caused by propyl hydroxybenzoate (Propylparaben) following the use of Varicosan bandages.

Benzylparaben

Tosti et al. (1989) reported the case of a 43-year-old woman with a 3-year history of relapsing erythematous nodules and small plaques on her trunk, but no history of other skin disease or atopy. She had a positive patch test to Benzylparaben. Because the patient had been using systemic and topical preparations in which Benzylparaben was the preservative, the authors concluded that this case was an example of a deeply located allergan that may cause dermal allergic contact dermatitis.

Würbach et al. (1993) reported a contact allergy to benzyl alcohol and Benzylparaben. A 50-year-old man, who developed a relapsing tinea-like contact dermatitis, had a positive patch test result to parabens mix. Symptoms disappeared with treatment (corticosteroids without preservatives) and discontinuance of daily showering with perfumed soap and foam bath preparations. The authors concluded that the patient's reaction to the paraben mix represented a reaction to benzyl alcohol, because Methylparaben, Ethylparaben, or Propylparaben alone did not produce a reaction.

Multiple Parabens

In the older literature, paraben hypersensitivity has been reported. In many, sensitization followed topical application of paraben medicaments to broken skin (Sarkany 1960; Schorr and Mohajerin 1966; Schamberg 1967; Reed 1969; Wulf and Memmesheimer 1969; Fisher 1975; Husain 1977; Simpson 1978; Henry et al. 1979). Other cases of sensitivity from parabens in anesthetic solutions injected intravenously are reported (Latronica et al. 1969; Aeling and Nuss 1974; Nagel et al. 1977).

Hjorth and Trolle-Lassen (1962) reported over 140 cases of paraben sensitivity. Epstein (1968) ascribed the incidence of sensitization, which appeared to be higher in Denmark than in the United States, to the use of higher concentrations of parabens in Denmark than in the United States. In their textbook, Rook et al. (1968) stated that sensitization reactions were reported as a result of paste-bandages containing parabens applied to venous stasis ulcer.

Case reports have continued to appear. Fisher (1982) reported three patients who acquired an allergic contact dermatitis from the application of Cortaid cream. Butylparaben and Methylparaben were the preservatives used in this product and it was to them that the three patients reacted in patch testing. One patient in particular was said to exhibit the so-called "paraben paradox." As a result of the use of the cream, the patient developed a widespread dermatitis of the left axilla, chest, and upper abdomen. Two weeks later, the patient used a deodorant spray, containing parabens. Only the left axilla flared; there was no reaction at the right axilla. This suggests the "paraben paradox" in which paraben-sensitive individuals can use paraben-containing topical applications providing the skin is not eczematized, nor has been the site of a previous dermatitis.

Fisher (1993, 1996) also published articles in which he suggested that the parabens are remarkably safe, effective preservatives and argued that no difficulty with sensitization or allergic contact dermatitis is being encountered from the presence of parabens in cosmetics which are in contact with the thin, delicate skin of the eyelids, a common site of allergic contact dermatitis from many other contactants.

Javors et al. (1984) and Schwartz et al. (1984) reported hypersensitivity reactions to parabens after barium enema examinations.

Fine and Dingman (1988) reported hypersensitivity dermatitis following suction-assisted lipectomy as a complication of paraben preservatives in the local anesthetic. Previous cases of allergic reactions to parabens in anesthetics were reported by Ivy (1983) and Wahl (1983).

Carradori et al. (1990) reported a case of systemic contact dermatitis due to parabens. A generalized eczematous eruption involving the trunk and limbs of a 65-year-old woman was observed after a 1-g intramuscular injection of ampicillin for a bacterial infection. Patch tests with a standard series identified positive reactions to balsam of Peru and paraben-mix at 48 and 72 h. Further patch tests showed reactions to Methylparaben and Ethylparaben present in the ampicillin at 18 mg/g. The authors suggested that sensitization to parabens was probably the result of previous use of topical agents for treatment of leg ulcers, although no specific agent that might have contained parabens was identified.

Verhaeghe and Dooms-Goossens (1997) reported a case of a 14-year-old female with a 5-year history of recurrent eczema on the palms and between the fingers. Patch testing had been positive to nickel sulfate and paraben-mix. Sources of the reactions appeared to include a toy play gel, water paints, topical medication, and a sunscreen. A list of paraben-free cosmetics and topical medications was provided, but the patient was back a week later after using a liquid soap that was not mentioned on the list, and turned out to contain parabens.

Cooper and Shaw (1998) reported a case of allergic contact dermatitis from parabens in a tar shampoo. A 74-year-old female presented with a 4-year history of dry, flaky scalp, diagnosed as pityriasis amianticea. After initially tolerating a tar shampoo, she reported severe itching and erythema of the face and scalp, with eyelid edema. The ingredient list on the shampoo included parabens. Patch testing revealed positive reactions to balsam of Peru and paraben mix. Further testing showed positive results with Ethyl-, Propyl-, and Butylparaben. The authors noted that an allergic contact dermatitis from parabens in a shampoo was unusual.

Mowad (2000) presented two case reports of allergic contact dermatitis caused by parabens. In addition, the author commented on paraben allergenicity, patch testing issues, and paraben paradoxes. One case report involved a 76-year-old woman with a long history of eczema (from childhood) who presented with an exacerbation of the rash on her face and neck. Patch testing revealed a positive reaction to paraben-mix. On discontinuation of paraben-containing products and use of paraben-free products, she cleared considerably. The other case report was a 40-year-old woman with no significant medical history who presented with a hand rash that had persisted for 2 years. Patch testing revealed a positive reaction to paraben-mix. A review of product usage revealed a moisturizer with parabens. With discontinuation of the product and avoidance of paraben-containing moisturizers, her hands cleared. She was able to use paraben-containing cosmetics (other than hand creams or cosmetics that are applied by hand, presumably) without difficulty.

The author noted the same paradoxes that Fisher described in his frequent articles, but went on to mention the issue of conflicting reports of cross-reactions among parabens and the so-called *para* group, which includes *p*-aminobenzoic acid (PABA) and *p*-phenylenediamine. For practical purposes, this author suggests that cross-reactions are not seen between parabens and *para* compounds. Overall, the author concluded that allergic contact dermatitis to parabens does occur, but given the widespread use of parabens, it is relatively uncommon (Mowad 2000).

Shaffer et al. (2000) reported the case of a 53-year-old female complaining that her large port-wine stain had recently become more irregular and thickened. Prior dermabrasion had been unsuccessful and the patient was relying on cover-up cosmetics for aesthetic purposes. Pulse dye laser treatment initially lightened the stain and treatments were continued. After the fourth month of laser therapy, the patient developed an itchy, erythematous, papular eruption on the left side of her face following each treatment. Progressive and more extensive eruptions occurred after subsequent laser treatments involving untreated areas. Patch testing showed positive reactions to balsam of Peru, neomycin sulfate, paraben-mix, and several cover-up cosmetics, of which parabens were believed to be relevant to the current extensive eruption. All of the cover-up cosmetics contained parabens. The authors noted that reports of laser-associated contact dermatitis are becoming more common.

Vilaplana (2000) reported a case of a 62-year-old male who developed contact dermatitis from parabens used as preservatives in eyedrops. The patient had used eyedrops containing Methyl- and Propylparaben for 1 year. At 11 months, he developed conjunctivitis and eyelid dermatitis. Patch testing was positive to the eyedrops and to paraben-mix. The eyedrops were negative in 25 control individuals. The patient was patch-tested 1 month later with Methyl- and Propylparaben at 3% in petrolatum and was positive to both, whereas the 25 controls were negative.

Clinical Treatment

Propylparaben

Ritzau and Swangsilpa (1977) studied the prophylactic effect of Propylparaben on alveolitis sicca dolorosa (ASD). 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% of the placebo group did. The prophylactic effect of Propylparaben was highly significant, and no side effects to treatment were reported.

Epidemiology

Mirick et al. (2002) reported a population based case-control study of breast cancer patients. The stated purpose of the study was to address the Internet rumor that antiperspirant use causes breast cancer. Women (20 to 74 years of age) with breast cancer first diagnosed between November 1992 and March 1995 were compared to control subjects identified by random digit dialing and matched by 5-year age groups. An in person interview was used to gather information on a large number of past exposures of interest. During the development of the questionnaire, the authors became aware of the Internet rumor that antiperspirants might contain harmful substances that could be absorbed via small nicks or abrasions caused by underarm shaving. The authors added a two-stage question to address this hypothesis. Individuals were first asked if they regularly shaved under their arms, and if the answer was yes, they were asked if they applied any product to control underarm perspiration, which products were used, and if the products were applied within 1 h of shaving.

Several measures of antiperspirant use were used, including ever/never regular use, exclusive use of antiperspirant (versus deodorant or talc), and application within 1 h of shaving. These three measures were captured for deodorant use as well. Other analyses included the use of a blade razor (nonelectric). Of the eligible cases, 813 (78%) agreed to participate. Of the controls, 793 (75%) agreed.

Nearly all case patients and control subjects had at some point in their life used at least one method of underarm hair removal (94% of cases and 93% of controls), with the most common method being razor shaving. Of the subjects who reported underarm hair removal, case patients were less likely than control subjects to have used antiperspirant regularly (50% versus 56%), exclusively (24% versus 26%), or to report application of antiperspirant within 1 h (36% versus 40%). There was no association between any of the three measures of antiperspirant use and the risk of breast cancer.

Deodorant use was more prevalent than antiperspirant use. Of the subjects who reported underarm hair removal, case patients were more likely than control subjects to have used deodorant regularly (71% versus 65%), exclusively (43% versus 38%), or to report application of antiperspirant within 1 hour (49% versus 43%). There was no association between any of

the three measures of deodorant use and the risk of breast cancer.

For both antiperspirant and deodorant use, the use of a blade razor was separately evaluated versus other methods of underarm hair removal and in subjects who used a blade razor and applied antiperspirant or deodorant within 1 h of shaving. In no case was there an association of any of these behaviors with the risk of breast cancer (Mirick et al. 2002).

Cosmetics Industry Complaint Experience

The cosmetics industry provided information on cosmetic product complaints. 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 a body scrub product, two suntan lotions, a hand lotion, and a bubble bath, each containing 0.2 % Methylparaben (CTFA 1981s).

Complaint experience data on a protective face cream containing 0.2 % Propylparaben shows three safety-related complaints in 3 years with an estimated 400,000 uses (CTFA 1981t). Two of these were listed as "allergy" and one as "burning sensation."

There were 35 safety-related complaints for a mascara containing both 0.2 % Methylparaben and 0.1 % Propylparaben with 4.6 million units sold: 20 "burning/stinging," 11 "irritated skin," and 4 "allergic reaction" (CTFA 1981u).

An aftershave lotion also containing 0.2% Methylparaben and 0.1% Propylparaben had one safety-related complaint with 170,000 sold (CTFA 1981v).

Complaint experience data on a mascara containing 0.2% Butylparaben shows 36 complaints with 2.3 million units sold; 33 of these were listed as "irritating/burning," 2 as "itching," and 1 "swelling" (CTFA 1981w).

OTHER PARABENS SAFETY REVIEWS/ASSESSMENTS

Methylparaben

Soni et al. (2002) published a safety assessment of Methylparaben, covering cosmetic, food, and pharmaceutical uses. Based on a NOAEL of 5500 mg/kg day^{-1} in rats and applying a factor of 10 for interspecies differences and 10 for intraspecies differences, the authors concluded that an increase in the ADI to 55 mg/kg day^{-1} for Methylparaben is justified.

Propylparaben

Soni et al. (2001) published a safety assessment of Propylparaben, covering cosmetic, food, and pharmaceutical uses. Based on a NOAEL of 5500 mg/kg day^{-1} in a 96-week feeding study using rats, and applying a factor of 100 for the extrapolation of this value from rats to humans, the authors recommend an increase in the current ADI from 10 to 55 mg/kg day^{-1}.

Multiple Parabens

The European Food Safety Authority (EFSA) Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food adopted an opinion on the safety of paraben usage in food (EFSA 2004). The opinion noted the earlier ADI of 0 to 10 mg/kg body weight, as the sum of methyl, ethyl, and propyl *p*-hydroxybenzoic acid esters and their sodium salts.

The Panel evaluated newly available developmental toxicity studies on Methylparaben in rats, mice, hamsters, and rabbits and found no evidence of developmental toxicity at the highest dose level of 300 mg/kg day^{-1} in rabbits or 550 mg/kg day^{-1} in rodents. Proliferative effects of parabens on rat forestomach cells were discounted as a threshold phenomenon and that human exposure resulting from use of parabens as food preservatives would be much below threshold levels.

While acknowledging estrogenic activity for parabens in vitro, the Panel cited the absence of estrogenic activity in vivo in classical uterotrophic assays using peroral or subcutaneous injections. The opinion did note that there were positive in vivo uterotrophic assay findings for Butylparaben and Isobutylparaben, but that these were not used as food preservatives. *p*-Hydroxybenzoic acid was not considered estrogenic.

For reproductive toxicity, the opinion cited reduction in daily sperm production in juvenile male rats fed Propylparaben at 10 mg/kg day^{-1} as the lowest observed adverse effect dose (even though no lower doses were tested) and contrasted these findings with the absence of effect for Methylparaben and Ethylparaben at doses up to 1000 mg/kg day^{-1}.

The opinion restated the 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, but failed to recommend an alternative ADI because of the lack of a clear NOAEL (EFSA 2004).

Cantox Health Sciences International (2004) prepared an assessment of the endocrine disrupting/estrogenic potential of parabens. This assessment noted that parabens do not have genotoxic, carcinogenic, or teratogenic potential and are rapidly hydrolyzed to *p*-hydroxybenzoic acid and excreted. They cited the same developmental toxicity data noted by the EFSA above for Methylparaben and Ethylparaben and additional data in support of the absence of developmental toxicity for Butylparaben.

The remainder of the assessment focused on endocrine disruption. The assessment noted that parabens are able to bind estrogen and androgen receptors, activate estrogen-responsive genes, stimulate cellular proliferation, and increase levels of estrogen receptor protein. To place the in vitro data in context, the assessment cited the comparisons of parabens activity with 17β-estradiol and DES (2 to 5 orders of magnitude lower) and phytoestrogens, including isoflavones (comparable or less).

In vivo uterotrophic assay findings depended on which paraben, dose, and route of administration. *p*-Hydroxybenzoic Acid was positive using mice in one laboratory at 5 mg/kg day^{-1} (subcutaneous) and negative in mice and rats in another laboratory at doses up to 100 mg/kg day^{-1} (oral or subcutaneous). Methylparaben was negative in rats and mice with oral and subcutaneous doses up to 100 mg/kg day^{-1}. Ethylparaben and Propylparaben were negative by both routes of administration at that dose, but were positive subcutaneously (but not orally)

starting at 400 mg/kg day^{-1}. Isobutylparaben increased uterine weights at estimated subcutaneous doses of ≥100 mg/kg day^{-1}. Again the assessment compared the findings for parabens with estradiol and found parabens to be 1000- to 10,000-fold less effective on a equimolar basis and the doses at which there were any positive findings were well above an estimated exposure to parabens of 1.3 mg/kg day^{-1}.

The 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.

Because skin and hair care products may be used on infants and children at concentrations up to 0.33%, a separate exposure analysis was performed. A range was determined between estimated dermal absorption values of 30% and 100%. The exposure estimate was adjusted by a factor of 1.7 to account for the difference between the surface/weight ratio of adults compared to children less than 1 year of age. The daily systemic exposure from cosmetic products used on infants and children ranged from 0.26 to 0.87 mg/kg day^{-1} (Cantox Health Sciences International 2004).

Golden et al. (2005) reviewed the endocrine activity of parabens and addressed the implications for risks to human health. These authors noted the reported effects, which include estrogenic activity in vitro, increased uterine weights, and male reproductive effects, and commented that not every effect is seen for every paraben. In addition, they noted that these estrogen active compounds exhibit activity that is several orders of magnitude less than that of estrogen itself. They argued that both the dose of endocrine active chemicals and their potency should be considered in attempting to extrapolate the findings to human health and that comparisons with existing human data are the most relevant.

These authors noted the considerable dose-response data in both humans and animals that demonstrate the effects in offspring of in utero exposure to diethylstilbesterol (DES). DES is a synthetic estrogenic compound known to be equal or more potent than estradiol. Using a human NOEL of 0.3 mg/kg day^{-1} for adverse effects on the male reproductive tract, a comparison was made to the lowest doses of Butylparaben and Propylparaben linked to decreased sperm production, 10 and 12 mg/kg day^{-1}, respectively. Potency comparisons were not possible because the male reproductive tract studies were not performed with estradiol as a positive control. Using the potency estimates from uterotrophic assays, it was determined that Butylparaben is at least 6000-fold below the dose of DES that might cause effects on the male reproductive tract. Then, based on an estimated daily dermal dose of 0.12 to 0.41 mg/kg day^{-1} of Butylparaben, the intake by pregnant women would be 15,000- to 50,000-fold below the equivalent amount of DES that may cause effects on the embryonic reproductive tract.

These authors also provided a perspective on potential risk of endocrine active chemicals such as parabens by comparing them to the daily intake of naturally occurring phytoestrogens. They described a hygiene-based margin of safety (HBMOS) as the estimated daily intakes weighted by the relative potency of the compound in question divided by the daily intake of a reference compound. The approach was developed for compounds with short half-lives, similar in concept to the rapid metabolism of parabens and dietary phytoestrogens. A daily exposure of 1 mg/kg day^{-1} of the phytoestrogen, daidzein, was combined with a marginal uterotrophic assay response to daidzein at 500 mg/kg day^{-1} to establish an exposure level considered to be nonhazardous; this became an HBMOS value of 1. To calculate the HBMOS for Butylparaben, for example, the daily intake of daidzein appears in the numerator, divided by the product of the Butylparaben daily intake times the relative potency of Butylparaben and daidzein. The Butylparaben daily dermal intake is estimated at 0.12 to 0.41 mg/kg day^{-1} and the relative potency is 500 mg/kg day^{-1} for daidzein divided by 1200 mg/kg day^{-1} for Butylparaben (from Routledge et al. 1998). Using the value of 1200 mg/kg day^{-1} for Butylparaben is not comparable to the daidzein value because it is a subcutaneous dose, but none of the oral doses of Butylparaben given in various studies produced a uterotrophic response. The resulting HBMOS ranges between 6 and 20. Again, given that the daily consumption of phytoestrogens is not likely to elevate risk, the daily exposure to Butylparaben is up to 20 times less likely to elevate risk.

Two of the authors acknowledge being compensated by CTFA for preparation of their review and the third noted previous work as a CTFA consultant, but all expressed the view that the interpretations and conclusions are solely their own (Golden et al. 2005).

The Scientific Committee on Consumer Products of the European Commission issued an opinion on parabens, underarm cosmetics, and breast cancer (European Commission 2005c). They concluded that there was no breast cancer risk from use of underarm deodorants. More specifically, they addressed breast cancer risk from underarm deodorants containing parabens. They stated that the estrogenic potential of parabens was very low, and concluded that there was no breast cancer risk from use of paraben-containing underarm deodorants. This group also issued an opinion on the overall safety of parabens (European Commission 2005a). They concluded that Methylparaben and Ethylparaben may be safely used in cosmetics at concentrations up to 0.4%. They stated, however, that the available data were insufficient to determine if Propylparaben, Butylparaben, or Isobutylparaben could be used safely in cosmetics. They asked for in vitro percutaneous absorption studies and reproductive and developmental toxicity studies, with a special focus on the male reproductive system. In an extended opinion, they added Isopropylparaben to the insufficient group, with the same data needs (European Commission 2005b).

Soni et al. (2005) published a safety assessment of parabens that summarized their previous reviews (Soni et al. 2001, 2002) of Methylparaben and Propylparaben and included data on other parabens. These authors suggest that the estrogenic potential of parabens to cause reproductive harm in humans is equivocal and recommend a multigenerational reproduction study using accepted protocols to resolve the concern.

SUMMARY OF SAFETY TEST DATA FOR BENZYL ALCOHOL, BENZOIC ACID, AND SODIUM BENZOATE

These data summaries were considered by the CIR Expert Panel specifically in evaluating the safety of Benzylparaben.

Benzyl Alcohol (Andersen 2001)[2]

Benzyl Alcohol is metabolized to Benzoic Acid, which is then conjugated with glycine and excreted as hippuric acid. EPA reviews of mouse and rat oral-dosing studies conducted by the Natinal Toxicology Program (NTP) determined subchronic and chronic oral reference doses for humans of 1 and 0.3 mg/kg/day, respectively for Benzyl Alcohol. Earlier, the World Health Organization (WHO) established an ADI of up to 5 mg/kg for Benzyl Alcohol. Investigators considered Benzyl Alcohol to be a moderate respiratory hazard and toxic when administered by the parenteral route. Benzyl Alcohol produced severe irritation when applied to the skin of nude mice.

In oral-dose teratogenicity studies using mice, Benzyl Alcohol was negative in one study (550 mg/kg/day), resulted in questionable results in another (750 mg/kg/day), and was a considered a suspect reproductive hazard in the third (750 mg/kg/day [which EPA extrapolated to a human dose of 58 mg/kg/day]).

Mutagenicity studies reported both positive and negative results. Benzyl Alcohol was negative for carcinogenicity when dermally tested on mice at 2.00% in a nonoxidative hair dye. NTP considered it negative for carcinogenicity following 2-years of oral dosing in rats and mice, but EPA considered the results equivocal.

In clinical settings, Benzyl Alcohol can produce nonimmunologic contact urticaria or non-immunologic immediate contact reactions. Benzyl Alcohol was not a sensitizer when tested in a maximization test at 10% in petrolatum, and demonstrated a low incidence of sensitization in provocation studies. Therapeutic ocular studies indicated that Benzyl Alcohol may be beneficial in the management of cataracts.

Benzoic Acid and Sodium Benzoate (Andersen 2001)[3]

Benzoic Acid is an aromatic acid that is used in cosmetics as a pH adjustor and/or preservative. Sodium Benzoate is its sodium salt and is used in cosmetics as a preservative. Both substances are GRAS ingredients. WHO established an ADI of up to 5 mg/kg. Benzoic Acid can be used in ointments and antifungal agents. Sodium Benzoate has been used clinically in the treatment of hyperammonemia. The benzoates are recognized hydroxy radical scavengers.

Benzoic Acid is rapidly absorbed following dermal application and its metabolism can deplete glycine supplies.

In animal multiple-dose oral toxicity studies, decreased feed consumption, depressed growth, and toxic effects were noted at doses of Benzoic Acid or Sodium Benzoate >1%. A neurobiological study was negative.

In oral-dose teratogenicity studies, Benzoic Acid (600 mg/kg) produced significant results in hamsters, but was negative in two rat studies (up to at least 500 mg/kg/day). Sodium Benzoate was negative for teratogenicity in mice and rats (175 mg/kg/day), hamsters (300 mg/kg/day), and rabbits (250 mg/kg/day).

Benzoic Acid was negative in mutagenicity studies. Sodium Benzoate was positive in assays done on the Chinese hamster ovarian (CHO) cell line, but negative in other studies. Benzoic Acid was negative for carcinogenicity when dermally tested on mice at 0.016% in a nonoxidative hair dye. Sodium Benzoate was negative for carcinogenicity when administered orally at up to 2% to rats (in feed for up to 2 years) or mice (in a life-time drinking water study).

In clinical studies, toxic symptoms were noted following doses far exceeding the ADI established by the WHO. The benzoates are recognized to produce nonimmunologic contact urticaria or nonimmunologic immediate contact reactions, but it is not clear whether the reactions are histamine- or prostaglandin-mediated. Dermal sensitization, phototoxicity, and photosensitization studies were negative.

SUMMARY OF PARABENS

Parabens are esters of *p*-hydroxybenzoic acid (PHBA). Parabens 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, as does the octanol/water partition coefficient. 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. Individual parabens and PHBA are easily separable using high-performance liquid chromatography and other separation techniques.

As reported by industry to FDA in 2006, parabens are used as preservatives in over 22,000 cosmetic formulations. This current use figure is an increase over the 13,282 uses reported to FDA in 1981. They are most commonly used at concentrations up to 0.8% (mixtures of parabens) or up to 0.4% (single paraben). Industry estimates of the daily use of cosmetic products that may contain parabens were 17.76 g for adults and 378 mg for

[2]This summary information is provided because Benzyl Alcohol is a metabolite of Benzylparaben.

[3]This summary information is provided because Benzoic Acid is a metabolite of Benzylparaben.

infants. Certain parabens are also used as preservatives in foods, pharmaceuticals, and other products.

Parabens in cosmetic formulations applied to skin penetrate the stratum corneum in inverse relation to the ester chain length. 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.

Ingested parabens are quickly absorbed from the 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.

The antimicrobial activity of parabens increases with increasing ester chain length, but water solubility decreases. Because microbial replication takes place primarily in the water phase of a cosmetic product, preservative effectiveness is a combination of antimicrobial activity and water solubility. Parabens are more active against fungi than bacteria and more active against gram-positive than gram-negative bacteria. 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.

Parabens can bind to proteins and, depending on the enzyme system, may inhibit and potentiate enzyme activity. They also compete with bilirubin for binding sites on serum albumin. Parabens can inhibit growth of cultures of animal and human cells, depending on concentration, and reduce biosynthesis of RNA and DNA in both bacterial and mammalian cell cultures. One study using human keratinocytes found that Methylparaben can potentiate the effects of UV radiation, but it was unclear that the wavelengths involved would be found in sunlight at the earth's surface.

Parabens have varying physiological and pharmacological effects. Parabens have been reported to have 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%), Propylparaben (10%), and Ethylparaben (100% and 10%) were, at most, mildly irritating when applied to rabbit skin. Benzylparaben applied directly (0.5 g) to rabbit skin produced no significant irritation. Methylparaben and Ethylparaben at 100% concentration were slightly irritating when instilled into the eyes of rabbits, but there were no adverse reactions to 0.1 g of Benzylparaben. Subchronic and chronic oral studies indicate that parabens are practically nontoxic. Practically all animal sensitization tests indicate that the Parabens are nonsensitizing.

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.

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. Isobutylparaben and Butylparaben were noncarcinogenic when given to mice in a chronic feeding study. Methylparaben was noncarcinogenic when injected subcutaneously in mice or rats, or when administered intravaginally in rats, and 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, and Ethylparaben was nonteratogenic in rats. 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; and decreased sperm number and motile activity in F_1 offspring of rats maternally exposed to 100 mg/kg day^{-1} were reported. Decreased sperm numbers and activity were reported in F_1 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 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 α 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.

Parabens are practically nonirritating and nonsensitizing in the population with normal skin. Paraben sensitization has occurred and continues to be reported in the case literature, however, principally when exposure involves damaged or broken skin. Even when patients with chronic dermatitis are patch-tested to a parabens mix, parabens generally induce sensitization in less than 4% of such individuals. 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 primary eye irritation study in humans showed Methylparaben to be nonirritating at concentrations up to 0.3%. Photocontact sensitization and phototoxicity tests on product formulations containing 0.1% to 0.8% Methyl-, Propyl-, and/or Butylparaben produced 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.

Several safety assessments of parabens have been prepared. One such assessment for Propylparaben in foods recommended an increase in the current ADI from 10 to 55 mg/kg day^{-1} and another on Methylparaben concluded that an increase in the ADI to 55 mg/kg day^{-1} for Methylparaben is justified.

The European Food Safety Authority opinion cited reduction in daily sperm production in juvenile male rats fed Propylparaben at 10 mg/kg day^{-1} 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}. The opinion restated the 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 NOAEL.

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 17β-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.

DISCUSSION

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.

TABLE 33

Margins of safety for parabens in cosmetics as a function of exposed population and single versus multiple paraben usage.

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

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 $\times$0.4% use concentration $\div$ 60 kg person $\times$ 50% absorption $\times$ 1000 mg/kg = 0.59 mg/kg day^{-1}

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

For infants, the relevant calculations are:

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

Systemic dose (multiple parabens) = 378 mg/day of product $\times$0.8% use concentration $\div$ 4.5 kg infant $\times$ 50% absorption = 0.336 mg/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 33.

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.

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.

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Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Parabens

COLIPA n° P82

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 9th plenary on 14 December 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

4-Hydroxybenzoic acid, its salts and esters ("parabens") are currently authorised in Annex VI, entry 12 of the Cosmetics Directive (76/768/EEC) at a maximum use concentration of 0.4% (acid) for one ester and 0.8% for a mixture of esters.

Between January 2005 and June 2008, the Scientific Committee on Consumer Products (SCCP) adopted four opinions on parabens:

- The first opinion (SCCP/0874/05) addressed parabens and breast cancer: "*Extended Opinion on parabens, underarm cosmetics and breast cancer"* and concluded that *according to the current knowledge, there is no evidence of a demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics*.
- The second opinion "(SCCP/0873/05) was "*An extended opinion on the Safety Evaluation of parabens*" with the following conclusions:

  "***Methyl- and ethylparaben***

  For the methyl and ethyl p-hydroxybenzoic acid esters, the maximum authorised concentrations remain unchanged.

  Propyl-, isopropyl-, butyl- and isobutylparaben

  As the present discussion is based solely upon data in the literature, it is the SCCP's opinion that more information is needed in order to formulate a final statement on the maximum concentration of propyl-, isopropyl-, butyl- and isobutylparaben allowed in cosmetic products. More specifically, the following data are requested before end of March 2005:

  - full descriptions of available in vitro percutaneous absorption studies;

  - a complete dossier with regard to the reproductive and developmental toxicity of propyl, isopropyl, butyl and isobutylparaben, with special focus on the male reproductive system."
- The third opinion (SCCP/1017/06) was adopted by the SCCP in October 2006 and concluded that the tests provided in Submission I of February 2006 contained too many shortcomings in order to be considered as scientifically valid and that the conclusion of opinion SCCP/0873/05 remained unchanged.
- After consultation of the SCCP, new data were submitted by Colipa, leading in June 2008 to adoption of the fourth SCCP opinion (SCCP/1183/08) concluding: "*As already concluded in earlier opinions, methylparaben and ethylparaben are not subject of concern.*

  The SCCP is of the opinion that, based upon the available data, the safety assessment of propyl- and butylparaben cannot be finalised yet. Parabens are important cosmetic preservatives and they have wide use in multiple product types.

  Since no unequivocal conclusion can be drawn with regard to the contradictory reproductive toxicity studies available, of which none appears to be scientifically acceptable, the SCCP welcomes the proposal made by industry to conduct further work in the field of skin penetration/metabolism and pharmacokinetics to further support existing data. It is, however, recommended to supplement the envisaged studies in the rat with toxicokinetic studies in human volunteers after dermal application of representative cosmetic products containing propyl- and butylparaben, since these may deliver essential information.

In case significant systemic exposure to propyl- and/or butylparaben is measured in the requested human volunteer study, a rodent 2-generation toxicity study may be unavoidable, although it is the opinion of the SCCP that this should only be performed as a last resort.

Safety data need to be provided for all authorised parabens, including iso-alkyl- and phenylparabens."

- In November 2009 Denmark submitted the report "Survey and Health Assessment of the exposure of 2-year-olds to chemical substances in Consumer Products" published by the Danish EPA (2009) for evaluation by the SCCS together with the expected new data from Colipa.
- In December 2009 Colipa submitted a pharmacokinetic study on methyl-, propyl- and butylparaben (Aubert 2009) together with the justification of the decision not to conduct a study on human volunteers. No data for other 4-hydroxybenzoic acid, its salts and esters ("parabens") such as iso-alkyl- or benzylparaben were submitted.
- In February 2010 the Danish Authorities submitted a report by the Danish National Food Institute, DTU: *Update on uptake, distribution, metabolism and excretion (ADME) and endocrine disrupting activity of parabens 2009*. In the meantime it has been published as an article of Boberg et al. (2010).

2. TERMS OF REFERENCE

1. *Does the SCCS consider the continued use of propyl- and butylparaben in a concentration up to 0.4% for one ester or 0.8% when used in combination in cosmetic products safe for the consumer taken into consideration the provided scientific data?*
2. *Does the SCCS consider the continued use of methyl- and ethylparaben in a concentration up to 0.4% for one ester or 0.8% when used in combination in cosmetic products is influenced in anyway taken into consideration the new provided scientific data?*
3. *Does the SCCS consider the continued use of isopropyl-, isobutyl- and phenylparaben in a concentration up to the existing 0.4% for one ester or 0.8% when used in combination in cosmetic products safe for the consumer taken into consideration that no scientific data has been provided?*

This opinion has been subject to a commenting period of four weeks after its initial publication. During this period, information was received from the Norwegian Scientific Committee for Food Safety (VKM) that the evaluation of parabens in cosmetic products by the Norwegian Institute of Public Health in 2003 (Paulsen and Alexander, 2003) was not considered valid anymore due to a misinterpretation of dermal absorption data contained in the applicant's dossier which had impacted the dermal absorption estimation. The evaluation has been superseded by a risk assessment carried out by VKM in 2006.

3. ISSUES

Considering the questions raised during the last six years on the safety evaluation of parabens, three separate issues need to be considered:

1) The relationship between the use of parabens in deodorants and the development of breast cancer.
2) The potential *in vitro* and *in vivo* endocrine modifying effects of parabens, in particular estrogenic/anti-androgenic activities and the NO(A)EL value to be used for the calculation of the MoS for the different paraben esters.
3) The toxicokinetics (dermal absorption and biotransformation) of the different paraben esters (in humans and rodents).

Each issue has been previously discussed and described in a number of publications and/or official reports. The following sections summarise the available data per issue with special emphasis on the remaining problem points.

The previous opinions of the SCCP on the subject of parabens, which provide additional information, can be found at:

SCCP/0873/05:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_019.pdf

SCCP/0874/05:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_00d.pdf

SCCP/1017/06:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_074.pdf

SCCP/1183/08:
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_138.pdf

3.1 THE RELATIONSHIP BETWEEN THE USE OF PARABENS AND THE DEVELOPMENT OF BREAST CANCER

With regard to their general toxicological profile, acute, subacute and chronic toxicity studies in rats, dogs and mice have proven parabens to be practically non-toxic, not carcinogenic, not genotoxic or co-carcinogenic, and not teratogenic (SCF 1994). Nevertheless, in 2004 a possible link between the use of underarm cosmetics and breast cancer was claimed in a number of scientific publications.
After thorough study of the available knowledge, the SCCP concluded that there was insufficient data to establish a link between the use of underarm cosmetics and breast cancer (SCCP/0874/05). Meanwhile, no additional data providing evidence to the contrary were encountered.

A more recent review article (Darbre and Harvey 2008) repeats the arguments that have all been refuted in SCCP/0874/05. It does not add new data nor adds any conclusive evidence. Therefore, this issue will not be reconsidered in the present opinion.

3.2 THE ESTROGENIC / ANDROGENIC PROPERTIES OF PARABENS

3.2.1 Data described in previous SCCP opinions

Two previous SCCP opinions (SCCP/0873/05, SCCP/0874/05) describe and discuss a number of *in vitro* and *in vivo* studies. A recombinant yeast estrogen screen showed parabens to be able to bind to the estrogen receptor, to activate genes controlled by these receptors, to stimulate cell growth and to increase the level of estrogen receptor protein. The estrogenic potency *in vitro* was shown to increase with increasing length of the linear alkyl chain and with increased branching of the alkyl chains, resulting in the following potency ranking order: methyl- < ethyl- < propyl- < butyl- < isobutylparaben. The potency, however, remained at all times 1,000 to 1,000,000 times below the potency of 17β-estradiol. p-Hydroxybenzoic acid (PHBA), the common metabolite of all parabens, was inactive in the *in vitro* assays presented in the 2005 opinion.

The *in vivo* estrogenic activities of parabens have been tested in uterotrophic assays employing female rodents, either immature or adult ovariectomised, after oral, subcutaneous or dermal administration. Butylparaben appeared to be more potent than propyl-, ethyl- and methylparaben, and again the values remained several magnitudes of order below the potency of 17β-estradiol.
Conflicting results, however, were reported for PHBA tested *in vivo*. One study claimed that it had no estrogenic effect, whereas another study gave potency values 1000-fold below the 17β-estradiol level (EFSA 2004, Anonymous 2004, Paulsen and Alexander 2003).

In summary, the *in vitro* data and *in vivo* rodent test results up to 2005 indicated that parabens can exert estrogenic activity, but with potency values that are 3 to 6 orders of magnitude lower than the potency of the positive control 17β-estradiol. The estrogenic activity of parabens appears to increase with increasing chain length.

3.2.2 Update on the hormonal (estrogenic / anti-androgenic) properties of parabens

Table 1 in the appendix to this opinion provides an overview of the most relevant studies, covering *in vitro* and *in vivo* assays with the linear paraben esters methylparaben (MePB), ethylparaben (EtPB), propylparaben (PrPB) and butylparaben (BuPB), but also with the branched esters isopropylparaben (IsoPrPB) and isobutylparaben (IsoBuPB), and with the less commonly used benzylparaben (BzPB, phenylmethyl 4-hydroxybenzoic acid). In some cases, the major metabolite p-hydroxybenzoic acid (PHBA) was also tested. For phenylparaben (PhPB, phenyl 4-hydroxybenzoic acid), no data are available.

3.2.2.1 *In vitro* experiments

In the ***in vitro* assays**, different hormonal-related mechanisms are examined:

- *Effects of 4 parabens and PHBA on the estrogen sulfotransferase (SULT) activity in cytosol from **human** skin and liver*:
  SULT activity appeared to be inhibited to various degrees by methylparaben, ethylparaben, propylparaben and butylparaben at micromolar concentrations, but not by PHBA. The potency and extent of SULT inhibition increased with increasing paraben ester chain length (Prusakiewicz et al. 2007).
- *The anti-androgenic potential of 3 parabens and PHBA by measuring inhibition of testosterone-induced transcriptional activity in a **human** embryonic kidney cell line:*
  Methylparaben, propylparaben and butylparaben inhibited an 0.1 nM testosterone-induced transcriptional activity at concentrations above 10 µM (max. 40% inhibition), whereas flutamide and vinclozolin (pos. controls) inhibited transcriptional activity induced

by a tenfold higher testosterone concentration at 10 to 100-fold lower levels. PHBA showed no effects (Chen et al. 2007).

- *The potential of 7 parabens and PHBA to induce proliferation in MCF-7 cells, a human breast cancer-derived cell line shown to be estrogen-responsive:*
  A weak potential was noted for all tested parabens (potency 5 to 6 orders of magnitude below that of 17β-estradiol) and PHBA was negative (van Meeuwen et al. 2008).
- *The ability of 7 parabens and PHBA to inhibit aromatase (enzyme converting androgens into estrogens) activity in human MCF-7 cells (indirect anti-estrogenic potential):*
  All parabens were capable of inhibiting aromatase *in vitro*, although effective concentrations (IC_{50} values) were far above the paraben levels detected in human samples. There was no link between aromatase inhibition and chain length. PHBA was negative (van Meeuwen et al. 2008).
- *The ability of ethylparaben or eutylparaben to interfere with steroidogenesis in a human adrenocortical carcinoma cell line:*
  Ethylparaben and butylparaben increased progesterone production at 30 µM, but had no effect on testosterone or estradiol production (Taxvig et al. 2008). No positive control was included.
- *The potential of butylparaben to act as a thyroid receptor agonist/antagonist in a rat pituitary cell line:*
  Butylparaben was considered a potential weak thyroid receptor agonist based upon increased cell proliferation at 3 µM. The effect was slightly more pronounced in the presence of triiodothyronine (T_3). No positive control was included (Taxvig et al. 2008).
- *The estrogenic potential of the ethylparaben and propylparaben based upon human MCF-7 gene expression related to estrogenic responses, making use of DNA microarray analysis:*
  A clear difference was noted in the expression profiles after treatment with ethylparaben and propylparaben. The activity showed a positive correlation with the chain length of esters. Gene expression profiles of propylparaben and butylparaben treated cells were, however, closer to each other than the profile of estrogen treated cells was to any of them (Terasaka et al. 2006).

Sub conclusion 1:

***In vitro* studies show the potential of endocrine modifying effects of parabens, with estrogenic activity as a function of chain length. PHBA, the common metabolite does not seem to exhibit endocrine modifying effects.**

3.2.2.2 *In vivo* experiments

The ***in vivo* experiments** cover different potential estrogenic/anti-androgenic mechanisms and involve oral or subcutaneous administration of sets of parabens to immature or pregnant **rats** and **mice**. Over the years, two important sets of *in vivo* studies were submitted to the SCCP/SCCS.

A first series of studies is described in four publications of the Tokyo Metropolitan Institute of Public Health. They contain the results of *in vivo* assays studying the effects on the male reproductive system of methylparaben, ethylparaben (Oishi 2004) and propylparaben (Oishi 2002a) in rats and of butylparaben in rats (Oishi 2001) and mice (Oishi 2002b). The author of these studies comes to the conclusion that exposure of post-weaning rats and/or mice to butylparaben at dosage levels down to about 10 mg/kg bw/day adversely affected the secretion of testosterone and the function of the male reproductive system. Combined with an earlier uterotrophic assay showing that dosage levels of 200 mg butylparaben/kg bw/day and higher, significantly increased the uterus wet weights in the female rats (Routledge et al. 1998), Oishi concluded that more research into the effects of parabens on the reproductive system was needed (Oishi 2004). For propylparaben, only minor effects were

noted at the 10 mg/kg bw/day level, which was further considered the NOAEL value for that paraben ester.

Methylparaben and ethylparaben were shown not to adversely affect the secretion of sex hormones or male reproductive function, up to dose levels of about 1000 mg/kg bw/day (Oishi 2004).

At the time of the 2005 SCCP opinion, the only *in vivo* study in which the lowest (and only) dosage level of butylparaben did not cause any adverse effect on the male reproductive parameters measured, was a rat assay in which the ester was subcutaneously administered to neonatal rats for 17 consecutive days. Out of this study a NOEL of 2 mg/kg bw/day could be extracted for butylparaben (Fisher et al. 1999).

Since Industry considered both the NOEL of 2 mg/kg bw/day for butylparaben (Fisher et al. 1999) and the NO(A)EL of 10 mg/kg bw/day for propylparaben (Oishi 2002) an overestimation of the reproductive hazard of the parabens under study, the applicant decided to repeat the Oishi assay in male rats with a more robust study design. Butylparaben and methylparaben were chosen as test compounds as they were considered to bracket the chain lengths of all parabens used and to allow interpolation of the results for ethylparaben and propylparaben. The full study report was submitted to the SCCP in 2006 and was later published (Hoberman et al. 2008).
After thorough examination, the SCCS identified some important shortcomings and concluded that the repeat studies were not scientifically acceptable (SCCP/1017/06 and SCCP/1183/08). The major comments are summarized below:

1) *Both repeat "reproduction studies" did not follow a well-established scientific protocol (e.g. OECD guideline, EC Regulation No 440/2008 standardised testing method).*

   The applicant argued that as the intention was to refute the results of Oishi, the same protocol was used instead of any officially issued OECD guideline.

   The SCCP accepted this argumentation.

2) *The raw data provided were considered to be insufficient. The study report mentioned that the 64 animals of the repeat assay were from 10 dams, but failed to provide further details (e.g. which pups came from the same dam).*

   Industry argued that cross-fostering at breeding increases diversity. Estimating that a minimum of 13 litters is represented, this is considered to be a large number for a study with 64 animals.

   The SCCP remark, however, was not focused on the number of dams, but specifically on the fact that the test description did not allow to determine which pups could be associated with the same dam. Viewing the suspected illness of the animals, the Committee thought that it could be possible that only a restricted number of dams was involved. By excluding these from the study, the results could have improved.

3) *The body weights of the animals varied considerably. Usually a variation of 20% in body weight is acceptable. The assays under consideration displayed deviations up to 48% within one dosage group.*

   The applicant explained that body weight variations of the laboratory animals were typical for this species strain and age. The animals were younger than those in traditional toxicity studies. The primary selection criterion for the study was for age, not for body weight.

   Independently of the fact that the age of the animals (22 days) and not their body weight was the selection criterion for the tests, a large variation range in body weight leads to a large variation range in the final dosages given to the animals (factor of at least 2). In the Oishi studies, for example, the animals were aged 19-21 days and showed much lower weight variation. It was further recognised, however, that the lack of raw data in these studies seriously hampered analysis of the data provided.

4) *In the methylparaben study protocol it was mentioned that testosterone, follicle-stimulating hormone (FSH) and luteinising hormone (LH) were measured in the blood. These values were not present in the raw data provided.*

   Industry explained that LH and FSH samples were only taken as a back-up in case the main sperm parameters would have shown an effect. Given that no effect was seen for methylparaben, these samples were not further processed.

   The Committee was of the opinion that, since the blood was collected and available, hormone levels should have been measured as it was done for butylparaben, which did not show reproductive effects either.

5) *Standard deviations of the hormone levels measured after butylparaben administration were large and exact sampling times for blood collection were not included in the raw data. This information was considered important as diurnal variations affect hormone levels.*

   Industry responded that standard deviations for hormone levels were typical and that the sampling period was within a specific 2-hour interval in the morning.

6) *26% of the animals displayed unexpected clinical signs such as chromorhinorrhea, chromodacryorrhea, etc., which raised questions about general animal husbandry.*

   Industry explained that the clinical signs were the result of frequent retro-orbital blood sampling for hormone determinations and that the symptoms observed were the typical result of careful, daily, cage side observations made in good laboratories.

   Blood sampling in experimental animals using retro-orbital bleeding, however, is no longer considered a humane method (Hui et al. 2007). In the hands of unskilled operators, side effects typically include blindness, ocular ulcerations, puncture wounds, loss of vitrous humor, infection or keratitis (Hoff 2000). In addition, increases in blood parameters (hormones, glucose, catecholamines) are described to be directly related to stressful methods of blood collection (Hoff 2000, Grouzmann et al. 2003). In case the animals are anesthetised before blood sampling, the interaction with the anesthetic needs to be documented (Hui et al. 2007). Therefore, the SCCP not only considered the observed chromorhinorrhea and chromodacryorrhea as insufficiently explained, but also expressed additional doubts on the relevance of the obtained hormone levels.

7) *Too many adverse effects with statistical significance were dismissed due to the lack of dose-dependency, abnormal high values in control animals, etc.*

   Industry emphasised that, although sporadic statistical changes were observed in their studies with methylparaben and butylparaben, none were dose-responsive, none were consistent over time, and none were corroborated by accompanying effects. One would expect a biologically significant reduction in testosterone concentration to be accompanied by a decrease in weight of testosterone-dependent tissues, or a perturbation in sperm parameters to be accompanied by a change in weight or presence of histopathology in the testis or epididymides. All effects seen were isolated and not dose dependent. They reflected normal variability in the parameters assessed.

   The SCCP, however, considered the numerous parameters affected a significant limitation of the reliability and relevance of the conclusions drawn from the study.

The Industry applicant stressed that there were indications that the Oishi laboratory lacked the expertise to appropriately evaluate the parameters being measured. More specifically, (i) the mean values for some parameters fell far outside the accepted historical control ranges and (ii) the standard deviations in the data were far less than the normal biological variability that has been observed by other groups (details can be found in SCCP/1183/08).

These doubts were shared by the SCCP. Unfortunately, although a formal request was made by the European Commission on behalf of the SCCP, the full protocols and raw data of the Oishi publications were not available.

The SCCP concluded that a) the quality of the Oishi studies could not be properly assessed as the full test description and the complete raw data packages were not available, b) with regard to the Industry repeat studies, although the full descriptions and raw data were available and although some of the questions raised by the SCCP were addressed during an Industry hearing, the remaining issues hampered their acceptance as unarguable refutation of the Oishi findings. This also meant that the NOEL of 2 mg/kg bw/day of butylparaben, obtained in the Fisher et al. (1999) study, was still considered as the NOEL to be used in further calculations.

Between 2008 and 2010, additional *in vivo* data on parabens were published. An overview of the most pertinent ones is given below:

- *Effects of ethylparaben and butylparaben on steroidogenesis in parental rats and offspring after subcutaneous administration to pregnant rats*:
  Ethylparaben and butylparaben (up to 400 mg/kg/day) showed no treatment-related effects on testosterone production, anogenital distance, or testicular histopathology. Butylparaben decreased ERβ mRNA expression in fetal ovaries, and mRNA expression of steroidogenic acute regulatory protein and peripheral benzodiazepine receptor in adrenal glands. However, these effects show no dose-dependency (Taxvig et al. 2008).
- *Effects of isobutylparaben on reproductive parameters and hormone levels after subcutaneous administration to pregnant rats:*
  Isobutylparaben decreased the plasma corticosterone concentration and increased the uterus weight in dams as well as the uterine sensitivity to estrogen in adult female offspring (Kawaguchi et al. 2009a). No dosage level was stated and no positive control was included.
- *Effects of isobutylparaben on emotional behaviour and learning performance in mature offspring after subcutaneous administration to pregnant rats:*
  Subcutaneous administration of isobutylparaben to dams increased anxiety, and specifically disturbed passive avoidance performance of offspring, although the effects were male-specific (Kawaguchi et al. 2009b). No exact dosage level was stated and no positive control was included.
- *Estrogenic effects of butylparaben, isobutylparaben and isopropylparaben measured through the uterotrophic assay (subcutaneous injection in immature female rats and Calbindin-D9-k (CaBP-9k) used as biomarker for estrogenic effects):*
  Butylparaben, isobutylparaben and isopropylparaben induced increased uterine wet weight at 1000 mg/kg/day, at dosage level 1000-fold higher than positive control effect level. The assay gives indication of estrogen-receptor and progesterone-receptor mediated pathways (Vo et al. 2009).
- *Effects of propylparaben and butylparaben on reproductive parameters and hormone levels after subcutaneous administration to pregnant mice:*
  Subcutaneous injection of dosages up to 950 mg/kg/day of propylparaben and butylparaben failed to affect number of pups born, litter weights, individual pup weight and pup survival, whereas 17β-estradiol terminated all pregnancies (Shaw and de Catanzaro 2009).
- *Uterotrophic assay with butylparaben through subcutaneous administration in two different mice strains:*
  Butylparaben does not affect uterine wet or dry mass at any dose in either strain. 17β-estradiol consistently increased uterine mass in both strains (Shaw and de Catanzaro 2009).
- *Studies on suppressive effects of 6 parabens on reproductive organs in female rats during the critical developmental stage:*
  At the highest dosage level (1000 mg/kg/day), each of the tested parabens (methylparaben, ethylparaben, propylparaben, butylparaben, isopropylparaben, isobutylparaben) induces one or more of the following effects: decreased ovary/kidney weight, increased thyroid gland/adrenal weight, reduced serum estradiol levels, decrease

of corporea lutea, increase in number of cystic follicles, myometrial hypertrophy. At lower dosage levels, no dose-dependent effects were noted. IC_{50} values for binding ERα and ERβ receptors are at least 3 orders of magnitude below the ones for 17β-estradiol and as far as their potencies are concerned, the parabens can be ranked as follows: isobutylparaben > butylparaben > isopropylparaben = propylparaben > ethylparaben > methylparaben (Vo et al. 2010).

Sub conclusion 2:

***In vivo* studies on parabens published between 2008-2010 showed effects with relatively high dosage levels (mainly about 1000 mg/kg bw/day) of paraben esters. The recent findings do not clarify the diverging results between the Oishi and Hoberman studies in male rats. The shortcomings of the Hoberman study prevent its acceptance. It cannot be used to refute the Oishi findings; these, in turn, cannot be properly assessed due to the unavailability of raw data.**

This means that the NOEL of 2 mg/kg bw/day for butylparaben, derived from the Fisher et al. (1999) study in the rat, is still considered as the NOEL to be used in further calculations.

For the iso-derivatives of butyl- and propylparaben, and for benzyl- or phenylparaben no suitable data are present.

3.3 DERMAL ABSORPTION AND OTHER TOXICOKINETIC DATA

3.3.1 Dermal absorption

3.3.1.1 Dermal absorption *in vitro*

The Norwegian Institute of Public Health published in 2003 a report (Paulsen and Alexander, 2003), briefly summarising the toxicity of the parabens and using in their calculation of the MoS a value of 3.5% dermal absorption, based on *in vitro* studies with human skin (Cross and Roberts 2000): This document was taken up in the 2005 SCCP opinion on parabens (SCCP/0873/05) and was considered to give a realistic value for dermal absorption. During the commenting period of the present opinion (SCCS/1348/10) however, the SCCS was informed that the value of 3.5% dermal absorption was based on a misinterpretation of the original study results contained in the applicant's dossier and should therefore not be used.

As discussed in SCCP/1017/06, four *in vitro* dermal absorption studies were submitted, one with methylparaben and butylparaben on split-thickness rat and human skin (Fasano 2004b), and three with butylparaben on full thickness **human** or **pig** skin (Fasano 2004a, 2005; Diembeck and Duesing 2005). These studies are summarised in Table 2 in the appendix to this opinion. The SCCP concluded that the studies displayed a number of shortcomings and that they appeared to show a significant dermal absorption of butylparaben in human skin.

The Fasano 2004b study with split-thickness skin indicated there was a higher level of absorption of parabens through **human** skin than through **rat** skin. The generated dermal absorption values were at the level of about 50% for methylparaben and 37% for butylparaben. The metabolism into PHBA more easily occurred in rat skin. This is not in line with the applicant's argument that all esters are quickly metabolised into PHBA in human skin. The cause for this apparent discrepancy may be the fact that the study was not performed with full thickness skin, but with dermatomed skin in which the metabolizing capacity is compromised. The latter view is supported by the findings in Fasano 2004a and 2005, where butylparaben appeared to be largely metabolised in the full thickness **human** skin samples, as mainly PHBA was measured in the receptor fluid. Taking both studies together, 0.23 to 0.67% butylparaben was measured in the receptor compartments of 6 out of the 16 skin samples (for the remaining 10 cells, the butylparaben concentration was below the detection limit). However, in these studies the metabolite distribution in the

different skin compartments and the solubility of both parabens in the receptor fluid was not determined.

Based upon a combination of the three Fasano (2004a,b and 2005) studies, the SCCS derived the value of 3.7% as a worst case assumption for the dermal absorption of unmetabolised butylparaben. This percentage originated from the mean dermal absorption of 37% measured in split-thickness skin (Fasano 2004b), using a correction factor of 10 to account for skin metabolism as seen in the full thickness skin experiments (Fasano 2004a, 2005). The factor of 10 is considered a conservative value as in these studies the measured butylparaben concentration in the receptor fluid was not 10, but 65 to 150 times lower than the metabolite (PHBA)concentration, meaning that butylparaben undergoes extensive metabolism in human skin.

Pape and Schepky (2009) recently re-analysed some existing 'preliminary' dermal absorption results (presumably the Diembeck and Duesing 2005 data) dealing with the penetration of butylparaben through 3 full thickness pig skin samples. The study is only briefly described and appears to show that in the epidermis, butylparaben was found unmetabolised, whereas in the dermis, 50% unmetabolised butylparaben and 50% PHBA were found. In the receptor fluid, mainly PHBA and less than 1% butylparaben were measured. Stability of butylparaben in the receptor fluid was not documented. The report is confusing, mixing percentages with amounts per cm^2, and results from a preliminary study. Finally, the authors mention that other paraben esters (methylparaben, ethylparaben, propylparaben) were also tested under the same conditions, but detailed data were not available to the SCCP/SCCS.

Sub conclusion 3:
The *in vitro* dermal absorption studies point towards a potential difference in dermal absorption and metabolism of higher chain parabens between rodents and humans. Studies with full thickness human skin showed that unmetabolised methylparaben and butylparaben were barely detectable in receptor fluid, whereas studies with split-thickness human skin reveal higher *in vitro* dermal absorption values for unmetabolised butylparaben. Unfortunately, none of the provided dermal absorption assays were of satisfying scientific quality. However, In the absence of new human dermal absorption data, as previously requested by the SCCP, and in the light of the fact that over the last years the weight of evidence approach in risk assessment is given more importance, the available *in vitro* dermal absorption studies on butylparaben were used to derive the value of 3.7%, which is considered to be a conservative estimate. Indeed, both in full thickness and, to a lesser extent, in split thickness human skin studies, a high level of biotransformation of butylparaben was observed although both *in vitro* models are not designed to obtain optimal biotransformation as is the case for freshly isolated human skin.

3.3.1.2 Dermal absorption *in vivo*

In human volunteers exposed for one week to a cosmetic formulation containing 2% of butylparaben, 2% of diethyl phthalate and 2% of dibutyl phthalate, serum measurements revealed that butylparaben was detectable. No effect was noticed on a number of relevant hormone levels: thyroid-stimulating hormone (TSH), luteinising hormone (LH), estradiol, Inhibin B, thyroxine (T_4) and free thyroxine (FT_4) (Janjua et al. 2007). Although these results are supportive for the safety of butylparaben, they do not exclude the possibility of endocrine effects for propylparaben.

Serum analysis showed the presence of unmetabolized butylparaben in the exposed human volunteers. The results were obtained from a combined test of butylparaben with two phthalates, which does not represent ideal test conditions to investigate the specific parabens concerned.

In the current submission, Industry acknowledges that the co-application of high concentrations in the Janjua 2007 study may have saturated skin esterases and produced an increased absorption of intact esters.

Sub conclusion 4:

One study with some shortcomings provides evidence for *in vivo* dermal absorption of butylparaben in the absence of notable effects on hormone levels. No data is available for the other parabens.

3.3.2 Additional toxicokinetic data

3.3.2.1 *In vivo* pharmacokinetic study in the **rat**

Industry proposed to perform an *in vivo* pharmacokinetic rat study through the oral, dermal and subcutaneous route with methyl- , propyl- and butylparaben and requested the approval of the SCCP. The SCCP declared that this study was welcomed, but that it should be supplemented with toxicokinetic studies in human volunteers after dermal application of representative cosmetic products containing propylparaben and butylparaben, since these could deliver essential information (SCCP/1183/08).

The current submission contains the *in vivo* pharmacokinetic rat study, investigating the absorption, plasma kinetics, body distribution, metabolism (determination of plasma metabolites) and excretion of [^{14}C]-labelled short-chain (methyl), medium-chain (propyl) and long-chain (butyl) parabens (Aubert 2009).

Dosage groups consisted of 12 male and 12 female Sprague Dawley rats who received single doses of 100 mg/kg ^{14}C methylparaben, propylparaben or butylparaben via the oral or dermal routes. An additional group of 12 male and 12 female rats were administered a single dose of 100 mg/kg [^{14}C]-butylparaben via the subcutaneous route.

Blood samples were collected from alternating 3 animals per sex and administration route at pre-dose, 0.5, 1, 2, 4, 8, 12, 22 and 24 hours after dosing or the start of dermal exposure, respectively. Blood/plasma samples were analysed for total [^{14}C]-radioactivity by liquid scintillation counting. After the last blood sample, the animals of the kinetic groups were sacrificed.

Plasma metabolic profiling was conducted in pooled samples per group that were collected between 0.5 and 8 hours for the orally, and between 0.5 and 4 hours for the subcutaneously treated groups. For the dermal route, samples were collected at t_{max}. Samples were analysed using a HPLC/UV/radioactivity monitoring system.

For the groups assigned to excretion balance determination, urine, faeces and cage washes were collected up to 168 hours. After this period, the animals were sacrificed, weighed, major organs and tissues were collected and stored frozen up to determination of radioactivity. The results of the study are summarised as follows:

(i) Pharmacokinetics:

Oral administration of methylparaben, propylparaben and butylparaben at 100 mg/kg resulted in high systemic uptake (based on radioactivity) with C_{max} values that generally occurred at 0.5 hrs (t_{max}) and tended to be higher in females than males, ranging from 11.4 (propylparaben, males) to 42.3 (propylparaben, females) µg-equivalents/ml. Corresponding plasma AUC_{0-t} values ranged from 58.3 (propylparaben, males) to 143.6 (methylparaben, females) µg-eq x hrs/ml. Blood levels declined rapidly and reached the limit of quantification at 8 to 22 hours.

Dermal administration of methylparaben, propylparaben and butylparaben at 100 mg/kg resulted in relatively low C_{max} values relative to those measured after oral administration, which ranged from 0.6 µg-eq/ml (propylparaben, males) to 3.1 µg-eq/ml (methylparaben, males) which occurred generally at 8 hrs (t_{max}). Corresponding

plasma AUC_{0-t} values ranged from 5.4 (propylparaben, males) to 20.4 (methylparaben, males) µg-eq x hrs / ml. A small, initial (1 hour time point) peak in the plasma levels in males was attributed to oral uptake, secondary to cage contamination, fur contact and oral uptake. This is, according to the authors, a common observation after open dermal treatment of rats, even in the presence of Elizabethan collars. Blood levels declined rapidly and reached the limit of quantification at 12 or 22 hours.

Subcutaneous administration of butylparaben at 100 mg/kg produced C_{max} values of 6.5 (males) or 12.2 µg-eq/ml (females) with corresponding plasma AUC_{0-t} values of 52.0 (males) or 88.9 (females) µg-eq x hrs/ml, respectively. C_{max} occurred after 2 and 4 hours after injection in males and females, respectively. Blood levels declined rapidly and reached the limit of quantification at 12 to 22 hours.

(ii) Plasma metabolite characterisation

Pooled plasma samples were collected and analysed by HPLC/[^{14}C]-detection. For the dermal route, only samples collected at t_{max} were analysed as other time points provided insufficient concentrations for analysis. In all plasma samples, independent of time of collection, paraben type and route of administration, only a single peak was found, which corresponded to PHBA. No evidence for the presence of parent parabens or other parabens-related metabolites was found. These results suggest that, in rats, after oral, dermal or subcutaneous administration of parabens, the principal systemic exposure agent is PHBA.

(iii) Excretion balance – oral administration

Following oral administration, the mean recovery of [^{14}C] in rats treated with methylparaben, propylparaben or butylparaben ranged from 89 to 95% of the applied [^{14}C], suggesting an adequate mass balance. Urinary excretion was the major pathway of elimination (range: 71 to 84% of the administered [^{14}C], suggesting similar bioavailability for all parabens, whereas faecal excretion was low to negligible, i.e. in the range of 1% of the administered [^{14}C]. The elimination of [^{14}C] via the urine was rapid and occurred mainly during the first 24 hours after administration. After sacrifice (168 hours), a very small amount of [^{14}C] was retained in the tissues and ranged from non-detectable to 2% of the administered dose. These data suggest rapid clearance of a single dose from the organism and absence of selective storage in organs or tissues.

(iv) Excretion balance – dermal administration

Following dermal administration, the mean recovery of [^{14}C] in rats treated with methylparaben, propylparaben or butylparaben ranged from 104 to 116% of the applied [^{14}C], suggesting an adequate mass balance. Most of the radioactivity was recovered in the swaps used for treated skin area and cage cleaning (upper part) at the end of the exposure period (range: 46 to 58% of the applied radioactivity).
Urinary excretion was the major pathway of elimination (range: 14.5 to 27.1% of the administered [^{14}C]) suggesting significant skin penetration and similar systemic availability for all parabens, whereas faecal excretion was negligible. The elimination of [^{14}C] via the urine was rapid and occurred mainly during the first 48 hours after administration. After sacrifice (168 hours), a very small amount of [^{14}C] was retained in the organs or the treated skin sites and ranged from non-detectable to 2% of the administered dose. The remainder of radioactivity was recovered in the carcasses (range: 21 to 37% of total radioactivity).
In the absence of significant skin or organ residues, these residues were attributed to the fur, muzzle and paws secondary to the open administration and subsequent cage contamination. Overall, these data suggest rapid clearance of a single dose from the organism and absence of selective storage in organs or tissues.

(v) Excretion balance – subcutaneous administration (butylparaben only)

Following subcutaneous injection, the mean recovery of [^{14}C] in rats treated with butylparaben was 84.0 and 82.7% of the administered [^{14}C] for males and females

respectively, suggesting an almost complete mass balance. Urinary excretion was the major pathway of elimination (range: 67 to 76% of the administered [^{14}C]), suggesting similar bioavailability, whereas faecal excretion was negligible. The elimination of [^{14}C] via the urine was rapid and occurred mainly during the first 24 hours after administration. After sacrifice (168 hours), a very small amount of [^{14}C] was retained in the tissues and ranged from non-detectable to 2% of the administered dose; a single carcass contained 2.3% of the applied radioactivity.
These data suggest rapid absorption and clearance of a single subcutaneous dose of butylparaben from the organism and absence of selective storage in organs or tissues.

Blood plasma analysis in all parabens-treated groups following all exposure routes showed only the presence of PHBA. For the dermal route, only samples collected at t_{max} were analysed as other time points provided insufficient concentrations for analysis. In all plasma samples, independent of time of collection, paraben type and route of administration, only a single peak was found, which corresponded to PHBA. No evidence for the presence of parent parabens or other parabens-related metabolites was found.
Plasma data after oral or subcutaneous, but not after dermal administration showed a trend towards higher systemic exposure values in females when compared with those in males. Overall, oral administration produced plasma values suggesting high systemic uptake for all parabens; after dermal administration, the systemic exposure was approximately an order of magnitude lower than that after oral dosing, whereas subcutaneous injection of butylparaben produced exposure patterns that resembled that of oral (similar C_{max} and AUC values) as well as dermal (delayed t_{max} values) administration.

Pharmacokinetic results showed plasma patterns typical for the different routes of administration: high C_{max} and AUC values were observed after oral dosing, after dermal administration the respective values were approximately one order of magnitude lower, whereas subcutaneous dosing produced similar, but somewhat lower values relative to those seen after oral administration. The principal route of excretion was via the urine and no selective organ / tissue storage was observed.

Sub conclusion 5:
The toxicokinetic study confirms that, in rats, short-, mid- and long-chain parabens are rapidly absorbed and eliminated after single oral or subcutaneous administration. After dermal administration, they are partly (15 to 27%) absorbed and rapidly eliminated. Blood analysis only showed the presence of PHBA.

3.3.2.2 Requested *in vivo* pharmacokinetic study in **human** volunteers

Although this study was requested, Industry chose not to perform it. The following argumentation was given:

The design of a comprehensive and relevant human clinical study would encounter significant problems. The choice of a relevant dose and vehicle would have to be carefully assessed. Trying to mimic a real life exposure dose from cosmetic products would probably produce very low plasma levels necessitating the use of extremely sensitive analytical equipment (LC/MS/MS). In order to show skin metabolism one would have to quantitatively characterise systemic metabolites. The principal metabolite of parabens, PHBA, is ubiquitous in plants and human nutrition and expected to naturally occur in humans. In addition, PHBA is a widely used preservative in consumer care products and food. Therefore, in order to distinguish systemic levels of PHBA resulting from topical exposure to parabens in cosmetics from those that result from food or other sources, such a study would require skin application of [^{14}C]-labelled parabens. However, ethical constraints limit the amount of [^{14}C] that may be applied to human skin.
The results of the rat study showed that, after dermal administration of high doses of [^{14}C]-parabens to rat skin, resulting plasma levels of [^{14}C]-PHBA were relatively low. Taking into account the sensitivity of [^{14}C]-detection and metabolite characterisation in rat plasma, the method permitted to track the major metabolite PHBA, but was not sufficiently sensitive to identify trace amounts of intact parabens.

Given that a human study would have to apply lower amounts of radioactivity and taking into account that human skin is less permeable than rat skin, a human study with [^{14}C]-parabens is expected to have even lower sensitivity and would not address the question of the ratio of parabens that reach the systemic circulation intact.
As a last resort, a human study would have to be conducted under total dietary control and analysis excluding food and other products that contain PHBA.
Theoretically, such data could be generated with large efforts in time and resources. However, considering the limited actual human exposure to long-chain parabens (Cowan-Ellsberry and Robison, 2009) and the current state of knowledge as well as the weight-of-evidence with regard to skin penetration/metabolism of parabens, and weighing it against the relatively limited new information that could be obtained in a new human PK study, the available information appears to sufficient for a human risk assessment.

Sub conclusion 6:

The requested *in vivo* pharmacokinetic data in human volunteers after exposure to paraben-containing cosmetic products are not available.

3.3.2.3 Paraben exposure in humans: additional data

As noted above, butylparaben has been detected in serum of volunteers who had been exposed to a cosmetic formulation containing 2% of butylparaben and 2% each of two different phthalates (Janjua et al. 2007). In a follow-up analysis, the authors analyzed also urinary concentrations of butylparaben and metabolites by LC-MS/MS in 24h urine collected before and after topical application (Janjua et al. 2008). All subjects showed increased levels in urine during treatment: butylparaben excretion was 2.6 ±1.1 mg/24h which corresponds to 0.32 % of the applied dose. This indicates that part of the dermally applied butylparaben is not hydrolyzed to PHBA.

A biomonitoring study examined urinary concentrations of free and conjugated methylparaben, ethylparaben, propylparaben, butylparaben (n- and iso-), and benzylparaben in a demographically diverse group of 100 adults in the US (Ye et al. 2006). Methylparaben and propylparaben were detected at the highest median concentrations (43.9 ng/ml and 9.05 ng/ml, respectively) in nearly all (> 96%) of the samples. The other parabens were detected in more than half of the samples (ethylparaben 58%; butylparaben 69%), and at much lower levels (1.0 ng/ml and 0.5 ng/ml, respectively). Although parabens in urine appear predominantly in their conjugated form (glucuronides, sulfates), free parent compounds were also detected. Similar median urinary levels of methylparaben and propylparaben seen in the Ye et al. (2006) study were reported in a recent biomonitoring study of methylparaben and propylparaben in 77 Harvard students (Carwile et al. 2009).

The concentration of five parabens, methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben in urine, serum and seminal plasma samples from 60 healthy Danish men were examined, using a sensitive and specific LC-MS/MS method for simultaneous determination of the five parabens in the three different matrices (Frederiksen et al. 2010). Highest concentrations of the parabens were found in urine, wherein methylparaben, ethylparaben, propylparaben and butylparaben were measurable in 98%, 80%, 98% and 83% of the men, respectively. Benzylparaben was only measurable in urine from 7% of the men. Serum and seminal plasma samples revealed the presence of mainly methylparaben and propylparaben, although in seminal plasma, butylparaben was also detected. Overall, urinary paraben concentrations correlated to the paraben concentrations in both serum and seminal plasma (Frederiksen et al. 2010).

Sub conclusion 7:

Human biomonitoring studies show the presence of parabens (free and conjugated species) in urine and/or serum and seminal plasma. Although these biomonitoring studies can neither discriminate between paraben exposure from oral intake or dermal application, nor between sources of exposure (medicinal

products, cosmetics, etc.), the presence of free and conjugated parabens in urine and/or serum and seminal plasma clearly indicates that –in contrast to the situation in rat– the compounds are not completely hydrolysed into the metabolite PHBA.

3.4 SUMMARY OF CONCLUSIONS RELATED TO ISSUES 3.1-3.3

1) Human-based *in vitro* data show an increasing potential for **endocrine modifying effects** with **increasing chain length**. **PHBA**, a common metabolite of all paraben esters, however, appears to exhibit **no endocrine modifying effects**.

2) The major repeated dose studies in rat (Oishi 2001 and 2002, Hoberman et al. 2008) are controversial and provide very **divergent critical effect levels** for butylparaben ranging from a **LOAEL** of **10 mg/kg bw/day** to a **NOAEL 1000 mg/kg bw/day**, respectively. Older data on butylparaben revealed a reproductive **NOEL of 2 mg/kg bw/day** in the rat (Fisher et al. 1999). The latter will be used as a conservative value in further calculations.

3) The presented in vivo pharmacokinetic studies on methylparaben, propylparaben and butylparaben in the rat (oral, dermal, subcutaneous administration) show that these parabens are rapidly absorbed and eliminated in this species.
Available in vitro dermal absorption study results point towards a **potential difference** not only in dermal absorption (Fasano 2004b, Pape and Skepky 2009) but also **in metabolism** of higher chain parabens (Ye et al. 2006, Janjua et al. 2007) **between rat and man**. Consequently the rat data as such cannot be simply extrapolated to the human situation without additional supportive data.
To this respect, no human study results for the parabens under discussion (with the exception of butylparaben) are available that show unchanged levels of hormones which are of importance for the ongoing discussion.
Furthermore, no metabolism studies have been submitted that clearly prove that no difference in metabolism exists between the rat and man. Such studies are needed to show that the higher chain parabens are completely metabolised into PHBA as claimed by industry. The biomonitoring studies presented in Section 3.3.2.3 indicate that in the **human body**, **parabens** may **not be completely hydrolysed into PHBA**. This means that the **necessary data needed to demonstrate that the available results for rats are also valid for humans are still missing**.

Until a properly conducted dermal absorption and toxicokinetic study in humans will allow the assignment of a more scientifically solid value, the SCCS will use a dermal absorption value of 3.7% in its MoS safety calculations. The value of 3.7% used in this opinion originates from a pragmatic approach combining three *in vitro* dermal absorption studies. The first one is a split-thickness *in vitro* study (i.e. a study lacking major skin metabolism), which shows a dermal absorption of butylparaben of 37% (Fasano 2004b). Two other studies were performed with full-thickness skin, which is better equipped for biotransformation. These studies show that butylparaben can be measured in the receptor fluid at concentrations which are 65 to 140 times lower than the metabolite (PHBA) concentrations, meaning that butylparaben undergoes extensive metabolism in human skin. Nevertheless, as the study does not provide individual butylparaben/PHBA concentration levels in the different skin compartments, the SCCS prefers to follow a conservative approach by applying a correction factor of 10 to the dermal absorption value obtained with butylparaben in the split-thickness skin study. The SCCS considers this corrected value to be a realistic high end value, which is more conservative than the value of 1% proposed by the Industry and the 2% value proposed by the Danish DTU (2010).

4. DISCUSSION

Not only Industry and the SCCS, but also other stakeholders expressed their views on the safe use of parabens in cosmetic products. In order to provide an as complete as possible picture on all the available information, the individual points of view of all parties are also summarized below.

4.1 VIEW OF THE INDUSTRY

The current Industry submission uses the following argumentation to declare all parabens safe for use:

1. The choice of the reproduction NO(A)EL value:
   Industry emphasizes that the Oishi (2001) study is not reliable and that the CTFA/Colipa study (Hoberman et al. 2008) is well performed. One of their arguments is that the SCCP (2008) acknowledged the scientific value of the new study.
2. Toxicokinetic aspects related to the risk assessment of parabens:
   Industry presents a large pharmacokinetic study in the rat using different routes of exposure (Aubert 2009). A major conclusion is that in the rat, independent of the route of exposure, parabens are quickly hydrolysed and only occur in the systemic circulation in the form of the metabolite PHBA. In addition, excretion is rapid and mainly occurs via the urine. Total dermal absorption (parent compound + metabolites) in the rat is estimated to be around 27%.
3. With regard to the requested human toxicokinetic study:
   Industry decided not to perform it (arguments stated under 3.3.2.2).
4. For the final safety assessment of the parabens, the following parameters are taken into account:
   - The NO(A)EL used for all paraben esters is the Hoberman et al. (2008) value of 1000 mg/kg/day.
   - For the calculation of the SED, the cumulative value of 17.4 g/day is used (SCCS Notes of Guidance, SCCS/1416/11), assuming that parabens may be used as a preservative in all cosmetic products.
   - Only 1% of the paraben level is assumed to become systemically available, due to the hydrolysis of the parent compound into PHBA (based upon Schepky et al. 2009).

   The MoS values obtained are 83,300 for the individual paraben esters and 41,600 for the paraben mixture. An additional calculation takes into account aggregate exposure through non-cosmetic use of parabens as described by Cowan-Ellsberry and Robison (2009), but this does not add to the current discussion.

4.2 VIEW OF THE COSMETIC INGREDIENT REVIEW PANEL (CIR)

In 2008, the CIR Expert Panel reviewed the safety assessment of methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutyl- and benzylparaben in cosmetic products (CIR, 2008). For their MoS calculations for the whole range of parabens, they used the NOAEL of 1000 mg/kg/day of the Hoberman et al. (2008) study, which was considered as the "*most statistically powerful and well-conducted study on the effects of butylparaben on the male reproductive system*".

4.3 VIEW OF THE EUROPEAN FOOD SAFETY AUTHORITY (EFSA)

The EFSA review panel used the 1000 mg/kg/day level for methyl- and ethylparaben, but considered more data necessary to determine a NO(A)EL value for propylparaben (EFSA, 2004).

4.4 VIEW OF THE DANISH NATIONAL FOOD INSTITUTE

As supplementary information for the drawing up of the current opinion, the European Commission provided the SCCS with the 'Update on uptake, distribution, metabolism and excretion (ADME) and endocrine disrupting activity of parabens', a report by the Danish National Food Institute, Technical University of Denmark (DTU 2010), later published as an article of Boberg et al. (2010).

This report summarises all available scientific literature on the subject (including SCCP opinions and literature data stated in the current opinion) and comes to the following major conclusions:

- Adverse effects were noted on sperm production and testosterone levels in young male rats exposed to butylparaben, isobutylparaben and propylparaben (Oishi publications).
- Parabens have been shown to be estrogenic *in vitro* and in uterotrophic assays *in vivo*, and estrogenicity appears to increase with side chain length.
- The ability of parabens to activate the estrogen receptor may not be the only mechanism of action, as they also show anti-androgenic effects, mitochondrial toxicity and ability to elevate endogenous estrogen levels via SULT inhibition.
- The use of the 1000 mg/kg/day value used by the CIR-panel is not supported by the DTU since this value was derived from an animal study with many shortcomings, as already pointed out by the SCCP in 2006 (SCCP/1017/06). The DTU refers to the LO(A)EL value of 10 mg/kg/day derived from a published Japanese study (Oishi 2002) with propylparaben.
- The maximal dermal uptake of intact parabens is estimated to be 2% (conjugated and free), based on the results of Janjua et al. 2008.
- The total dermal uptake of parabens and metabolites amounts to 80%. Higher uptake and less metabolism were measured in human skin than in the applied rat models. However, more studies are needed to examine human levels of parabens and metabolites and to compare these levels to those obtained in experimental animal studies. It needs to be determined whether the endocrine disrupting effects seen in experimental animals are due to the (low) levels of intact parabens, or whether metabolites such as PHBA may play a role.

Finally, the DTU included a list of data gaps on parabens, among which reproduction studies on both long- and short-chain parabens, extended toxicokinetic studies (*in vitro* and *in vivo* combination assays) and studies exploring novel endpoints such as mammary development.

4.5 VIEW OF THE DANISH ENVIRONMENTAL PROTECTION AGENCY (EPA)

As supplementary information, DG SANCO provided the SCCS with the 'Survey and Health Assessment of the exposure of 2 year-olds to chemical substances in Consumer Products' by the Danish EPA (2009). The latter reports on a large-scale project investigating the exposure of 2 year-olds to chemical substances through contact with consumer products, carried out in Denmark from July 2008 to September 2009. A total of 12 product groups were included in the survey phase. Several substances were selected because of their endocrine modifying effects in animal studies. Among these chemicals were propylparaben, butylparaben and isobutylparaben.

For the individual risk assessments, however, the report refers to all SCCP opinions on parabens and the remaining uncertainties/open questions. In the report it is concluded that the amounts that 2 year-olds absorb from propylparaben and butylparaben can constitute a risk for estrogen-like modifications of the endocrine system. This contribution originates predominantly from cosmetic products such as oil-based creams/moisturising creams/lotions and sunscreens and was dealt with earlier (see Notes of Guidance).

4.6 VIEW OF THE SCCS

In light of the available data, including the latest Industry submission, the following conclusions can be made:

- The potential of butylparaben and propylparaben to modify the endocrine system is the major concern related to the use of parabens in cosmetics. Therefore, the availability of a sound *in vivo* reproductive toxicity study is essential in the hazard assessment of the different esters. However, no unequivocal conclusion can be drawn from the available male reproductive toxicity studies of Hoberman et al. (2008) and Oishi (2001; 2002a,b; 2004) with butylparaben and/or propylparaben. They deliver contradictory results and neither of them is considered to be scientifically acceptable. Therefore the SCCS cannot determine an adequate NO(A)EL-value for the paraben esters under consideration from these studies. Consequently, the **NOEL** value of **2 mg/kg bw/day**, based on Fisher et al. (1999) and also mentioned by Oishi (2001), remains the **conservative choice** for the calculation of the MoS of butyl- and propylparaben. The Committee acknowledges the fact that the Fisher et al. (1999) study involves subcutaneous instead of oral administration, but emphasizes that 2 mg/kg bw/day clearly represents a NOEL instead of an NOAEL and that another study shows butylparaben to cause similar effects at about the same dosage levels after subcutaneous or oral administration (Routledge et al. 1998).
- With regard to the toxicokinetic aspects related to parabens, the SCCP not only requested sound *in vitro* dermal absorption data, but also the performance of a human study in order to obtain adequate and detailed information on the absorption and metabolism of paraben esters in human skin. This request was based upon the fact that the observed *in vitro* (human and rat cell lines)/*in vivo* (rats and mice) endocrine modifying effects caused by parabens were attributed to the parent compounds and not to their common metabolite PHBA.

  Industry uses the argument that paraben esters are quickly and nearly completely hydrolyzed into PHBA after dermal application to human skin, so their systemic toxicity becomes negligible. The SCCS, however, is aware of studies indicating that the biotransformation of the different paraben esters into PHBA is not as efficient as claimed. The weight of evidence in this matter is described in Section 3.3.

  The available set of *in vitro* dermal absorption studies is considered of poor scientific quality and the results of biomonitoring studies show the presence of unmetabolised parabens in the plasma of human volunteers. This emphasizes the importance of sound *in vivo* human data, obtained by administration of parabens through the dermal route. To this respect, the applicant cites a human study (Janjua et al. 2007) in which three putative estrogens, among which butylparaben, were together in a cream applied to the skin of 26 volunteers. The fact that three substances were combined in this assay and that no metabolite measurements were performed decreases the scientific value of the results obtained for the present risk assessment.

  Considering these points together, the SCCS is of the opinion that the issues raised earlier by the SCCP (SCCP/1183/08) have not been sufficiently addressed. Although the provided data is quite informative, there still is the missing link between the rat and human dermal absorption, especially of the absorption and metabolism of the parent compound in the skin. According to the applicant, the metabolism of the absorbed parabens through the skin is complete, but no study performed on human volunteers provides conclusive results.

  As dermal absorption is prone to species variability (especially between humans and rats), the rat toxicokinetic study, as currently presented, does not provide a conclusive answer.

  Industry's argumentation that 'real life exposure would probably produce very low plasma levels necessitating the use of extremely sensitive analytical equipment

(LC/MS/MS) .....' is not considered valid, as such equipment is now state-of-the-art in all modern analytical laboratories, and it has been applied successfully in measuring numerous parabens in human urine and/or plasma samples (Ye et al. 2006, Janjua et al. 2007).

In addition, the applicant explains that 'the principal metabolite of parabens, PHBA, is ubiquitous in plants and human nutrition and expected to occur naturally in humans'. Therefore Industry considers that 'in order to distinguish systemic levels of PHBA resulting from topical exposure to parabens in cosmetics from those that result from food and other sources, such a study would require skin application of [^{14}C]-labelled parabens and raises ethical constraints'.

The SCCS is aware of the problem that non-cosmetic exposure to PHBA could invalidate an interpretation of results that are based on metabolite analysis. However, the main point of interest is dermal absorption of unmetabolised parabens after topical application, and this would not necessarily require the use of radiolabeled compounds. Parabens are apparently not completely hydrolyzed to PHBA as indicated in several human studies (Ye *et al.* 2006, Janjua *et al*. 2007, Janjua *et al*. 2008, Carwile *et al*. 2009, Frederiksen *et al*. 2010).

As long as properly conducted dermal absorption and/or toxicokinetic studies in humans are not available, the Committee chooses to use a pragmatic approach and to base its calculations on the 3.7% dermal absorption value derived from the results of three in vitro dermal absorption studies (full rationale under 3.4). The limited data available for human in vivo studies support the assumption of an absorption value for unmetabolised parabens in the lower one-digit percentage range. This value is a more conservative estimate than the 1% proposed by Industry and the 2% value proposed by the Danish Technical University (2010)

- The MoS calculation as proposed by Industry, based upon the Hoberman et al. (2008) NO(A)EL value and a 1% dermal absorption is not acceptable for the following reasons:
  - the 1% value of systemic availability results from a re-analysed 'preliminary' dermal absorption study (Pape and Schepky 2009), of poor quality. In case parabens are completely hydrolyzed into PHBA, the latter will become systemically available.
  - the reproductive toxicity NO(A)EL is based on a study with insufficient scientific reliability. Using the *in vivo* estrogenicity studies and applying additional safety factors is not feasible either, as all studies are performed either through subcutaneous or oral route, meaning that skin metabolism is avoided. Therefore, with the current level of knowledge, their relevance for this risk assessment is not clear.

  Of the three assumptions present in the MoS calculation proposed by the Industry, being the dermal absorption value, the NO(A)EL value and the finished product exposure level, only the latter seems acceptable.

As explained before, the SCCS uses the following parameters for the final calculation of the MoS of butylparaben:

Dermal absorption:	3.7%
Intended concentration in finished product:	0.4%
Typical body weight:	60 kg
Cumulative exposure to preservatives:	17.4 g/day
NOEL (subcutaneous, rat, 17 days):	2.0 mg/kg bw/day

$$SED = \frac{17400 \text{ mg/day} * 0.4/100 * 3.7/100}{60 \text{ kg}} = 0.043 \text{ mg/kg bw/day}$$

MoS = NOEL / SED = 46.6

This means that, in order to obtain a MoS ≥ 100, **the concentration of butylparaben in the finished cosmetic product needs to be reduced to 0.19%.**

5. OPINION

With respect to the safe use of parabens as cosmetic ingredients, concern was expressed as to the potential endocrine modifying effects of parabens of higher chain length including propylparaben, butylparaben and related *iso* compounds. Benzylparaben was also of concern. Based upon the currently available *in vitro* data and *in vivo* rodent test results, the SCCS agrees that the estrogenic properties displayed by parabens appear to increase with increasing chain length. Nevertheless, the SCCS stresses that the displayed potency levels remain about 3 to 6 orders of magnitude lower than the potency of the positive controls.

It is difficult to determine an adequate NO(A)EL value for the observed reproductive effects of butylparaben or propylparaben in rodents, as each of the two available key (sets of) oral studies suffered serious shortcomings. Industry attempted to resolve this issue by providing data to suggest the complete skin metabolism of parabens into the non-endocrine modifying and non-reproductive toxic metabolite p-hydroxybenzoic acid (PHBA).

Unfortunately, this data consisted of pharmacokinetic results from rodent studies only, whereas other reports clearly pointed towards a potential difference in dermal absorption between rats and humans (Fasano 2004b, Pape and Schepky 2009) and to differences in metabolism of the compounds concerned. Substantial amounts of unmetabolised parabens were detected in human/pig skin samples (Janjua et al. 2007, Ye et al. 2006, Fasano 2004a) and in urine of exposed volunteers (Carwile et al. 2009). Thus, for human skin, no clear demonstration is given of fast and complete metabolism of higher chain length parabens into the common and inactive metabolite PHBA, as is the case in rats.

Therefore, the SCCS cannot ascertain that butylparaben and propylparaben are completely metabolised into PHBA after application to human skin, and still considers the parent compounds as potentially systemically available, however not to an unlimited extent. Due to the lack of properly conducted dermal absorption and/or toxicokinetic studies in humans, the SCCS derived the conservative value of 3.7% dermal absorption for butylparaben. This leads to a MoS of 47 for both butylparaben and propylparaben at the intended use concentration of 0.4% (applying a read-across approach for these two esters).

As the two male reproductive toxicity studies in rodents are of insufficient scientific quality, the NOEL of the Fisher 1999 study (2 mg/kg bw/day) is used as the most conservative value by the SCCS.

Based upon the above, the SCCS considers 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%. This conclusion is based on the lack of scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular with regard to the metabolism of the parent compound in the skin. The latter can only be addressed through additional human data.

With regard to methylparaben and ethylparaben, the previous opinion, stating that the use at the maximum authorized concentrations can be considered safe, remains unchanged.

Finally, the SCCS emphasizes that the studies submitted to the Committee primarily concerned propyl- and butylparaben. Limited to no information was submitted for the safety evaluation of isopropyl-, isobutyl-, and phenylparaben. Therefore, for these compounds, the human risk cannot be evaluated.

The same is true for benzylparaben and pentylparaben (the latter not mentioned earlier in SCC(NF)P/SCCS opinions), two esters for which there are indications that they might be used in cosmetic products for 'other purposes', e.g. for their anti-microbial activity. None of them is listed in Annex VI of the Cosmetics Directive, as they do not fall under the indicated 'esters of 4-hydroxybenzoic acid' of entry n°12. The SCCS wishes to draw the attention of the Commission services to this anomaly, which may have effects on consumer safety.

6. MINORITY OPINION

Not applicable

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APPENDIX

Table 1: Literature overview on estrogenicity-related properties of parabens

Test substances	Test system	Test principle(s)	Result(s)	Reference
***In vitro* assays**				
MePB EtPB PrPB BuPB	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Principle of gene expression profiling based on DNA microarray analysis with 120 genes selected as showing greater statistical reliability for estrogen-responses.	Clear difference in expression profile between EtPB and PrPB. The activity showed a positive correlation with the chain length of esters. Clear correlation between profiles of PrPB and BuPB. Nevertheless, profiles of PrPB and BuPB were closer to each other than the estrogen profile was to any of them.	Terasaka et al. 2006
MePB EtPB PrPB BuPB PHBA	Skin and liver cytosol and **human** epidermal keratinocytes	Parabens elevate estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin	SULT activity was inhibited in skin cytosol by MePB, EtPB, PrPB, BuPB, **not** by PHBA. Potency increased with chain length (IC_{50} BuPB = 37 µM). No inhibition of androgen sulfation. In the human epidermal keratinocytes, BuPB displayed an IC_{50} of 12 µM. No pos. control was included.	Prusakiewicz et al. 2007
MePB PrPB BuPB PHBA flutamide vinclozolin	a stably transfected **human** embryonic kidney cell line that lacks critical steroid metabolizing enzymes	Investigate anti-androgenic activity by measuring inhibition of 0.1 nM testosterone (T)-induced transcriptional activity	MePB, PrPB, BuPB inhibited 0.1 nM T-induced transcriptional activity at concentrations above 10 µM (max. 40% inhibition). PHBA was negative. Pos. controls (flutamide and vinclozolin) inhibited 1nM T-induced signal at concentrations of 0.1 to 10 µM (11 to 90% inhibition).	Chen et al. 2007
MePB EtPB PrPB BuPB IsoPrPB IsoBuPB BzPB PHBA 17β-estradiol	MCF-7 cells (**human**-breast cancer derived cell line shown to be estrogen responsive)	Investigate estrogenic effects of mixtures of parabens on cell proliferation; investigate anti-estrogenic effect through inhibition of aromatase, the enzyme that converts androgens into estrogens	EtPB, PrPB, BuPB, IsoPrPB, IsoBuPB and BzPB induced cell proliferation with EC_{50} values between 0.5 and 10 µM. PHBA was negative. Assays with mixtures of PB showed an additive effect. Potency of PB remains 5 to 6 orders of magnitude below that of 17β-estradiol. Parabens inhibited aromatase with IC_{50} values between 3.5 and 26.4 µM, but there was no link between chain length and IC_{50}. PHBA was negative. Authors note that typical human PB concentrations (10-80nM) are much lower than EC_{50} and IC_{50} values encountered here.	van Meeuwen et al. 2008

Test substances	Test system	Test principle(s)	Result(s)	Reference
EtPB BuPB	Human adrenocortical carcinoma cell line **rat** pituitary GH3 cell line	H295R assay evaluating the ability to interfere with steroid hormone biosynthesis and T-screen assay to define whether the compound is either a thyroid hormone receptor agonist or antagonist by investigating binding and activation of the thyroid receptor (TR), resulting in GH3 cell proliferation	Progesterone production was increased in H295R assay at 30 µM EtPB and BuPB. No effect on testosterone or estradiol production. No positive control included. BuPB increased cell proliferation in GH3 rat cells at 3 µM; considered potential weak TR-agonist. No positive control included.	Taxvig et al. 2008
***In vivo* experiments**				
MePB BuPB	Alpk:AP **rat**	Uterotrophic assay with both immature and ovariectomized rats. MePB and BuPB were administered at the following dosage levels: - MePB orally at 40, 400 and 800 mg/kg/day - MePB subcutaneously (sc) at 40 and 80 mg/kg/day - BuPB orally at 4, 40, 400, 800 and 1200 mg/kg/day - BuPB subcutaneously at 40, 200, 400, 600, 800, 1000 and 1200 mg/kg/day	MePB administered sc or orally failed to increase uterus weights up to 800 mg MePB/kg/day. BuP given orally increased uterus wet and dry weights at dose levels ≥ 800 mg BuPB/kg/day, whereas subcutaneous administration increased uterus wet weights at dosages ≥ 400 mg/kg/day. The lowest dosage level inducing any uterotrophic response was 200 mg BuPB/kg/day. The positive control estradiol exerted its adverse effects at 0.04 mg/kg/day (sc).	Routledge et al. 1998
BuPB	Wistar **rat**	Effects of neonatal exposure to BuPB on development of rat testis after subcutaneous administration of 2 mg BuPB/kg/day for 17 days (postnatal days 2-18).	No detectable effect on any of the measured reproductive parameters (testis weight and histological examination).	Fisher et al. 1999
BuPB	Wistar **rat**	Study of the potential reproductive effects of BuPB on male rats (19-21 days old), receiving BuPB through the oral route for 8 weeks at dosage levels of 10.4, 103 and 1026 mg/kg/day.	There were no treatment-related effects on testes, ventral prostates and preputial glands in any of the groups. Decreases in cauda epididymal sperm reserve, sperm count, daily sperm production and in serum testosterone concentration were observed from 10.4 mg/kg/day onwards.	Oishi 2001
PrPB	Wistar **rat**	Study of the effects of PrPB on general function of the male rat reproductive system. Rats (19-21 days old) received PrPB through the oral route for 4 weeks at dosage levels of 12.4, 125 and 1290 mg/kg/day.	There were no treatment-related effects on testes, epididymides, ventral prostates, seminal vesicles and preputial glands in any of the groups. At all three dosage levels, however, a decrease in cauda epididymal sperm reserve, sperm count and daily sperm production was observed and from 125 mg/kg/day on, serum testosterone concentration was decreased.	Oishi 2002a
BuPB	CD-1 ICR **mice**	Study of the effects of BuPB on general function of the male mouse reproductive system. Mice (25-27 days old) received BuPB through the oral route for 10 weeks at dosage levels of 14.4, 146 and 1504 mg/kg/day.	Administration of BuPB at 146 and 1504 mg/kg/day caused an increase in epididymal weights, a decrease in testis spermatid count and in serum testosterone concentration. The NOAEL is stated to be 14.4 mg/kg/day.	Oishi 2002b

Test substances	Test system	Test principle(s)	Result(s)	Reference
IsoBuPB	CD1 **mice**	Uterotrophic assay with IsoBuPB in the mouse at following subcutaneous dosage levels (supposing a mouse of 18 days old weighs about 30g) of: - 40 mg/kg/day (1.2 mg/mouse) - 400 mg/kg/day (12 mg/mouse)	Wet uterine weight was increased at both dosage levels. Positive control 17β-estradiol exerted comparable effects at 167 ng/kg/day (5 ng/mouse).	Darbre et al. 2002
BuPB	Sprague Dawley **rats**	Study of the effect of BuPB on the development of the reproductive organs of F1 offspring when pregnant rats are subcutaneously injected with 100 or 200 mg BuPB/kg/day from gestation day 6 to postnatal day 20 (lactation period).	At both dosage levels, the weights of testes, seminal vesicles and prostate glands were decreased, together with the sperm count and the sperm motile activity in the epididymis. Testicular expression of estrogen receptor (ER)-α and ER-β mRNA was significantly increased at the highest dosage level.	Kang et al. 2002
MePB EtPB	Wistar **rat**	Study of the effects of parabens on testosterone secretion and the function of the male reproductive system in rats receiving the test substances orally at dosage levels of ± 100 and 1000 mg/kg/day. Rats were 25-27 days old and received the parabens for 8 weeks.	MePB and EtPB did not affect the male reproductive system including anti-spermatogenic activity to about 1000 mg/kg/day.	Oishi 2004
EtPB BuPB	Wistar **rat**	Study of the effect of parabens on the steroidogenesis in rats and their offspring when dams are subcutaneously exposed to either: - 400 mg EtPB/kg/day; or - 200 - 400 mg BuPB/kg/day from gestation day 7 to 21.	Neither EtPB nor BuPB showed any treatment-related effects on testosterone production, anogenital distance, or testicular histopathology. BuPB caused a significant decrease as well in the mRNA β-ER expression level in fetal ovaries, as in mRNA expression of steroidogenic acute regulatory protein and peripheral benzodiazepine receptor in the adrenal glands. However, these effects show no dose-dependency.	Taxvig et al. 2008
IsoBuPB	Sprague Dawley **rats**	Study designed to clarify the estrogenic effects during gestation and lactation on the endocrine systems of dams and offspring by measuring - in dams: plasma hormone concentrations and organ weights - in offspring: ratio of male pups, anogenital distance, organ weights and plasma hormone concentrations, puberty, estrous cycle and response of organ weight and plasma hormone concentrations to estrogen in adult females, and reproductive and adrenal function in adult males. Exposure occurred via silastic capsule implanted subcutaneously. No dosage level(s) stated.	Maternal exposure to IsoBuPB showed to decrease the plasma corticosterone concentration and to increase the uterus weight in dams as well as the uterine sensitivity to estrogen in adult female offspring. All other indices examined were unaffected by the treatment. No positive control was included.	Kawaguchi et al. 2009

Test substances	Test system	Test principle(s)	Result(s)	Reference
IsoBuPB	Sprague Dawley **rats**	Study designed to analyze the effects of maternal IsoBuPB treatment on the emotional behavior and learning performance in mature offspring. Exposure occurred via silastic capsule implanted subcutaneously. No dosage level(s) stated. 'Estimated dose' is 4.36 mg/kg/day	Early exposure to IsoBuPB may increase anxiety, and specifically disturb passive avoidance performance, although the effects are male-specific. Other parameters were unaffected and no signs of overt toxicity were noted.	Kawaguchi et al. 2009b
PrPB BuPB IsoPrPB IsoBuPB 17α-ethinyl estradiol	Sprague Dawley immature female **rats**	Uterotrophic assay. Subcutaneous injection of 62.5-250-1000 mg/kg/day of paraben for 3 days. Investigation of Calbindin-D9-k (CaBP-9k), biomarker for estrogenic effects.	Sc injection of 1000 mg/kg/day induced increased uterine wet weight for BuPB, IsoBuPB and IsoPrPB (also for pos. control at 1 mg/kg/day). The effect was blocked by addition of anti-estrogen fulvestrant, indicating estrogen receptor-dependent pathway. At the highest dosage level, parabens also increased the expression levels of uterine CaBP-9k through progesterone-receptor involved pathways.	Vo and Jeung 2009
BuPB PrPB 17β-estradiol	CF-1 and CD-1 female **mice**	Subcutaneous injection of 0-1.4-14-271-407-542-813-949 mg BuPB/kg/day, of 0-949-1084 mgPrPB/kg/day on day 1 to 4 of gestation. Additional uterotrophic assay with BuPB at 0-20-200-949 mg/kg/day in two different mice strains. 14 mg/kg/day 17β-estradiol was administered as positive control in both assays.	Sc injection of BuPB did not affect any of the measured parameters, such as the number of pups born, litter weights, individual pup weight and pup survival. Sc injection of PrPB did not affect any of the measured parameters, including the number of intrauterine blastocyst implantation sites. 17β-estradiol terminated all pregnancies. The uterotrophic assay revealed that BuPB did not affect uterine wet or dry mass at any dose in either strain. 17β-estradiol consistently increased uterine mass in both strains.	Shaw and deCatanzaro 2009

Test substances	Test system	Test principle(s)	Result(s)	Reference
MePB EtPB PrPB BuPB IsoPrPB IsoBuPB 17α-ethinyl estradiol	Mated Sprague Dawley female **rats**	*In vivo* assay to investigate whether long-term exposure to PB may induce suppressive effects on reproductive organs in female rats during the critical developmental stage. Oral administration of 62.5-250-1000 mg/kg/day of paraben from postnatal day 21 to 40. Investigation of Calbindin-D9-k (CaBP-9k), biomarker for estrogenic effects.	<u>1000 mg/kg/day:</u> MePB, IsoPrPB: decreased ovary weight MePB, EtPB, PrPB: increased adrenal weight EtPB, IsoPrPB: decreased kidney weight, reduced serum estradiol levels MePB, BuPB: increased thyroid gland weight IsoBuPB: decrease of corporea lutea, increase in n° of cystic follicles, myometrial hypertrophy PrPB: myometrial hypertrophy <u>All dosage levels:</u> BuPB: increased liver weight (no dose-response relationship) BuPB, IsoBuPB: decrease of corporea lutea, increase in n° of cystic follicles, myometrial hypertrophy (no dose-response relationship) All PB: changes in T_4 serum levels (no dose-response relationship) <u>IC_{50} values for binding ERα and ERβ receptors:</u> 17β-estradiol: 3.10^{-9} M IsoBuPB: 2.10^{-6} M BuPB: 5.10^{-6} M IsoPrPB: 2.10^{-5} M PrPB: 2.10^{-5} M EtPB: 5.10^{-5} M MePB: too low to be calculated	Vo et al. 2010

Table 2: Overview of dermal absorption studies with parabens submitted to the SCCP

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
***In vitro* assays**				
BuPB	Full thickness **human** skin (1000 µm) 6 samples	Measurement of dermal absorption through human skin of BuPB at 0.4% in an o/w emulsion, applied at 8-10 mg/cm² and left in contact with skin for 24h.	Absorbed dose (%): Receptor fluid: 21.01 ± 6.95 Receptor wash: 0.49 ± 0.16 Skin (excl. tape strips): 36.92 ± 4.97 TOTAL: 58.42 ± 10.39 The authors state that the principle metabolite, PHBA, was detected in de the receptor fluid and that unmetabolised BuPB could only be detected in 1 of the 6 samples at a concentration below 0.67%. SCCP major comments: - insufficient skin samples used - only one concentration tested - ratio metabolised / unmetabolised Butylparaben only measured in receptor fluid, not in skin compartments - solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated	Fasano 2004a

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
BuPB	Full thickness **human** skin (1587-1983 µm) 10 samples from 2 donors	Measurement of dermal absorption through human skin of BuPB at 0.4% in an o/w emulsion, applied at 8-10 mg/cm² and left in contact with skin for 24h.	<u>Absorbed dose (%):</u> Receptor fluid: 14.90 ± 3.73 Receptor wash: 0.32 ± 0.14 Skin (excl. tape strips): 14.80 ± 4.67 TOTAL: 30.10 ± 7.08 The authors state that the principle metabolite, PHBA, was detected in de the receptor fluid and that unmetabolised BuPB could only be detected in 5 of the 10 samples with a mean concentration of 0.225%. <u>SCCP major comments:</u> - insufficient skin samples used - ratio metabolised / unmetabolised Butylparaben only measured in receptor fluid, not in skin compartments - only one concentration tested - solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated	Fasano 2005

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
BuPB MePB	**Rat** and **human** skin (450 µm) 10 samples from ≥ 3 donors	Measurement of dermal absorption through rat and human skin of MePB and BuPB in an o/w emulsion, at 0.8% and 0.4% respectively, applied at 8-10 mg/cm² and left in contact with skin for 24h.	<u>Absorbed dose rat skin (%):</u> MePB / BuPB Receptor fluid: 54.94 ± 5.92 / 54.23 ± 5.92 Receptor wash: 0.43 ± 0.20 / 0.44 ± 0.20 Skin (excl. tape strips): 12.23 ± 5.57 / 13.01 ± 5.57 TOTAL: 67.61 ± 6.06 / 67.69 ± 9.06 52-54% of penetrated amount accounted for PHBA, whereas 24% (MePB) or 5.5% (BuPB) accounted for the unmetabolised paraben. EtPB was, in both cases, also measured in the receptor fluid. <u>Absorbed dose human skin (%):</u> MePB / BuPB Receptor fluid: 79.36 ± 15.62 / 73.51 ± 10.34 Receptor wash: 0.46 ± 0.11 / 0.72 ± 0.21 Skin (excl. tape strips): 4.88 ± 2.01 / 6.92 ± 1.77 TOTAL: 84.69 ± 15.46 / 81.15 ± 10.65 33-35% of penetrated amount accounted for PHBA, whereas 60% (MePB) or 50% (BuPB) accounted for the unmetabolised paraben. EtPB was, in both cases, also measured in the receptor fluid. <u>SCCP major comments:</u> - insufficient skin samples used - only one concentration tested - solubility of BuPB in receptor fluid (HEPES buffer + 3.75% BSA) not demonstrated	Fasano 2004b
BuPB	Full thickness **pig** skin N° of skin samples not stated	Measurement of dermal absorption through pig skin of BuPB in an o/w lotion at 0.5%, applied at 8-10 mg/cm² and left in contact with skin for 24h.	Epidermis: unmetabolised BuPB measured Dermis: 50% unmetabolised BuPB + 50% PHBA Receptor fluid: only PHBA measured. <u>SCCS major comments:</u> - description of test is not detailed enough - only one concentration tested - no data on solubility of BuPB in receptor fluid - confusing report, mixing percentages with amounts/cm²	Pape and Schepky 2009

Test substances	Test system	Test principle(s)	Result(s) and major SCCP/SCCS comment(s)	Reference
***In vivo* experiments**				
BuPB, combined with diethyl and dibutyl phthalate	**Human** male volunteers	7 day daily whole body topical 2 mg/cm² application of a skin cream containing 2% BuPB, 2% DEP and 2% DBP. BuPB levels measured in serum, together with reproductive hormones: - follicle stimulating hormone (FSH) - lutenising hormone (LH) - testosterone - estradiol - inhibin B And thyroid hormones: - thyroid stimulating hormone (TSH) - free thyroxine (FT_4) - total triiodothyroxine (T_3) - total thyroxine (T_4)	BuPB was detected in serum after 1 hour (rapid uptake with peak of 135 µg/l after 4h), but no effect was noticed on a number of relevant hormone levels, such as TSH, LH, estradiol, Inhibin B, T_4 and FT_4. SCCP major comment: The results are obtained from a combined test of BuPB with two phthalates, which does not represent ideal test conditions to investigate the specific paraben concerned.	Janjua et al. 2007
MePB PrPB BuPB	Sprague Dawley **rats**	Study of the absorption, plasma kinetics, body distribution, metabolism (determination of plasma metabolites) and excretion of [^{14}C]-MePB, -PrPB and –BuPB. Oral and dermal administration of 100 mg/kg of MePB, PrPB and BuPB and sc administration of 100 mg/kg of BuPB.	Oral administration High and rapid (C_{max} at 0.5 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 8 to 22 hrs. Dermal administration Relatively low and slower (C_{max} at 8 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 12 to 22 hrs. Sc administration High and relatively rapid (C_{max} at 2-4 hrs) uptake of radioactivity in serum for all three parabens. Elimination after 12 to 22 hrs. Plasma metabolite characterisation revealed only one metabolite, namely PHBA, independent of time of collection, paraben type and route of administration. The study revealed that the principal route of excretion was via the urine and that no selective organ / tissue storage was observed.	Aubert 2009

EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

Clarification on Opinion SCCS/1348/10

in the light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age

Scientific Committees

on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion by written procedure on

10 October 2011

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

On 21 March 2011, the Commission received the notification of a decision taken by the Minister of the Environment of Denmark to ban propyl and butyl paraben, their isoforms and their salts in cosmetic products for children up to three years of age, in light of article 12 of the Cosmetics Directive[1]. The ban entered into force on 15 March 2011.

According to article 12 (2), the Commission "*shall as soon as possible consult the Member States concerned, following which it shall deliver its opinion without delay and take the appropriate steps*".

The Commission's services have already written to the Member States in order to inform them and ask for any further information they may have and will discuss the issue at the next Working Party on Cosmetic Products in June 2011. However, in order to deliver an opinion and take appropriate steps, which may include amending the annexes to the Cosmetics Directive, the Commission's services would like to request the assistance of the SCCS.

Denmark's decision makes reference to the scientific data presented in the SCCS opinion published for public consultation in December 2010 and finally adopted on 22 March 2011[2]. While fully agreeing with the contents, the approach, argumentation and the conclusions of the SCCS, the Danish authorities take a precautionary approach for children under three years of age relying heavily on the inherent endocrine properties of these parabens which have shown experimentally *in vitro* and the lack of high-quality *in vivo* data. In its opinion, the SCCS concluded there were no reasons for concern as it took a risk assessment approach based on the inherent properties of parabens and the anticipated consumer exposure levels from the use of parabens in cosmetics. Furthermore, the opinion, however, did not highlight specific concerns for the health of children or other population subgroups. More details about the justification of this decision are to be found in the documents attached.

On the basis of the SCCS opinion the Commission's services were already preparing a proposal reflecting the conclusion of the SCCS opinion on parabens before being informed of Denmark's clause of safeguard. The measure under consideration included a reduction to 0.19% of the allowed maximum concentration of propyl- and butylparabens, used individually or combined, and a ban of five parabens, for which there was insufficient information to conduct a safety assessment (isopropyl -, isobutyl -, pentyl -, phenyl - and benzyl esters of 4-hydroxybenzoic acid and their salts).

Denmark's decision to ban parabens in products intended for children under three years of age opened the question of whether the same measure should be taken at EU level or not. In order to propose an appropriate risk management measure, the Commission's service would like to request the position of the SCCS on the scientific justification for the Danish measure.

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1 Council Directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC), OJ L 262, 27.9.1976, p. 169.

2 SCCS/1348/10 Revision 22 March 2011.

2. TERMS of REFERENCE

1. Can the SCCS confirm that its opinion of 22 March 2011 (SCCS/1348/11) on parabens addresses all safety concerns (including potential for endocrine disruption) which may be associated with the use of propyl and butyl parabens in cosmetics at levels of 0.19% for all consumers including children under the age of three and other potentially sensitive consumer sub populations (e.g. pregnant women)?

2. If this should not be the case, does the SCCS consider that parabens (and in particular butyl- and propylparaben) present a risk when used in products intended for children under three (or other specifically vulnerable groups)?

3. Opinion

3.1. INTRODUCTION

This document responds to the scientific rationale given by the Danish authorities for the ban of propyl- and butyl parabens in products intended for use in children under three years of age. The concern of the Danish authorities relates to potentially increased susceptibility and exposure of children to certain endocrine disrupters such as propyl- and butylparaben compared to adults. In the scientific argumentation on possible effects of endocrine disrupters in children, general reasons with regards to young children have been stated, which can be summarized as follows:

- Different absorption and distribution factors due to the immaturity of physiological functions of young children may cause ineffective inactivation and elimination kinetics and thus higher internal exposure to the same external dose of certain chemicals in young children compared to adults (Makri et al. 2004; Renwick et al. 2000; Schwenk et al. 2003).

- In addition, infants have a higher body surface area to body mass ratio compared to older children and adults (US EPA 2009), which may be a cause of higher exposure per kg body weight to dermal applied compounds (Makri et al. 2004).

- Potentially enhanced target organ sensitivity in the young organism and effects induced in childhood may result in increased severity compared to adult effects, as impaired development of an organ may be irreversible and therefore more severe (Renwick et al. 2000).

Apart from the above general reasons, the specific reasons raising concern with regards to parabens by the Danish authorities can be summarized as follows:

- Parabens affect reproductive or endocrine endpoints in both male and female immature rats and mice, and both boys and girls at exposure may be at risk of endocrine disruption. Furthermore, the estrogenicity of parabens and their metabolites in vivo has not been fully determined and will also need to be compared to the possible risk of exposure to other sources of estrogens (Boberg et al. 2009 and 2010).

- For parabens (including butyl- and propylparaben), no adequate reproductive and developmental studies are available, and the possible effects of parabens may be of an irreversible nature. Therefore, according to Renwick et al. 2000, an additional uncertainty factor for children might be necessary.

- In addition to a high body surface area to body mass ratio of young children, during the summertime, children in the age up to 3 years spend many hours outside and therefore are exposed to a high amount of sunscreen products likely to contain propyl- and butylparaben (Danish EPA 2009).

3.1.1. SCCS opinion SCCS/1348/10 on parabens

In its Opinion SCCS/1348/10, the SCCS reiterated its previous conclusion that the continued use of methylparaben and ethylparaben as preservatives in cosmetics at the maximum authorized concentrations (0.4% for one ester or 0.8% when used in combination) is considered safe for human health.

Concerns were expressed with respect to the potential endocrine modifying effects of propylparaben, butylparaben and their related iso compounds and benzylparaben, as these properties appeared to increase with increasing chain length. For the frequently used compounds, propylparaben and butylparaben, considered as having a weak endocrine modifying potential, the deduction of an adequate NO(A)EL value was hampered by

considerable shortcomings of the reproductive toxicity studies carried out in rodents. In rats it was found that longer chain parabens are metabolized in a fast and complete way into p-hydroxybenzoic acid (PHBA) which is considered to be an inactive metabolite (rationale is given in the document). In humans, on the other hand it is possible that parent (unmetabolized) parabens become systemically available, even if in limited amounts. As properly conducted dermal absorption and/or toxicokinetic studies in humans were lacking, a quantitative risk assessment was carried out incorporating several layers of conservatism. The risk assessment was done for the most lipophilic compound butylparaben using the very low NOEL value of 2 mg/kg bw/day derived from a study where juvenile rats were exposed after subcutaneous administration of 2 mg butylparaben/kg/day for 17 days (postnatal days 2-18), a high dermal absorption value of 3.7% and a cumulative human exposure value of 17.4 g/day to cosmetic products containing lipophilic parabens. As a consequence, the use of propylparaben and butylparaben as preservatives in cosmetic products was considered as safe to the consumer as long as the sum of their individual concentrations does not exceed 0.19%. This conclusion was drawn in a conservative way due to the lack of scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular in relation to the metabolism of the parent compound in the skin. The latter can only be addressed through additional human data. As no or only limited information was available for their safety evaluation, human risk could not be evaluated for isopropyl-, isobutyl-, phenyl-, benzyl-and pentylparaben.

3.1.2. Relevant age groups to be considered

"Children" are developing organisms at various stages of immaturity and maturation up to nearly two decades with age-dependent different susceptibilities and sensitivities[3] compared to adults. In the Danish document no clear definition of the term "*children"* has been given and there is no universally agreed age range for what constitutes childhood. Article 1 of the United Nations Convention on the Rights of the Child defines "children" as persons up to the age of 18. However, in many reports of the United Nations (UN) and the World Health Organization (WHO), the term "children" refers to persons up to the age of 14 years (e.g. UN 2010, WHO 2010). The term "infant" often refers to children between the ages of 1 month and 12 months (Berk 2009, WHO 2010b); however, other definitions vary between birth and 3 years of age. The term "toddler" refers to children who are learning to walk, so it is typically used for children aged 1 to 2 years (Berk 2009), but sometimes also up to 3 years.

Essential functional changes occur in the period between the first week and the first few months after birth (Makri et al. 2004; Lemper et al. 2009; Scheuplein et al. 2002). It seems therefore necessary to use a discriminating terminology for this period of childhood. From a survey of the literature, it appears that a variety of age-related terms are commonly used. The SCCS will use the following terminology in the further discussion[4]:

- Full-term neonate (<1 week)
- Newborn 1 wk–2 months
- Early infant 2–6 months
- Crawlers/toddler (6 months–2 years)
- Preadolescent (2–12 years)
- Adolescent (12–18 years)

[3] According to Makri et al. (2004), "*susceptibility* is defined as a capacity characterized by biological (intrinsic) factors that can modify the effect of a specific exposure, leading to higher health risk at a given exposure level. The term *sensitivity* is used to describe the capacity for higher risk due to the combined effect of susceptibility (biological factors) and differences in exposure."

[4] Note that premature babies are not considered in this opinion

- Adult

This terminology reflects the normal changes in development of the child, in particular the skin maturation and dietary changes. Up to the age of 2 years, the occluded nappy area is frequently exposed to topically apply cosmetic products. Episodic acutely inflamed skin, nappy dermatitis, may occur particularly when the diet switches from solely milk, breast or formula, to the introduction of solid food; reports of potential effects of teething are not consistent. This could increase skin absorption from this area. Nappy dermatitis is treated with topical pharmaceutical creams or ointments in addition to cosmetics. After the first months, nappy dermatitis is less common. The SCCS considers the suggested terminology reflects more accurately the rapid physiological changes between neonates and newborns, early infants, crawlers/toddlers and other "children" up to 3 years.

It is difficult to follow the rationale of the Danish authorities to include in the ban of parabens all age groups of children up to three years without further differentiation, as no reasons for choosing this age range are given. It might be in analogy to a previous ban on phthalate esters in children's toys and childcare articles, where the threshold of 3 years was chosen because of the specific behavioural habits (hand-mouth contacts, sucking and chewing on toys etc.) in this particular age group, which would result in high exposure to the phthalate esters. These behavioural habits, however, are not applicable in the case of parabens in cosmetics, and hence choosing the age group of 3 years for parabens appears quite arbitrary from a scientific point of view.

The SCCS recognises the Danish argument that high exposure to sunscreens for the general age group of children up to 3 years can occur as a result of repeated use. However, children of this age group should not be exposed to direct sunlight, and if exposed, should be covered by appropriate clothing[5]. Sunscreens then need only to be applied on those areas that are exposed to sun and that cannot be protected by clothing. The SCCS considers the scenario of over-exposure to sunscreens as the result of product misuse and hence not applicable to risk assessment which considers normal uses of a product.

3.2. GENERAL CONSIDERATIONS

3.2.1. General susceptibilities/sensitivities of children – need for an extra safety factor?

Several reviews have dealt with the potential physiological differences between children of different age groups, and between children and adults. Moreover the various methodological difficulties in determining toxic effects and assessing risks in a continuum of developmental stages, functions and susceptibilities as well as sensitivities (e.g., enhanced exposures due to age-specific behavioural habits) have been considered. In these articles, in the light of the available data, the necessity of an additional safety factor for children or subgroups of children (e.g., SF 10) beyond the usual factor of 100, covering intra-and interspecies differences, was extensively discussed (Makri et al. 2004; Renwick et al. 1998 and 2000). Likewise, authorities have developed guidance how to deal with deficiencies or uncertainties in the database of chemical substances or with genotoxic and carcinogenic exposures in childhood (US-EPA 2005). For instance the REACH guidance document on information requirements (ECHA 2010) recommends that:

"A higher intraspecies assessment factor for children (US-EPA 1996, recommends from 10 up to 100 when assessing pesticides in relation to food safety) should be considered when the following two criteria are both fulfilled:

[5] http://ec.europa.eu/health-eu/news/sun_uv_en.htm

- There are indications, obtained from, for example, experiments in adult animals, epidemiological studies, *in vitro* experiments and/or SARs (structure activity relationships), of effects on organ systems and functions that are especially vulnerable under development and maturation in early life (in particular the nervous, reproductive, endocrine and immune systems and also the metabolic pathways), and

- There are deficiencies in the database on such effects in young animals."

Dermal exposure of the newborn and early infant: general differences and potential risk factors compared to adults

In general, a full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. As outlined in **Annex 1**, the dermal absorption in newborn skin is similar to that observed in adult skin. For babies during their first weeks and months, however, a number of typical differences and potential risk factors exist which are not present in the adult. These are:

(i) The surface area/body weight ratio is 2.3-fold higher in newborns than in adults, changing to 1.8- and 1.6-fold at 6 and 12 months, respectively. This ratio is covered by the intraspecies factor of 10 used in exposure-based risk assessment (in MoS)

(ii) Toxicokinetic parameters differ between various age groups of children and adults and can result in reduced clearance and/or longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies. Depending on the specific substance under consideration, half-lives in premature and full-term newborns might be three-to nine times longer compared to adults (Renwick et al. 2000)

(iii) *In –use* conditions of topical products also play a role since baby skin care products are often applied to most of the body surface compared with selective sites in adults. This should be considered in exposure-based risk assessment of the finished product.

(iv) The nappy area: the nappy area and non-nappy regions are indistinguishable at birth but show differential behaviour over the first 14 days, with the nappy region having a higher pH and increased hydration. With respect to *skin hydration* in the nappy zone, newborns tend to have somewhat higher water content in the horny layer and a greater variation than newborns, infants and crawlers up to one year. The pH is stabilized at a slightly acidic range of 5-6, but is not much different from the adult. However, the buffering capacity is smaller in the newborn making baby skin more susceptible to pH changes, in particular in the case of rash and damaged skin. The latter may occur in particular during the first months by so-called nappy dermatitis, which consists of episodic acute skin inflammation (mean duration 2 to 3 days) caused by physical, chemical, enzymatic, and microbial factors in the nappy environment, for example it is seen with diet switches *(*breast feeding, bottle feeding, solid food).

According to the SCCS Notes of Guidance, with respect to points (i) - (iii) above, there is no need for a general additional uncertainty factor for children when intact skin is involved. There might be the need for an additional safety factor if substance-specific data clearly demonstrate that inter-individual variability would result in a value higher than 10.

Cosmetic products used in the nappy area

The special circumstances associated with the nappy area (resulting from the close confining clothes and nappies, uncontrolled urination and defecation and resulting problems with potential damage of the skin in the nappy zone) are outlined in **Annex 1**. Modern nappy technology has shown to provide increasingly good skin compatibility, leading to a decline in the frequency and severity of nappy dermatitis. However, irritant nappy

dermatitis cannot be completely avoided and might enhance dermal absorption of substances.

Baby cosmetics can be subdivided in 2 groups: cleansing and protecting cosmetics:

Baby cleansing products consist of bath products, shampoos, soap bars and syndets (synthetic detergents), cleansing milk, baby wipes. Baby protecting cosmetics consist of face/body creams and body lotions, powder and sunscreens.

Protective creams for the nappy zone are preventive or protect against damage from urine, feces and their interactions. O/W creams are the first choice when no damage is present, but in cases of skin damage, mostly W/O creams or even water-free ointments on the basis of ZnO are used. As cosmetic products are meant to be used on intact skin medical consultation is necessary in the case of real skin damage and pharmaceutical products (and not cosmetics) should be used.

For the development of baby cosmetics, and the risk assessment of products intended to be used in the nappy area, the potential for irritation in this area which may lead to higher absorption needs to be considered by the manufacturers who are responsible for the final quantitative risk assessment of their products.

3.2.2. Sub-conclusions regarding general aspects of susceptibility/sensitivity of neonates/newborns and infants up to 6 months - need for an extra safety factor?

From the above, the following two main conclusions can be drawn:

- The skin structure of full-term neonates/newborns and early infants is similar to that of adult skin and the dermal absorption is comparable. However, distinction should be made between the skin of the nappy zone and the rest of the baby skin, since for this particular area risk factors exist which are not present for the rest of the body. Therefore, the nappy zone should be further considered, independent of the substance(s) under question.

- The SCCS is of the opinion that in general no additional safety factor needs to be included for ingredients used in children's cosmetics used on intact skin as an intra-species assessment factor of 10, covering the toxicokinetic (3.2) and toxicodynamic (3.2) differences between children and adults, is already included in the MoS calculated for individual ingredients (Notes of Guidance, 2010).

3.3. SPECIFIC ISSUES REGARDING PARABENS

3.3.1. Endocrine modifying effects by parabens (and their metabolites)

The position of the Danish authorities has been based on a recent review of Boberg et al. (2010). The SCCS has considered the main arguments of the authors and has come to the following conclusions:

Possible effects on the developing organism

The toxicity of parabens, in particular butylparaben, has been investigated in previous and more recent studies, with exposure in utero, during lactation and in juvenile animals[6]. The lowest available critical effect level (NOAEL) chosen in the safety assessment (opinion SCCS/1348/10) was based on such studies.

The study chosen by SCCP/S is that of Fisher et al. (1999) with a NOEL for butylparaben of 2 mg/kg bw/day (no other doses studied) in male juvenile rats.

In this study, male rats received subcutaneous injections on postnatal day 2, 4, 6, 10 and 12 of either diethylstilbestrol (DES), ethinylestradiol (EE), bisphenol A, genistein, octylphenol or butylparaben. Numerous parameters were assessed during and after treatment (up to 75 days). The more potent estrogens (DES, EE) caused (dose-related) changes in testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts and expression of aquaporin-1; minor effects were seen with the less potent estrogenic compounds. However, administration of butylparaben (2 mg/kg bw/day) had no detectable effects on any parameter on day 18.

In other studies in female and male rodents often (much) higher dose levels (several hundred mg/kg bw) were administered (e.g. Kang et al. 2002; Taxvig et al. 2008). This (and not the lack of any studies) makes it difficult to derive a NO(A)EL. Although a multigeneration OECD guideline study is missing, the main endpoints of reproductive toxicity are covered by the available studies.

The SCCS considers that the question of possibly increased susceptibility of children is sufficiently covered by the available data on reproductive toxicity. Potential remaining uncertainties have been addressed by introducing several layers of conservative assumptions in the assessment (summarized in the final conclusions).

Safety assessment based on endocrine activity

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The approach taken by Boberg et al. (2010) to use values from uterotrophic assays for NOAEL/LOAELs derivation (for MOS calculations, section 8.3) is problematic for two reasons:

i. Only one study in immature mice is referred to (Lemini et al. 2003), but other data from similar uterotrophic assays by other groups (e.g. Shaw & deCatanzaro 2009) which indicate higher values are neglected.

ii. It is inappropriate to refer to these results as NOAEL/LOAEL, since the endpoint provides data on estrogenic activity/potency; but this cannot simply be "translated" to an adverse effect. Note that widely accepted definitions of an "endocrine disruptor" or a "potential endocrine disruptor" make a clear distinction.

In the review on possible endocrine disrupting activity of parabens the „estrogenic burden" of parabens was estimated based on estrogenic potency (*in vitro* and in uterotrophic assays) and human blood concentration of estradiol, parabens and PHBA (Boberg et al. 2009, Table 6; Boberg et al. 2010 Table 5). The comparison is worth discussing, however, it is weakened by the following facts: a) the data base on child estradiol levels is poor (see Bay et al. 2004), b) the human plasma levels of butylparaben were determined following application of a cream containing 2% butylparaben whereas only 0.4% is currently permitted and c) the assumption that 5 mg/kg/d of PHBA is effective is questionable (see below and **Annex 2**).

Exposure to endocrine active substances such as parabens should be assessed by comparison with exposure to other endocrine active compounds in the diet (Bolt et al. 2001). By use of estimated daily intakes and the relative potencies, a hygiene-based margin of safety may be derived for endocrine active compounds. Taking the phytoestrogen daidzein as reference and assuming a daily systemic intake of about 0.1 mg/k bw butylparaben the estrogenic burden of daidzein is about 20 times higher (CIR 2008).

Estrogenicity of the common metabolite PHBA

Concerning the assumed estrogenic activity of PHBA, the experimental results of PHBA showed no endocrine activity *in vitro* while the results *in vivo* are contradictory. PHBA was tested negative in most uterotrophic bioassays with mice and rats (Hossaini et al. 2000; Twomey et al. 2000; Lemini et al. 2003) with subcutaneous administration of doses up to 100 mg/kg bw/d and more; only one study (Lemini et al. 1997) reports a positive response at 5 mg/kg bw/d. This unusual finding for PHBA may be due to differences in rodent chow and experimental procedures (Shaw & de Catanzaro 2009; references and details in Annex 2). PHBA is the common metabolite of all parabens. The different parabens exhibit large differences in estrogenic activity *in vitro* (see Table in Annex 2) and *in vivo* and also in toxicity. When assuming endocrine activity also for the main metabolite PHBA, such differences are not plausible.

The weight of evidence supports the generally accepted view that the metabolite PHBA lacks estrogenic activity and does not contribute to endocrine activity of parabens.

Estrogenicity of paraben conjugates

According to Boberg et al. (2010), conjugated parabens are assumed to be rapidly excreted, but it has not been clarified whether these conjugated parabens may have any potential endocrine disrupting effects.

Regarding the potential effects of conjugated parabens, no experimental data is available. The observed structure-activity relationships of estrogen receptor activation for a series of parabens (e.g. Byford et al. 2002; Okubo et al. 2001; Gomez et al. 2005; van Meeuwen et al. 2008) and molecular modeling of their receptor binding (Byford et al. 2002) may serve as an argument against the view that paraben conjugates may be biologically active in terms of estrogenicity. Similarly, the conjugated metabolites of bisphenol A are devoid of

binding to the nuclear estrogen receptor *in vitro* whereas this estrogenic activity has been demonstrated for the parent compound (Matthews et al. 2001; Shimizu et al. 2002). On the basis of the available scientific evidence, the SCCS concludes that an estrogenic activity by paraben conjugates is highly unlikely, particularly since the steroid conjugates themselves are inactive at the receptor.

Inhibition of sulfotransferases

Boberg et al. (2010) argue that inhibition of sulfotransferases in human skin and liver by parabens may contribute to the estrogenic effects of parabens.

The influence of parabens including butylparaben and PHBA on estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin was studied using skin and liver cytosol and human epidermal keratinocytes (Prusakiewicz et al. 2007). SULT activity (estradiol, estrone) was inhibited in skin cytosol by methyl-, ethyl-, propyl-, and butylparaben, but **not** by PHBA. Potency increased with chain length (IC_{50} butylparaben in skin: 37 µM = 7.2 µg/ml; IC_{50} propylparaben: about 0.2 mM = 36 µg/ml). With methyl- and ethylparaben, the inhibition in skin was weak, so that no IC_{50} value could be derived. No inhibition of androgen sulfation was detected. In human epidermal keratinocytes, butylparaben displayed an IC_{50} of 12 µM (2.3 µg/ml). No positive control was included.

Although exact concentrations of parabens in human skin cells after topical application are not known, it would seem scientifically plausible that the concentrations of free propyl- and butylparaben could cause a marked inhibition of estrogen SULT (if any) only in cells of the skin area of the topical application. Available data indicate that concentrations of free propyl– and butylparaben in human body fluids (serum, seminal fluid and urine) are on average 1-3 orders of magnitude lower, 95-percentiles at least 1 order of magnitude lower than IC_{50} values of the parabens (see **Annex 4**). As IC_{50} values in human liver were similar to those in human skin, a marked inhibition of systemic estrogen sulfotransferases by long-chain parabens is regarded as not likely.

3.3.2. Metabolism and toxicokinetics of parabens

The SCCS has re-assessed the role of metabolism of parabens, as there is increasing evidence that rat and humans markedly differ in this respect and that the rat appears to be a model of limited value when predicting the toxicokinetics of parabens in humans (reviewed by Boberg et al. 2009, 2010 and in the SCCS Opinion 2011).

While parabens are efficiently hydrolysed to PHBA in the skin (and possibly in the systemic circulation) of rats, free and predominantly conjugated parabens (glucuronides and sulfate esters) can be detected in human serum or urine after dermal application. The extent of hydrolysis to PHBA has not been quantified in these studies (Janjua et al. 2007 and 2008). From *in vitro* studies with human skin from adults, an uptake of about 3.7% free butylparaben has been derived (although the studies have some shortcomings; see the discussion in SCCS/1348/10). It is assumed that the parabens dermally taken up into the systemic circulation are further metabolized to PHBA and parabens conjugates in the liver and other organs of the human body before the remaining free parabens and their metabolites are excreted into the urine.

As the efficiency of the metabolic pathways determines the level of free parabens in the body, in the first postnatal months (neonates/newborns and infants) the immaturity of drug metabolising enzymes involved in the metabolism of parabens in humans (carboxylesterases, UDP-glucuronosyltransferases and sulfotransferases) may influence the level of unconjugated parabens circulating in the human body (for details see **Annex 3**).

3.3.2.1. Role of esterases and hydrolysis of parabens

Human skin expresses carboxylesterases hCE1 and hCE2 at a much lower level when compared to liver. Other forms of carboxylesterases may also be expressed in humans, but are less well characterised. Both hCE1 and hCE2 are developmentally expressed in the human liver. If this developmental expression is also evident in skin, it can be assumed that expression of both hCE1 and hCE2 is lower in the skin of children when compared to adults. The difference is more pronounced for hCE1 which preferentially metabolises methylparaben and ethylparaben. For hCE2, which preferentially metabolises propyl-, butyl- and benzylparaben, the age difference is less pronounced.

For hepatic hCE1and hCE2, age differences were most pronounced between adults and children under the age of 1 year. No further differentiation between the first 12 months of life has been made in this study. Thus, if age dependency of carboxylesterases as observed in the liver holds also true for skin, ester cleavage of parabens can be assumed to be lower in the skin of children age <1 year when compared to adults.

3.3.2.2. Role of glucuronidation and sulfation of parabens

In neonates/newborns and early infants up to 6 months, glucuronidation activity is known to be reduced, whereas older children mostly have similar activities compared to adults (see Annex 3 for details).

It has been shown *in vitro* that several UDP-glucuronosyltransferase (UGT) isoenzymes are capable of glucuronidation of parabens in the liver of adult humans. Although glucuronidation of parabens in human skin appears possible, the contribution of glucuronidation to the inactivation of parabens in adults, neonates and infants remains to be elucidated. In addition, there is only little information available on the ontogeny and development of the UGT isoenzymes conjugating parabens in neonates, newborns and early infants below six months (**Annex 3**).

Of the sulfotransferase (SULT) isoenzymes accepting exogenous phenols as substrates, SULT1A1 is the only SULT enzyme form with proven (although low or moderate) catalytic activity towards one of the parabens, namely butylparaben, and is considered so far as the only established defence among the SULT isoenzymes against this member of the parabens in adults, neonates and infants. The role of sulfation of parabens in human skin and systemic circulation remains to be elucidated.

Overall, the existing data suggest that the glucuronidation of parabens may be reduced in neonates and infants at least up to six months of age. Of the sulfotransferases, only SULT1A1 has been shown to convert parabens to sulfate esters *in vitro* so far. Because of the patchy data in neonates and infants, it is questionable whether sulfate ester formation of parabens by SULT isoenzymes can counterbalance the reduced glucuronidation. Hence, neonates, newborns and early infants exposed to parabens might have higher internal exposures than adults and thus be potentially at higher risk (at comparable dermal/external exposure) due to reduced glucuronidation and prolonged half-lives of parabens circulating in the body.

Consistent with a reduced metabolic capacity in very young children, in spot urine samples of hospitalized preterm neonates/newborns, 3- to 5-fold higher proportions of free methylparaben or propylparaben (about 10-15% of the total parabens fraction, free and conjugated) were found compared to 2-5 % in adults. The preterm neonates/newborns in the study had an assumed gestational and postnatal age of less than 44 weeks and had an active (although probably immature) UGT1A1 because individuals with hyperbilirubinaemia had been excluded from the study (Calafat et al. 2009). Although the paraben conjugates were considered stable under controlled conditions of storage for several years, according to

the authors the estimated urinary concentrations of the free parabens must be interpreted with caution.

Conclusions

The level of free parabens in the body is determined by the efficiency of the drug metabolising enzymes involved in the metabolism of parabens in humans (carboxylesterases, UDP-glucuronosyltransferases and sulfotransferases). The UDP-glucuronosyltransferase enzyme family is not fully developed until an age of 6 months and data suggest reduced carboxylesterase expression in children below 1 year. Therefore it cannot be excluded that the internal dose and the half-life of the unmetabolised parabens may be higher in children up to 6 months of age when compared to adults after topical application of cosmetics containing parabens.

Whether such enhanced internal exposures to parabens also imply enhanced risks to neonates/newborns and early infants remains uncertain and has yet to be determined. In any case, the missing data regarding parabens metabolism in adult humans, neonates/newborns and early infants require particular consideration in the risk assessments.

Compared to neonates/newborns or early infants, the unborn foetus will be better protected by the relatively efficient systemic parabens inactivation by the mother than the neonate/newborn or early infant exposed dermally to parabens.

The SCCS emphasizes that relevant human data regarding metabolism, required for reducing uncertainties and for a sound risk assessment of parabens, is missing so far. This data could be gained for instance by a human toxicokinetic study *in vivo* (e.g., by use of deuterated parabens) or by an approach combining *in vitro* data on the metabolism of parabens and toxicokinetic modelling, similar to the case of bisphenol A (Mielke and Gundert-Remy 2009; Mielke et al. 2011). For toxicokinetic modelling of parabens metabolism in humans of different age groups, relevant *in vitro* data regarding hydrolysis and phase II metabolism of parabens in human skin and liver would be needed.

3.3.3. Dermal absorption and exposure of parabens

Based on the exposure calculation made for adults in opinion SCCS/1348/10, an extrapolation can be made for children on the basis of the body surface area, assuming a concentration of 0.19% for butylparaben in the finished cosmetic product.

The cumulative exposure to preservatives used in all cosmetic product categories is considered to be 17.4 g/day on a surface of 1.75 m^2 for an adult. For a child of 3 months of age (5.3 kg and a surface area 0.31m^2)[7] the cumulative exposure would then result in 17.4 *0.31/1.75= 3.08 g/day.

Accordingly, the MOS would then be:

Dermal absorption: 3.7%

Intended concentration in finished product: 0.19%

Typical body weight: 5.3 kg

Cumulative exposure to preservatives: 3.08 g/day

NOEL (subcutaneous, rat, 17 days): 2.0 mg/kg bw/day

SED = 3080 mg/day * 0.19/100 * (3.7/100* 5.3) kg = 0.0408mg/kg bw/day

[7] http://www.rivm.nl/bibliotheek/rapporten/320005005.pdf

MoS = NOEL / SED = 49

However, it is not realistic to assume that children are exposed to all the cosmetic products that adults use. Therefore, this exposure calculation needs to be refined, using appropriate exposure information (data on amounts applied and use frequency) for children. Unfortunately, reliable information is not available.

COLIPA[8] was requested to provide exposure data for children existing in the cosmetics industry, but reported that data for children on use frequencies and amounts are currently not available. However, COLIPA suggested correcting the use data for adults for body weight of children.

One set of data was provided by the French Authorities which had been received from representatives of the cosmetic industry. The SCCS has no further information on how this data was generated.

According to these data, the following quantities of products are used daily for children:

- for leave-on products:

0.063 g/d for body care leave-on products,

1.34 g/d for leave-on products for nappy area,

0.55 g/d for wipes for nappy area

- for rinse-off products:

1 g/d for rinse-off products for body care

2.4 g/d for rinse-off products for nappy area,

This results in the following exposure, considering a child 3 month of age (5.3 kg bw):

Leave-on products			
	Body care products	**Products for buttock area**	
		Cream and other products	**Wipes**
Dermal absorption	3.7%	>3.7%	>3.7%
concentration	0.19%	0.19%	0.19%
Daily amount	0.063 g	1.34 g	0.55 g
Body weight	5.3 kg	5.3 kg	5.3 kg
SED (mg/kg/day)	0.000836	0.0177	0.0076
NOEL=2 (mg/kg/day)			
MOS	2393	<112	<275

[8] The European Cosmetics Association

Leave on body care products:

The MOS calculated for the body care products is considered acceptable. However, there is uncertainty with regard to the exposure data. The daily amount for body care products used by children was reported to be 0.063 g (according to the representatives from the French cosmetic industry) but no justification for this value was given.

An alternative approach would be to correct the amount of body lotion used by adults for a body weight of a child as suggested by COLIPA. For body lotion the value of 123.20 mg/kg/day is given[9]; resulting in a daily applied amount of 123.2X5.3= 0.6 g, i.e. 10 fold higher than the value used in the present calculation using the French data. The amount of body lotion used on children can also be calculated by correction for body surface area. This would result in an amount of 8 g * 0.31 /1.75= 1.4 g per day and a MOS of 107. As stated before, it is not clear whether it is appropriate to extrapolate from adult use to children. The range of results obtained by the different approaches demonstrates the uncertainty in the exposure data and urges the need for children specific exposure information. A realistic exposure is expected to be inside this range and the MOS is considered sufficient despite the uncertainties with regard to the metabolic capacity of the skin of newborn and early infants, as the value for the dermal absorption and the NOEL are conservative.

Leave-on products used in the nappy area:

A specific calculation has been made for products used for the nappy area. For this area it is expected that, especially in the case of irritated skin (see specific section on cosmetics products used in the nappy area above (section 3.2.1) the dermal absorption might be higher than the 3.7% used in the calculation above. In combination with the uncertainty associated with the exposure data, the likely simultaneous use of wipes and cream on the nappy area, and the fact that for children under 6 months of age the metabolic system in the skin may be immature, the calculated MOS is not considered acceptable for this age group.

Rinse- off products		
	Body care products	**Products for buttock area**
Dermal absorption	3.7%	> 3.7%
concentration	0.19%	0.19%
Retention factor	0.01	0.01
Daily amount	1 g	2.4 g
Body weight	5.3 kg	5.3 kg
SED (mg/kg/day)	0.0001326	0.000318
NOEL=2 (mg/kg/day)		
MOS	15078	<6282

[9] SCCS Notes of Guidance, § 4-2, Tab 3, http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_004.pdf

Rinse-off-products:

For rinse-off products, the MOS is considered sufficient both for body care products and for products for the nappy area.

Paraben levels in urine and plasma

Information on exposure to parabens can be derived from human biomonitoring studies.

Concentrations in human biological fluids (human biomonitoring) account for both dietary intake (e.g. from foods with paraben preservatives) and dermal application of products with parabens; according to Soni et al. (2005) the latter is considered to be the major contributor. Thus, such measurements are of interest as they provide information on the frequency and the magnitude of an overall exposure.

Urinary paraben concentrations were assessed in the U.S. general population (adults and children above age 6 years) by Ye et al. (2006) and by Calafat et al. (2009, 2010), in U.S men attending an infertility clinic (Meeker et al, 2011) and in young Danish men (Frederiksen et al. 2011) in addition to premature infants (Calafat et al., 2009)..

There are also data on serum levels in consumers, one from a small sample size in the U.S. (Ye et al. 2008), and two from larger sample sizes in Danish males (Frederiksen et al. 2011) and in Norwegian females (Sandanger et al. 2011).

The results of these studies (see Annex 4 for details and references) indicate that the (average) systemic exposure dose is considerably lower than estimated in the previous paraben opinion for adults who use all types of cosmetic products with parabens at the authorized concentrations.

Exposure estimates based on biological monitoring data are considered by SCCS as useful additional information in their overall evaluation on the safety of parabens.

4. CONCLUSIONS

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. The risk assessment in opinion SCCS/1348/10 was carried out for the most lipophilic compound, butylparaben, using the very low NOEL value of 2 mg/kg bw/day in juvenile rats, a high dermal absorption value of 3.7% and a cumulative human exposure value of 17.4 g/day to cosmetic products containing lipophilic parabens. This approach is confirmed to be very conservative by recent human biomonitoring data from Europe and the United States (for adults and children above 6 years) suggesting that systemic exposure doses are considerably lower than estimated in the paraben opinion.

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.

Scientifically sound data on the pivotal link between dermal absorption in rats and humans, in particular with regard to the metabolism of the parent parabens in the skin and specific exposure information for cosmetic products used for children would allow a refinement of the above assessment.

With regard to pregnant women, the unborn foetus 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.

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ANNEX 1 - Dermal exposure of the newborn and early infant: differences and risk factors compared to adults immature skin: leading to enhanced absorption of chemicals?

In general, a full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. The skin of the newborn could be considered as an "unripe" skin which progressively adapts during the first weeks and months of life. These adaptations lay at the origin of the physiological differences observed between baby and adult skin (1).

On the basis of the functional measurements of TEWL (trans-epidermal water loss is an indirect measurement of the barrier function) and dermal absorption studies, term infants seem to possess a fully developed stratum corneum with adult barrier properties. Other parameters such as skin thickness, skin pH, stratum corneum hydration also show that neonatal skin is adjusting very well to the extra uterine environment (2). Thus the dermal absorption in newborn skin is similar to that observed in adult skin. For babies during their first weeks and months, however, a number of typical risk factors exist (3-5) which are not present in the adult. These are:

(i) **The surface area/body weight ratio** is 2.3-fold higher in newborns than in adults, decreasing to 1.8- and 1.6-fold at 6 and 12 months, respectively (6).This ratio is taken up in the intraspecies factor of 10 used in exposure-based risk assessment (in MoS).

(ii) **Pharmacokinetic parameters** differ widely between babies and adults and result in reduced clearance and/or longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies. Premature and full-term neonates newborns tend to show a three-to nine times longer half-life than adults. However, these differences do not necessarily apply and are strongly dependent on the substance in question. Moreover, once the neonatal period is over, often a greater elimination and higher clearance are observed compared with adults bringing back the normal equilibrium (6, 7). This neonatal period coincides with the lactation period (6-10).

(iii) ***In –use*** **conditions of topical products** also play a role since baby skin care products are often applied on relatively larger surfaces than usually is done in adults. This factor is considered in exposure-based risk assessment.

(iv) **The nappy area**: the nappy area and non-nappy regions are indistinguishable at birth but show differential behaviour over the first 14 days, with the nappy region having a higher pH and increased hydration (11).

Cosmetic products used in the nappy area:

The nappy area shows a higher pH and increased hydration. Indeed, special circumstances arise because of the close confining clothes and nappies and the uncontrolled urination and defecation. The close-fitting nappy provides a warm nutritive environment for the proliferation of bacteria (12). Because of the interaction between the urine and the faeces, urease becomes activated and converts urea into ammonia, giving rise to alkaline skin pH. As a consequence, fecal enzymes such as lipases and proteases become activated and damage the skin in the nappy zone. Despite modern nappy technology, which has shown to provide increasingly good skin compatibility profile reducing the frequency and severity of nappy dermatitis (13, 14), irritant nappy dermatitis cannot be completely avoided, favouring dermal absorption of substances. A number of molecules are historically known to induce systemic toxicity in such a way, including hexachlorophene, dichlorophene, corticosteroids, boric acid, ethanol and others (4). These of course are forbidden or should be only used when medically indicated. In practice, when baby cosmetics are developed for use in the napkin area, the manufacturer often incorporates a 100% dermal absorption of

the "actives" in the risk assessment carried out for the manufacturer, bringing in these particular cases a safe product on the market.

TEWL measurements show values for newborn skin of 6-8 g/m² (15), which are similar as for adults. This value, however, increases when skin damage occurs as can happen in the nappy zone when nappy dermatitis is present (16). It is also used to measure the capability of a nappy to keep the skin dry (17).

With respect to skin hydration in the nappy zone, newborns tend to present somewhat higher water contents in the horny layer and a greater variation than adults up to one year (18, 19).

The pH is stabilized at a slightly acidic range of 5-6, but again that is not much different from the adult. However, the buffering capacity is smaller in the newborn making baby skin more susceptible to pH changes, in particular in the case of rash and damaged skin.

Cosmetic products for babies

From the anatomical/physiological differences between baby skin and adult skin, it can be learned that frequent contact with xenobiotics should be avoided since they could damage the barrier function and change skin pH, which may be at the basis of dermal absorption, an increased TEWL and the onset of infections (15, 16). Therefore, exposure-based risk assessment for baby products in a so-called Technical Information File (TIF) is key to bringing safe baby cosmetics to the market. This is the responsibility of the manufacturer, first importer or marketer of the product under consideration (Dir 76/768/EEG).

Baby cosmetics can be subdivided in 2 groups: cleansing and protecting cosmetics. Baby cleansing products consist of bath products, shampoos, soap bars and syndets, cleansing milk, baby wipes. Baby protecting cosmetics consist of face/body creams and body lotions, powder and sunscreens. Protective creams for the nappy zone are preventive or protect against aggressions from urine, faeces and their interactions. O/W creams are the first use when no damage is present, but in case of starting skin damage mostly W/O creams or even water-free ointments are used on the basis of ZnO. As cosmetic products are meant to be used on intact skin, in the case of real skin damage, medical consultation is necessary and pharmaceutical products (and not cosmetics) should be used.

For the development of baby cosmetics, a number of criteria should be taken into consideration by the manufacturer such as using high quality raw materials, no use of irritant ingredients, no known sensitizers, limiting promotional additives, adjusting the pH to a skin friendly value, adding anti-oxidants whenever necessary and preservatives in well-determined correct amounts, etc. (1)…...The manufacturer is responsible for the final quantitative risk assessment that brings the cosmetic finished product safely on the EU market.

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ANNEX 2 - Estrogenicity of p-hydroxybenzoic acid (PHBA), the common metabolite of parabens

There is a proposal to ban the use of butylparaben and propylparaben in the EU for the use for children less than three years of age. In the argumentation for increased sensitivity of children to certain endocrine disrupters compared to adults it was argued that the estrogenicity of parabens and their metabolites *in vivo* is not fully determined, and that the common metabolite of all parabens, p-hydroxybenzoic acid (PHBA), contributes considerably to an endocrine activity ("estrogenic equivalency"). This statement was based on reviews of the Danish Environmental Protection Agency (Boberg et al. 2009 and 2010).

In all *in vitro* tests investigated (yeast screen, MCF-7 cells, estradiol binding in uterine cytosol, sulfotransferase inhibition in skin cytosol) PHBA reacted negative. Endocrine activity *in vivo* was negative in fish. Uterotrophic assays were performed in ovariectomized and immature mice and immature rats after oral and s.c. administration. Two publications (Hossaini et al. 2000 and Twomey 2000) reported no activity in both species with both routes. One group (Lemini et al. 1997, 2003) reported negative effects in rats but positive ones in mice. According to these authors the lowest effective dose was 5 mg/kg/d (1997) or 150 mg/kg/d (2003) using s.c. administration whereas 50 mg/kg/d were negative (2003) which is considered non-consistent. Shaw & deCatanzaro (2009) discuss as possible reasons for the discrepant findings differences in phytoestrogen content of rodent diets and in experimental procedures (vaginal smearing).

Conclusion

The experimental results of PHBA *in vitro* showed no endocrine activity while the results *in vivo* are contradictory. PHBA is the common metabolite of all parabens. The different parabens exhibit big differences in endocrine activity *in vitro* (see Table **A2-1**) and *in vivo* and also in toxicity. When assuming endocrine activity also for the main metabolite PHBA, such differences are not plausible. The weight of evidence supports the generally accepted view that the metabolite PHBA lacks estrogenic activity and does not contribute to endocrine activity of parabens.

Table A2-1: Summary of *in vitro* potency data of parabens in MCF-7 cells compared to estrogen (molar ratio); from *Golden et al. 2005*

Studies	Detection of competitive ligand binding to estrogen receptor	Regulation of CAT gene expression in transfected MCF-7 cells [1]	Proliferation
Byford et al. 2002	Estrogen (1) MePB (1,000,000)	Estrogen (1) MePB (10,000)	Estrogen (1) MePB (1,000,000)
Darbre et al. 2002, 2003	EtPB (1,000,000) PrPB (100,000)	EtPB (10,000) PrPB (10,000)	EtPB(1,000,000) PrPB (100,000)
Okubo et al. 2001	BuPB (100,000) i-BuPB (100,000) Benzyl (1000)	BuPB (1000) i-BuPB (1000) Benzyl (1000)	BuPB (100,000) i-BiPB (100,000) Benzyl (100,000)

[1] Chloramphenicol acetyl transferase gene expression after 7 d

Studies on endocrine activity *in vitro* of PHBA

Routledge et al. 1998

The yeast estrogen screen assay with the parabens MePB, EtPB, PrPB and BuPB as well as PHBA was used. All parabens were tested positive, BuPB was 1/10.000 less effective than estradiol. In contrast, PHBA was negative.

Byford et al. 2002

MePB, EtPB, PrPB, BuPB and PHBA were investigated in MCF-7 cells (human-breast cancer derived cell line) and measured a) competitive inhibition of estradiol receptor binding, b) CAT gene expression and c) cell proliferation. The results were as follows:

a) molar ratio to estradiol PrPB and BuPB 1/100.000, MePB 1/1.000.000

b) MePB and EtPB 1/10.000; PrPB and BuPB 1/1.000

c) MePB 1/1.000.000; EtPB, PrPB and BuPB 1/100.000

PHBA was tested negative.

Lemini et al. 2003

A competitive estradiol receptor binding assay was used with cytosol from uteri of immature rats. All parabens investigated (MePB, EtPB, PrPB and BP) were able to displace estradiol, except MePB and PHBA, the relative binding activities were about 1/100,000 compared to estradiol.

Pugazhendhi et al. 2005

Using the same techniques as Byford et al. (2002), the study compared the estrogenicity of MePB and PHBA in MCF-7 cells (human-breast cancer derived cell line) by measuring a) competitive inhibition of estradiol receptor binding, b) CAT gene expression and c) cell proliferation. Despite a similarity in oestrogen receptor binding between both compounds, the activity of PHBA in whole cells was clearly lower than that of MePB for all endpoints up to concentrations of 5×10^{-4} M. The authors interpret the findings as indicative of estrogenic activity of PHBA in these assays.

Gomez et al. 2005

This study investigated the activity of various parabens and PHBA in HeLa cell derived reporter cell lines expressing ERalpha or ERbeta and an ER negative cell line to account for non-specific binding: Estrogenic activity of parabens was ranked as BuPb > PrPb > EtPb, and similar for ERalpha and ERbeta. MePB and PHBA did not activate estrogenic responses up to 10^{-5} M. With the other parabens the magnitude of an estrogenic response increased with the alkyl group size, and at 10^{-6} M the ranking was EtPb < PrPb < BuPb.

Prusakiewicz et al. 2007

The influence of Parabens (MePB, EtPB, PrPB, BuPB) and PHBA on estrogen levels by inhibiting estrogen sulfotransferases (SULT) in skin was studied using skin and liver

cytosol and **human** epidermal keratinocytes. SULT activity (estradiol, estrone) was inhibited in skin cytosol by MePB, EtPB, PrPB, BuPB, **not** by PHBA. Potency increased with chain length (IC50 BuPB = 37 µM). No inhibition of androgen sulfation was detected. In human epidermal keratinocytes, BuPB displayed an IC50 of 12 µM. No positive control was included.

Studies on endocrine activity *in vivo* of PHBA

Hossaini et al. 2000

Uterotrophic assays were performed in immature mice (B6D2F1 strain) and rats (Wistar strain). In **mice** MePB, EtPB, PrPB and BuPB as well as a mixture of MePB+EtPB+PrPB either at 100 mg/kg/d were administered s.c., PHBA doses were 5 and 100 mg/kg/d. In addition, oral doses of MePB 1 - 1000 mg/kg/d, PrPB 1 - 100 mg/kg/d and a mixture MePB+EtPB+PrPB 100 mg/kg/d were studied. No uterotrophic effect was reported for any of the parabens alone or in combination, either by oral or subcutaneous injection at levels up to 100 mg/kg/d. PHBA at 5 and 100 mg/kg/d sc reacted negative.

In **rats** BuPB was administered s.c. at 100, 400, and 600 mg/kg/d, PHBA at 5 mg/kg/d. An increase in wet and dry uterine weight at 600 mg/kg/d BuPB was observed. PHBA at 5 mg/kg sc reacted negative.

Twomey 2000 as cited in CIR 2008

Alpk:AP *f* CD-1 immature female mice (20-21 days of age) were used in an uterotrophic assay. PHBA single sc doses were injected at dose levels 0.5, 5.0, 50.0, and 100.0 mg/kg/d for three consecutive days, 10 animals/ group. As vehicle control arachis oil was given, as positive control group diethylstilbesterol at 0.01 mg/kg/d was used. Blotted uterus weights in animals administered diethylstilbesterol were significantly increased compared to controls. Uterus weights in animals administered PHBA were significantly decreased compared to controls, although no dose-response was reported.

Lemini et al. 1997

PHBA was investigated in an uterotrophic assay with both immature and ovariectomized CDI mice, positive control was estradiol. SC administration of 0.05, 0.5 and 5 mg/kg/d PHBA.

PHBA reacted positive at 5 mg/kg/d (both in ovariectomized and immature mice), the relative potency to estradiol was 0.0011 and 0.0018.

Lemini et al. 2003

PHBA was investigated in an uterotrophic assay with both immature and ovariectomized CDI mice and immature Wistar rats using SC dosages of 50 and 150 mg/kg/d. PHBA reacted positive in immature mice at 150 mg/kg/d and negative in immature rats.

Shaw and deCatanzaro 2009

The authors conducted an *in vivo* study with subcutaneous administration of butylparaben in early pregnancy and uterotrophic assays in two mouse strains (CF-1 and CD-1). The results indicate that the estrogen-sensitive period of implantation is not vulnerable to butylparaben exposure (up to 35 mg/kg/d), and that the *in vivo* estrogenicity may not be as potent as previously reported.

Studies on endocrine activity in fish of PHBA

Pedersen et al. 2000

Induction of yolk precursor protein vitellogenin in trouts was used to test EtPB, PrPB, BuPB and PHBA for oestrogenicity. All tested parabens were oestrogenic in doses 100 – 300 mg/kg while PHBA showed no activity.

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ANNEX 3 - Metabolism of parabens in humans after dermal exposure

Introduction

Parabens topically applied to the human skin are absorbed, partly/predominantly metabolized in the skin and during systemic circulation (mainly liver) and rapidly excreted into the urine predominantly as p-hydroxybenzoic acid (PHBA) and probably to a relevant part as glucuronides and sulfate esters. Some other minor conjugate metabolites as well as minor amounts of the parent parabens are also excreted into the urine. In addition, PHBA conjugates with glycine (p-hydroxyhippuric acid), glucuronide, and sulfate ester were formed after oral applications in humans, rats and rabbits (Andersen 2008) at mid and high doses. The PHBA conjugates were also formed in the rat after i.v. or duodenal application of 2 mg/kg b.w. ethylparaben (Kiwada et al. 1979 and 1980). Whether PHBA conjugates are also formed during low-dose dermal exposures in humans has yet to be determined. Overwhelming evidence indicates that the common metabolite of parabens, p-hydroxybenzoic acid, has no endocrine modulating activity. This is also assumed for the glucuronides and sulfate esters of parabens and the minor conjugates of PHBA. The interplay between the three main metabolic inactivation pathways (ester hydrolysis, glucuronidation and sulfation of the parent parabens), determines the level of free parabens in the body (see the metabolic scheme, **Fig. A3-1**). It is expected that the level of systemic exposure to free parabens determines the endocrine modulating activity of these compounds. Insofar, the main inactivating metabolic pathways may play a critical role in the availability of free parabens in the body of adults, neonates/newborns and infants.

Toxicokinetic animal studies, biomonitoring studies in humans, and investigations *in vitro* indicate that the metabolism of parabens differs between rats and humans (reviewed by Boberg et al. 2010 and in the SCCS Opinion 2011). In rats, after dermal exposure, parabens are efficiently hydrolysed to p-hydroxybenzoic acid in skin (and possibly in the systemic circulation) and no parent parabens (free or conjugated) were detected in serum or urine. In contrast, in humans, studies with dermal application of parabens revealed parabens in free and predominantly conjugated form (as glucuronides and sulfate esters) in serum or urine whereas the proportion of hydrolysis to p-hydroxybenzoic acid has not been determined in these studies and thus remains unclear. Concerning specifically butylparaben, absorption studies using rat skin *in vitro* showed a rapid hydrolysis of butylparaben by esterases, which was apparently more efficient than in human skin. Although the studies with human skin displayed a number of shortcomings they appeared to show a significant dermal absorption of parent butylparaben (Janjua et al. 2007 and 2008). These observations are supported by other studies in vitro showing that parabens are hydrolysed in human skin by up to three orders of magnitude slower than in rat skin (Harville et al. 2007).

Main metabolic pathways of dermally applied parabens (PB) in humans and rats

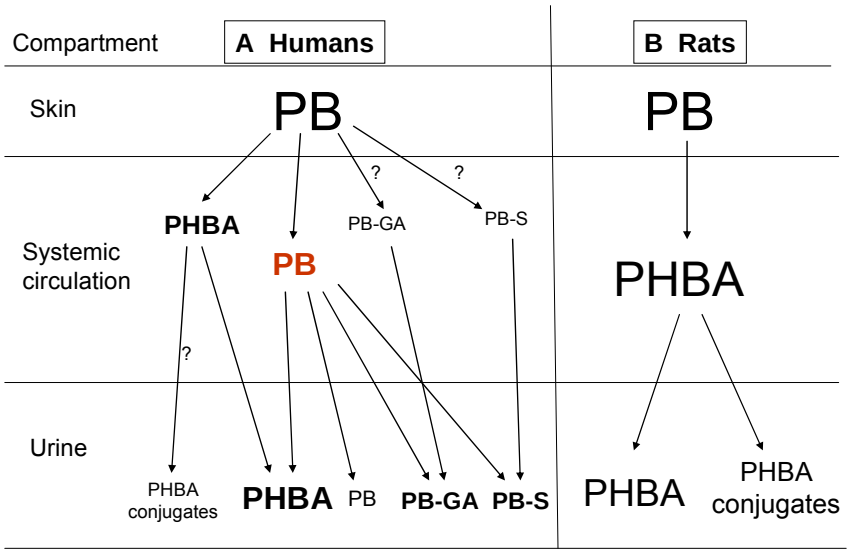

Fig. A3-1: Paraben metabolism in human and rat
PB, paraben; PHBA, p-hydroxybenzoic acid, PB-GA, paraben glucuronide; PB-S, paraben sulphate ester. PHBA conjugates in the rat: PHBA glycine, PHBA glucuronide, PHBA sulphate ester (amounts formed in decreasing order).

Humans in their early life may be considered as susceptible groups to endocrine modulating substances such as parabens (although their estrogenic activity is very low compared to endogenous estrogens). There is already some evidence that the metabolism of exogenous substances may be immature in neonates, newborns and early infants.[10] Therefore, the role of the main metabolizing enzymes involved in the inactivation of parabens in neonates, newborns and early infants is reviewed in order to determine whether and to which extent differences in paraben inactivation between adults and children of different age groups might be quantified or whether there are uncertainties and gaps of knowledge that hamper a sound risk assessment.

Carboxylesterases in human skin

There are five carboxylesterase genes listed in the human genome organization database, from which several variants may result, respectively. Their protein products have partially been characterized (Sanghani et al. 2009).

Lobemeier et al. (1996) identified three carboxylesterases of B-type in human skin, which were capable of hydrolysing parabens, and characterized their substrate specificities regarding parabens. Paraben esterase I is located in subcutaneous fat tissue and appears to correspond to the most prominent unspecific carboxylesterase in subcutaneous fat. It prefers methylparaben as substrate and its activity decreases with increasing chain length of the alcohol moiety. Paraben esterase II is also present in subcutaneous fat tissue and prefers butylparaben over methylparaben. Paraben esterase III was found in transformed keratinocytes (HaCaT cells) and also prefers butylparaben as substrate. Its activity decreases with decreasing chain length of the alcohol moiety. Another paraben esterase IV considered as an impurity in skin homogenates is probably an enzyme in human blood and was not further characterized.

[10] For definitions see response to DK

By using human skin from three individual female donors (age 28, 35 and 37 years), Jewell et al. (2007) demonstrated the presence of human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2) in human skin by investigating hydrolysis of different parabens (methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben) in skin microsomes, skin cytosol and during skin penetration.

The authors confirmed earlier findings revealing that hCE1 preferentially hydrolyses substrates with small alcohol groups whereas hCE2 preferentially hydrolyses lipophilic substrates with large alcohol and small acyl groups. Thus, methylparaben was preferentially hydrolysed by hCE1 and butylparaben was preferentially hydrolysed by hCE2.

The involvement of hCE2 in the metabolism of butylparaben, benzylparaben and (partly) propylparaben was confirmed using the hCE2 specific inhibitor loperamide. A further finding of the study was that the expression of both hCE1 and hCE2 is by far higher in human liver when compared to human skin (activity approximately several 100-fold lower in skin).

It is nearby to assume that paraben esterase I in the study of Lobemeier et al. (1996) corresponds to hCE1 in the Jewell et al. (2007) study and that paraben esterase III in the Lobemeier et al. (1996) study corresponds to hCE2 in the Jewell et al. (2007) study.

Age dependency of Carboxylesterases

By using a small number of samples, Pope et al. (2005) observed that the expression and hydrolytic activity of carboxylesterases in the liver differs between children and adults. Yang et al. (2009) investigated the age dependency of carboxylesterases in human liver by using a larger number of individual liver samples from three different age groups (48 fetuses (gestation days 82 – 224), 34 children (age 0- 10 years) and 22 adults (> 18 years)).

The individual and/or pooled liver samples were investigated for the expression patterns of hCE1 and hCE2 by using RT-qPCR, Western Analysis (protein analysis) and enzymatic assays (cleavage of typical substrates for hCE1 and hCE2 such as aspirin, pyrethroids and oseltamivir).

The authors could demonstrate that at the mRNA, protein and enzyme activity level age differences in the expression of hCE1 and hCE2 exist. Age differences were more pronounced for hCE1 when compared to hCE2. For example, at the mRNA level, adults had an approximately 50% higher level of hCE1 when compared to children. The mRNA level of hCE2 in adults was about 40% higher in adults when compared to children.

An attempt has been made to compare mRNA levels with age in the group comprising all children. As correlation was not statistically significant, the group of children was further subdivided into smaller age groups. A statistically significant correlation between mRNA for both carboxylesterases and age was observed for the group between 0 – 1 years.

The observations of age differences between adults, children and fetuses were also confirmed by Western analysis and hydrolysis of substrates for hCE1 and hCE2.

As a further observation from the study, high interindividual variability in enzyme expression was observed in the different age groups (this might be due to the heterogeneity of the samples (with respect to age, sex and ethnicity) but in the case of hCE2 also to the polymorphic expression of the enzyme).

Conclusions on carboxylesterases and hydrolysis of parabens

Among carboxyl esterase enzymes in human skin, most information concerns the major forms hCE1 and hCE2. Scientific literature reveals that at lest one further form is expressed in human skin, but no statements can be made about its developmental regulation. Also in

the liver a third carboxylesterase is expressed (Sanghani et al. 2004, cited in Yang et al. 2009), but whether parabens represent substrates for this third hepatic carboxylesterase and whether the expression of this carboxylesterase is developmentally regulated, remains to be established as well as its concomitant expression in human skin.

Human skin expresses hCE1 and hCE2 at much lower level when compared to liver. Maybe other forms of carboxylesterases might be expressed in humans.

Both hCE1 and hCE2 are developmentally expressed in the human liver. Assumed that this developmental expression is also present in skin, it can be assumed that expression of both hCE1 and hCE2 is lower in the skin of children when compared to adults. The difference is more pronounced for hCE1 which preferentially metabolises methylparaben and ethylparaben. For hCE2, which preferentially metabolises propyl-, butyl- and benzylparaben, the age difference is less pronounced. A good correlation between hCE1 and hCE2 mRNA levels and age was only found in the subgroup 0-1-year-old indicating that age difference is highest for children under the age of 1 year. Thus, as an approach to quantify the difference between adults and children age <1 in carboxylesterase expression, the mean value of mRNA levels in adults is compared to the lowest level observed in children (table 2 from Yang et al. 2009) which leads to a 87 fold higher hCE1 level in adults compared to children age <1 and to a 12.8 fold higher level of hCE2 in adults when compared to children age <1.

Thus, under the condition that age dependency as observed in the liver holds also true for skin and based on the assumption that parent parabens are responsible for the endocrine modulating activity, metabolism (cleavage) of parabens can be assumed to be lower in the skin of children age <1 when compared to adults. As metabolism by ester cleavage is regarded as inactivation of parent parabens, children at the age <1 year are at higher risk compared to adults from the ester cleavage point of view based on the information available on hCE1 and hCE2.

However, the interplay between all paraben-inactivation pathways has to be considered when addressing a potential higher risk of children towards the endocrine modulating effects of parabens. Thus the main alternative pathways, glucuronidation and sulfate ester formation (sulfation), and their role and age-dependency in the inactivation and elimination of free parabens in skin and systemic circulation of humans including neonates and infants have to be considered.

Glucuronidation and sulfate ester formation of parabens in humans

The data on glucuronidation and sulfate ester formation (sulfation) of parabens in humans is scarce. In most of the available biomonitoring studies, only the fractions of the parent parabens (free and/or conjugated) were determined in human serum or urine samples. Mostly, the frequently used parabens methyl-, ethyl, n-propyl- and butylparaben were determined (Boberg et al. 2010; Calafat et al. 2009; Calafat et al. 2010). In a population study, Ye et al. (2006) determined the paraben glucuronides and sulfate esters separately, besides free, unconjugated parabens, in spot urine samples from individuals with variable but unknown environmental exposures to parabens. They found that the relative proportions of the glucuronides and sulfate esters were similar and did not much differ when considering the whole range of exposures to parabens that were assessed by the concentrations of the parabens fraction (free and conjugated) found in urinary samples. The combined conjugates amounted to 95% or more and free, unconjugated parabens to 2 - 5% of the total parabens fractions. After topical application of butylparaben to adults, a similar proportion of free butylparaben in urine was determined (2.1%) (Janjua et al. 2008). In urinary spot samples of hospitalized preterm infants, 3- to 5-fold higher proportions of free methylparaben or propylparaben (about 10-15% of the total parabens fraction, free and conjugated) were found compared to 2-5 % in adults. The preterm infants in the study had an assumed gestational and postnatal age of less than 44 weeks and had an active

(although probably immature) UGT1A1 because individuals with hyperbilirubinaemia had been excluded from the study (Calafat et al. 2009). Although the paraben conjugates were considered stable under controlled conditions of storage for several years, the estimated urinary concentrations of the free parabens must be interpreted with caution, according to the authors.

The data available may raise concerns about free parabens circulating in the human body and potentially exerting endocrine modifying effects in susceptible groups such as neonates or infants. In neonates and infants up to 6 months, glucuronidation activity is known to be reduced, whereas older children mostly have similar activities compared to adults (Allegaert et al. 2008; Edginton et al. 2006; Gow et al. 2001; Miyagi and Collier 2007; Renwick et al. 2000; Zaya et al. 2006). Hence, the question has to be solved whether neonates and infants exposed to parabens are at higher internal exposures than adults and therefore potentially at higher risk (at comparable dermal/external exposure) due to reduced glucuronidation and prolonged half-lives of parabens circulating in the body. In addition, the question has to be considered to what extent sulfation of parabens can counterbalance the reduced glucuronidation in neonates and infants.

It will be shown below that data on the conjugation of parabens in neonates and infants are patchy so far. Therefore, predictions on the fate of parabens in neonates and infants and on the degree of protection by conjugating enzymes are very difficult. As an approach to bridge the gaps, it is nearby to identify the isoenzymes of UDP-glucuronosyltransferase (UGTs) and sulfotransferase (SULTs) capable of conjugating parabens in adults, neonates and infants and to pursue their development and roles regarding parabens conjugation around parturition and early life after birth. The identification of these isoenzymes and the knowledge on their ontogenetic and kinetic properties may contribute to better assessments of the fate of parabens in neonates and infants or even enable more precise predictions when using suitable assessment tools such as PBPK modelling (de Zwart et al. 2004; Edginton et al. 2006).

In addition to the differences between rats and humans in the metabolism of parabens described above, the UGTs and SULTs responsible for glucuronidation and sulfate ester formation of exogenous substances including parabens often develop differently in humans and laboratory animals during intra-uterine life and after parturition (Coughtrie 2002; de Wildt et al. 1999; Gammage et al. 2006; Hines 2008; McCarver and Hines 2002). Because of the well-known ontogenetic differences between developing humans and laboratory animals regarding drug metabolizing enzymes, the emphasis of this review is on human data.

High inter-individual differences have been shown for UGT and SULT enzyme expression and enzyme activities in vitro and in vivo (see for instance Renwick et al. 2000). This is not unusual since such variability has been observed with many of the phase I and II enzymes of drug metabolism. Partly these differences can be explained by proven genetic polymorphisms. Prominent examples are some allelic variants in the UGT1A family and of the SULT1A1 isoenzyme. Apart from these genetic differences, other factors on the level of regulation not well understood so far may contribute to enzyme expression and activity. Therefore, inter-individual variations in glucuronidation and sulfate ester formation will not be considered here. In the future, inter-individual differences of drug metabolism will become an increasing challenge to risk assessors.

Human UDP-glucuronosyltransferases (UGTs)

UDP-glucuronosyltransferase enzymes (UGTs) (EC 2.1.4.17) are located in the membrane of the endoplasmatic reticulum of cells and exhibit distinct but overlapping substrate specificities. Two UGT gene families were found in humans. Nine functional genes exist in the UGT1 family, UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9 and 1A10, and seven within

the UGT2 family, UGT2A1, UGT2B4, 2B7, 2B10, 2B11, 2B15 and 2B17. The majority of these enzyme forms are located in liver but most of them are also found in extrahepatic tissues, normally at lower expression levels compared to liver (Tukey and Strassburg 2000). Several of the isoenzymes also conjugate endogenous signalling substances such as steroid or thyroid hormones and thereby probably serve for the control and balance of endogenous hormone concentrations.

Identification of UGT isoenzymes conjugating parabens

Available data on parabens glucuronidation in humans is mainly derived from biomonitoring studies and is limited as delineated above. Apart from parabens (free and conjugated) in urinary samples from preterm infants, data on the glucuronidation of parabens in neonates and infants are missing so far. Abbas et al. (2010) have recently published an *in vitro* study on glucuronide formation and ester hydrolysis in liver samples from adult humans. The authors used commercially available human recombinant UGT isoenzymes and several parabens (methyl, ethyl, propyl, butyl, benzyl) and showed that these parabens are mainly conjugated by the UGT isoenzyme forms 1A1, 1A8, 1A9, 2B7, 2B15, and 2B17 (however, with different specific activities). Other isoenzymes investigated displayed lower or even very low specific glucuronidation rates, namely the UGTs 1A3, 1A4, 1A6, 1A7, 1A10, and 2B4. The authors concluded that the parabens are readily metabolized in human liver through glucuronidation by several UGT isoforms as well as by esterase hydrolysis and suggest according to their results that these parabens do not accumulate in human tissues. Apart from human liver samples, data on the glucuronidation of parabens from other relevant human extrahepatic organs such as gut, kidney, lung or skin are not available.

UGT isoenzymes capable of conjugating parabens in human skin

Glucuronidation of parabens in human skin has not been investigated in published studies. Existing information on UGT isoenzymes which are capable of forming glucuronides of parabens in human skin is scarce (Oesch et al. 2007). From the UGT1A family members, only UGT1A1 with bilirubin as a probe substrate and potentially another "phenol-UGT" have been detected in skin from adult humans (Peters et al. 1987; Pham et al. 1990) and human keratinocytes, respectively (Vecchini et al. 2005). In addition, the UGT2B family members UGT2B4, UGT2B11, UGT2B15 and UGT2B17 have been detected as gene transcipts in human skin (Lévesque et al. 1997; Lévesque et al. 1999; Luu-The et al. 2007; Tukey and Strassburg 2000).

Taken together, in adult human skin, only UGT1A1, UGT2B15 and UGT2B17 with proven catalytic activity towards parabens in liver have been detected, in part only as gene transcripts. It can be concluded that glucuronidation of parabens in adult human skin is possible but the contribution of glucuronidation to the inactivation of parabens in human skin remains to be determined.

Ontogeny of UGT isoenzymes in human development

Most of the human UGT isoenzymes conjugating parabens are not well expressed at birth up to several months or even some years of age. Strassburg et al. (2002) could not detect any gene transcripts of the UGT enzyme forms in human liver of two foetuses of week 20 of gestation. For most UGT isoenzymes capable of conjugating parabens, there is little knowledge on their development at birth and infancy (**Table A3-1**). Similar gaps of knowledge exist for the other UGT enzyme forms that are less relevant for the glucuronidation of parabens in humans (see reviews of de Wildt et al. 1999; Hines 2008; McCarver and Hines 2002).

Table A3-1: Ontogeny of human UGT isoenzymes capable of forming paraben glucuronides

UGT isoenzyme (marker substrate)	**Onset, gene transcript or protein levels, and enzyme activities in development compared to adult levels.**	**Remarks**	**References**
1A1 (bilirubin)	Onset of activity at birth; the activity is fully developed to adult levels after 3-6 months of age.	Frequently jaundice (icterus) in newborns due to unconjugated hyperbilirubinaemia during first days after parturition	de Wildt et al. 1999; Hines 2008; Tukey & Strassburg 2000
1A8 (various phenols)	Ontogeny and activity at birth and during the first months of age are unknown. The gene transcript is fully developed to adult levels after 6 months of age.	Extrahepatic UGT isoenzyme. Predominantly located in gastrointestinal tract. Relevant only in case of oral parabens exposure	Strassburg et al. 2002
1A9 (estrogens, paracetamol)	Ontogeny and activity at birth and during the first months of age are unknown. The gene transcript of this isoenzyme reaches 30-40% of the adult activity after 6-12 months and 60-70% after 1-2 years of life.		Strassburg et al. 2002
2B7 (morphine)	With morphine as substrate, an onset of activity within the second trimester and adult levels of activity after 2-3 months of age were reported. With epirubicin as substrate: < 10% of adult levels at less than 1 year of age, 50-70% at an adolescent age and interjacent levels at 1 to 11 years.	Early development was not confirmed by a more recent study of Zaya et al. (2006). See text.	de Wildt et al, 1999; Hines 2008; Zaya et al. 2006
2B15 (propofol: testosteron**)**	Ontogeny and activity at birth and during the first months of age are unknown. The gene transcript is fully developed to adult levels after 6 months of age.		Strassburg et al. 2002
2B17 (androgenic steroids)	Enzyme activity of less than 10% in foetal liver samples and about 10% in neonates compared to adult levels was reported.		de Wildt et al. 1999; Hines 2008

Regarding UGT2B7 with morphine as a typical substrate, the early foetal development previously described was not confirmed by a more recent study of Zaya et al. (2006) who investigated another substrate, the drug epirubicin, and both the expression of the UGT2B7 protein and its catalytic activity. They reported a much slower increase of this enzyme form and levels of enzyme activity in adolescent age coming closer to adult levels (Table 1).

Strassburg et al. (2002) observed that gene transcripts and proteins of all except two of the UGT isoenzymes investigated were shown to have reached adult levels after 6 months of age. However, UGT enzyme activities towards various substrates tested in vitro were low and did not correlate to the appearance or content of the UGT isoenzyme proteins. Understanding of the ontogeny and development of the UGT isoenzymes is complicated by the fact that after appearance of the gene transcripts and the proteins, UGT enzymes may need further post-translational maturation during the development of the neonate or infant (and in particular cases beyond) until adult levels of enzyme activity are reached.

Human sulfotransferases (SULTs)

Sulfate ester formation is also an important and potentially critical pathway of the inactivation and elimination of parabens from the human body as delineated above and is catalyzed by sulfotransferase (SULT) enzymes (EC 2.8.2.1); those involved in drug metabolism are located in the cytosol. In humans, four different SULT enzyme families are known, SULT1, SULT2, SULT4 and SULT6, comprising at least twelve distinct members of isoenzymes (Blanchard et al. 2004; Gamage et al. 2006, Lindsay et al. 2008). Similar to the UGTs, SULT isoenzymes exhibit distinct but overlapping substrate specificities towards exogenous substances. Several of the SULT isoenzymes also conjugate, e.g. endogenous steroid or thyroid hormones and thereby play a role in the control and balance of endogenous hormone concentrations.

Identification of human SULT isoenzymes conjugating parabens

Available evidence on sulfate ester formation of parabens in humans is primarily derived from biomonitoring studies and is limited as delineated above. Prusakiewicz et al. (2007) reported that butylparaben sulfate was formed *in vitro* in human liver and skin cytosols, and when using the recombinant human allozyme SULT1A1*2, respectively. They concluded that butylphenol is "not a very good SULT substrate". SULT1A1 is an isoenzyme mainly located in human liver and small intestine and also present in smaller amounts in other extrahepatic tissues (Riches et al. 2009). The allozyme SULT1A1*2 normally has lower specific activity than the wild type enzyme. In the past often termed as "phenolsulfotransferase", SULT1A1 has been characterized to possess broad substrate specificity towards many exogenous and endogenous phenolic substrates (Gamage et al. 2006; Lindsay et al. 2008). It is not known which of the other SULT isoenzymes are capable of forming sulfate esters of parabens. In addition to human SULT1A1, SULT1A3, SULT1B1 and SULT1C2 have also been shown to sulfate exogenous phenols of different structures (Lindsay et al 2008). In tissues from adults, SULT1A3 is present as a major SULT isoenzyme in small intestine but could not be detected in liver whereas human foetal liver and small intestine contain SULT1A3 in appreciable amounts (Riches et al. 2009; Stanley et al. 2005). SULT1B1 consisting of two isoenzymes, 1B1_a and 1B1_b, is predominantly expressed in small intestine and kidney but also found in liver. The role of SULT1B1 in drug metabolism is unclear so far: Although it has a broad spectrum of substrates similar to SULT1A1, the substrate affinities are in general much lower.

SULT isoenzymes conjugating parabens in human skin

No information on sulfate ester formation of parabens in the skin of neonates and young infants is available in the published literature. Sulfate ester formation of parabens in human skin *in vitro* has not been investigated in detail so far. Prusakiewicz et al. (2007) reported that butylparaben sulfate was generated in small amounts in human skin cytosol (about 10% compared to human liver). On the other hand, they found that butylparaben along with other parabens inhibits the sulfonation of estradiol (with an IC_{50} of 37 µM).

In human skin and keratinocytes cultures, the following SULT isoenzymes have been detected or investigated: SULT1A1, 1A3, 1E1, and 2B1 (Falany et al. 2006; Svensson et al. 2009).

Apart from SULT1A1, from these isoenzyme forms, only SULT 1A3 can currently be assumed to conjugate parabens although it cannot be excluded that SULT1E1 and/or SULT2B1 isoenzymes are also capable of conjugating parabens in human skin. It is concluded that sulfation of parabens in human skin from adults in vitro occurs albeit to a much lower extent than in human liver. The contribution of sulfation to the inactivation of parabens in human skin after dermal exposure remains to be determined.

Ontogeny of SULT isoenzymes in human development

The ontogeny and development of SULT isoenzymes has been investigated and compared in human tissues of foetal origin, from neonates, infants and adults (reviewed by Hines 2008). This compilation is restricted to SULT1A1, SULT1A3, SULT1B1 and SULT1C2. SULT1A1 is expressed in human liver of all age groups investigated (including foetuses, neonates and infants) in substantial and comparable amounts whereas different trends were observed in extrahepatic tissues. Hepatic SULT1A3 was expressed at high levels in foetal tissue of about the second trimester, but substantially decreased in neonatal or infant liver to about 10% or less of the foetal level and was essentially absent in the adult liver. Similar trends of SULT1A3 activities were observed with lung and kidney tissues. SULT1A3 levels in foetal gut tissue were highest and essentially not different from adults (Adjej et al. 2008; Richard et al. 2001; Stanley et al. 2005). SULT1B1 was only found in foetal small intestine. The protein expression of SULT1C2 was much higher in foetal small intestine than in foetal kidney or liver and was found to be barely expressed in human colon or liver of adults suggesting that SULT1C2 is more widely expressed in the foetus than in the adult (Stanley et al. 2005). Although these three SULT isoenzymes discussed appear to tend towards higher levels in the human foetus than in adults, activities or protein levels also tend to decrease in neonates and infants. The decrease of SULT1A1 enzyme activity is less marked than the interindividual differences in foetal, early postnatal and adult liver samples and may be considered slight. Nevertheless, the data on the sulfation of parabens in neonates and young infants are poor so that no firm conclusions regarding the role of sulfate ester formation of parabens can be drawn in these age groups.

Conclusions

In biomonitoring studies, only small proportions of free parabens were detected whereas conjugates of parabens consisting of glucuronides and sulfate esters predominated both in serum and urinary samples of adults. Higher proportions of free parabens were determined in urinary spot samples from preterm infants compared to adults. In contrast, in rats, only p-hydroxybenzoic acid and no free or conjugated parabens were found after dermal or oral exposure due to rapid hydrolysis of parabens to p-hydroxybenzoic acid. Thus, the rat is a model of limited value when predicting the toxicokinetics of parabens in humans. Because of proven internal exposure of humans to free parabens, the question has to be solved whether neonates and infants when dermally exposed are at higher internal exposure of free parabens than adults given the immature and thus reduced functions of UDP-glucuronosyltransferases (UGTs). A related issue is whether sulfate ester formation of parabens by sulfotransferases (SULTs) can counterbalance the reduced glucuronidation in neonates and infants.

It has been shown in vitro that several UDP-glucuronosyltransferase (UGT) isoenzymes are capable of glucuronidation of parabens in the liver of adult humans. Although glucuronidation of parabens in human skin appears possible, the contribution of glucuronidation to the inactivation of parabens in adults, neonates and infants remains to be elucidated. In addition, there is only little information available on the ontogeny and development of the UGT isoenzymes conjugating parabens in neonates and young infants up to six months.

Of the sulfotransferase (SULT) isoenzymes accepting exogenous phenols as substrates, SULT1A1 is the only SULT enzyme form with proven (although low or moderate) catalytic activity towards one of the parabens, namely butylparaben, and is considered so far as the only established defence among the SULT isoenzymes against this member of the parabens in adults, neonates and infants. SULT1A1 and the three SULT isoenzymes potentially forming sulfate esters from parabens (SULT1A3, SULT1B1, and SULT1C2) are differentially expressed in foetal tissues and show different expression profiles in human development and in adults. The role of sulfation of parabens in human skin and systemic circulation remains to be elucidated.

Taken together, in humans including neonates and infants, glucuronidation and sulfate ester formation play a critical role of in the inactivation and elimination of free parabens in skin and systemic circulation, different from rats. Existing data suggest that the glucuronidation of parabens is reduced in neonates and infants at least up to six months of age. Of the sulfotransferases, only SULT1A1 has been shown to convert parabens to sulfate esters *in vitro* so far. Because of patchy data in neonates and infants, it is questionable whether sulfate ester formation of parabens by SULT isoenzymes can counterbalance the reduced glucuronidation. Thus, for neonates and infants up to 6 months of age, it cannot be excluded that the internal dose and the half-life of the unmetabolised parabens may be higher than in adults after topical application of cosmetics containing parabens. However, based on the limited information available, no quantitative conclusions can be drawn for differences in glucuronidation and sulfation between adults and children.

The SCCS emphasizes in the Opinion of March 2011 that relevant human data regarding metabolism of parabens is missing so far, which is required for reducing uncertainties and for a sound risk assessment. This data can be gained by either a human toxicokinetic study in vivo (e.g., by use of deuterated parabens) or by an approach combining *in vitro* data on the metabolism of parabens and toxicokinetic modelling, similar as in the case of bisphenol A (Mielke and Gundert-Remy 2009; Mielke et al. 2011). However, relevant in vitro data regarding hydrolysis and phase II metabolism of parabens in human skin and liver is missing; these data is a prerequisite for toxicokinetic modelling of parabens metabolism in newborns, infants and adults.

References Annex 3

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ANNEX 4 - Biomonitoring of parabens in humans

The main measurements of parabens[11] in human sera/plasma, seminal sera, and urine presented in the papers discussed below are presented in Table **A4-1**.

Serum/plasma

Ye et al. (2008) measured methyl-, ethyl- and propylparaben in 15 commercially available serum samples collected between 1998 and 2003 from 4 male and 11 female donors. The serum samples were frozen on dry ice and shipped to the laboratory, where upon receipt the samples were stored at −70 °C. Both free and total parabens (sum of unconjugated, deglucuronidated and desulfated parabens) were measured in serum. Free PP was detected in 47% of the samples. The median level of free PP was below the limit of detection (<LOD) and the maximum level was 2.3 ng/ml. Total PP was measured to 1.4 ng/ml (median) with a maximum level of 67.4 ng/ml. For free MP the median value was 0.2 ng/ml with a maximum value of 9.8 ng/ml.

Frederiksen et al. (2011) measured parabens in urine, blood and semen samples obtained from 60 young and healthy Danish men (average age 19.7 years, samples collected 2006). Urine, serum and seminal plasma were analyzed and the total levels of parabens were determined. It is noted that the paraben levels, with exception of the maximum level of EP, were considerably lower than in the study of Ye et al. (2008). The difference is probably due to the fact that the study of Frederiksen et al. (2011) was performed on young men, while Ye et al. (2008) studied commercial sera from 11 women and 4 men.

Sandanger et al. (2011) measured parabens (methyl-, ethyl-, propyl-, butyl-, and benzylparabens) in plasma from 322 women (blood drawn in 2005). All blood samples were frozen within 3 days of collection. Butyl- and benzylparabens were not detected. PP was detected in 29% of the group (median < 2 ng/ml). It is stated in the report that PP "was only detected above MDL (method detection limit) in women who used body lotion ''once a day'' (2.2 ng/ml) and ''twice or more per day'' (4.0 ng/ml. The maximum level of PP measured was 43.9 ng/ml.

The authors have not hydrolyzed any of the plasma samples and state: "The high concentration of native parabens identified in this study is not likely caused by hydrolysis of conjugates as paraben conjugates in human serum have been shown to be stable over 30 days when stored at 37 °C (Ye et al. 2009). The contribution of conjugate hydrolysis is therefore considered negligible to the values reported." SCCS does not find this argumentation convincing, as Ye et al. (2009) studied the stability of paraben conjugates in serum that had been prepared and frozen at -70° C before it was thawed and used for stability studies. Sandanger et al. (2011) used blood samples that had been kept for up to 3 days (temperature not given) before being frozen. The stability of the conjugated parabens may obviously differ in full blood and sera that have been frozen.

The medium level for free MP reported by Sandanger et al. (2011) was nearly as high as found by Ye et al. (2008) for total MP and the maximum level of free MP found by Sandanger et al. (2011) was nearly 15 times higher than the corresponding level found by Ye et al. (2008). The medium level of free PP was below LOD both in the study of Sandanger et al. (2011) and Ye et al. (2008), while the maximum value was found by Sandanger et al. (2011) was nearly 20 times that reported by Ye et all. (2007) and nearly as high as they reported for total PP.

Janjua et al. (2007) studied the systemic uptake of some phthalates and butylparaben following whole-body topical application. Twenty-six healthy male volunteers (mean age 26

[11] Abbreviations used: MP = methylparaben, EP = ethylparaben, PP = propylparaben, BP = butylparaben

years old) participated in the study. The subjects were only allowed to use a phthalate and BP free moisturizer and deodorant supplied by us one week before the study and during the study. The study lasted two consecutive weeks: a control week followed by a treatment week. A cream containing 2% (800 mg/person, 10 mg/kg bw based on measured body weights) of BP (together with 2% diethylphthalate and 2% diethylmethylphthalate) was applied every day for 5 days. The test persons waited 20 min to let the cream absorb into the skin before dressing. Blood samples were centrifuged and aliquots for chemical analysis were acidified with to inhibit endogenous enzyme activity. The aliquots were stored at -20 C until analysis. It is not stated if free or total BP was studied; however as no use of enzymes were reported and the sera were acidified it is assumed that the authors analyzed free BP. The level of BP increased to about 100 ng/ml after 1 hour. A maximum (mean) of 135 ng/ml BP was found 3 hours after applying the cream. Subsequently the level decreased. At 24 hours after the first application the level of BP was 18 ng/ml. The level was then constant for the next 4 days. The authors estimated that (0.135 mg/l x 6 l) 0.8 mg of butylparaben was in circulation at the time of peak concentration corresponding to 0.1% of parent compound. It is not clear if the reduction in the level of BP observed at 24 hours was due to further distribution in the body, enzymatic conjugation or hydrolysis to p-hydroxybenzoic acid.

SCCS notes that the doses applied are much higher than the worst-case doses that the consumer receives and that the study clearly demonstrates that BP does not accumulate.

Seminal plasma

The presence of parabens in the seminal plasma found by *Frederiksen et al. (2011)* is of considerable interest. The authors point out that they cannot say whether the parabens measured in seminal plasma are derived from fluids coming from the testis together with the spermatozoa and thus reflect a direct exposure of the testis or whether they derived from seminal fluid coming from the accessory glands. But irrespective of the route, the authors consider it may be of concern that the medium level of PP in seminal plasma (0.68 ng/ml) is 3 times higher than measured in serum (0.32 ng/ml).

Urine

Ye et al. (2006) measured the urinary concentrations of methyl-, ethyl-, propyl-, butyl (n- and iso-)-, and benzylparabens in a demographically diverse group of 100 anonymous adults with unknown exposure to parabens. The samples were collected from 2003 to 2005 at different times throughout the day. The authors detected MP and PP in > 96% of the samples. The other parabens were detected in more than half of the samples. It was found that parabens in urine appear predominantly in their conjugated forms. The high correlation (Pearson correlation coefficient $r = 0.92$, $p < 0.0001$) between total urinary concentrations of MP and PP suggests that human exposures to MP and PP most likely share common sources. The authors did not differentiate between samples from men and women. *Calafat et al. (2009)* measured the concentrations of free and total (free plus conjugated) MP and PP in urine collected from 42 premature infants in two neonatal intensive care units in the Boston area in 2003. The parabens were detected in all of the samples. The authors point out that their findings suggest that infants may be exposed during critical periods of their development to several potential reproductive and developmental toxicants at levels higher than those reported for the general population.

Calafat et al. (2010) report concentrations of parabens measured in 2548 urine samples from the US National Health and Nutrition Examination Survey (NHANES) 2005-2006 study. The urine specimens were collected from a one-third subset of participants > 6 years of age. Participants provided one spot urine sample during one of three daily examination sessions. The samples were shipped on dry ice to CDC's National Center for Environmental Health and stored at temperatures below -20 ^{o}C until analyzed. The samples were analyzed for total parabens. MP and PB in urine were detected in > 92% of the samples examined; EP and BP

in about 50%. The high frequency of detection of MP and PP most likely resulted from their wide use in food products and in common personal care products (e.g., lotions, cosmetics, hair preparations). The range of urinary concentrations spanning up to three orders of magnitude may be related to lifestyle factors, including diet, that result in exposure differences and/or to individual variations in bioavailability, distribution kinetics, or metabolism of the parabens. The concentrations of parabens were higher in women than in men.*Meeker et al. (2011)* collected urine samples from males attending an infertility clinic in USA between 2000 and 2004. The study involved 194 men between 18 and 55 years of age. The urine was analyzed for total (free plus conjugated) MP, PP, BP, and bisphenol A (BPA). Associations with serum hormone levels (n = 167), semen quality parameters (n = 190), and sperm DNA damage measures (n = 132) were assessed using multivariable linear regression. The urine samples were divided in aliquots and frozen at -80 °C. Detection rates in urine were 100% for MP, 92% for PP, and 32% for BP. Paraben exposures in the present study were likely representative of those found among men in the U.S. general population as the numbers were similar to those reported in males participating in the NHANES for 2005-2006 (Calafat et al. 2010). It should be noted that paraben exposure was much higher among women than among men in NHANES.

With the exception of a suggestive inverse association between MP and TSH (thyroid-stimulating hormone), and a suggestive positive association between BP and FAI (free androgen index), no evidence for a relationship between MP, PP, or BP and altered hormone levels or conventional semen quality parameters was found. For sperm DNA damage, a suggestive inverse association between PP and TDM (tail distributed moment), a suggestive positive association between MP and Tail%, and a statistically significant positive association between BP and Tail% was found. *Frederiksen et al. (2011)* measured parabens in urine samples obtained from 60 young and healthy Danish men (average age 19.7 years, samples collected 2006). Urine was analyzed and the total levels of parabens (sum of unconjugated, deglucuronidated and desulfated parabens and metabolites) were determined. The authors point out that compared with previous studies of urinary concentration of parabens in US male and female adults (Ye et al. 2006) the median urinary concentration of the parabens were in general about 2.5-fold lower in Danish men, with the exception of EP, which was twice as high in the Danish men. This may represent a country difference in use of parabens between the USA and Denmark. However, the US study also included women, whereas the Danish study did not, and thus the difference in excretion pattern may also reflect a difference in exposure between women and men.

Table A4-1: Levels of parabens measured in human serum/plasma, seminal plasma, and urine.

Paraben	Study (parabens analyzed as free and/or total)	Medium (ng/ml)	95 percentile (ng/ml)	Maximum (ng/ml)
Serum/plasma				
Methylparaben	Ye et al. 2008 (free/total); adults	0.2/10.9 (2%)*		9.8/301 (3%)
	Frederiksen et al. 2011 (total); male	1.53		59.6
	Sandanger et al. 2011; female	9.4		142.9
Ethylparaben	Ye et al 2008 (free/total)	<LOD**/0.2		<LOD/5.4
	Frederiksen et al. 2011 (total); male	<LOD		20.8
	Sandanger et al. 2011; female	<0.3		45.9
Propylparaben	Ye et al 2008 (free/total)	<LOD/1.4		2.3/67.4(3%)

	Frederiksen et al. 2011 (total); male	0.32		5.50
	Sandanger et al. 2011; female	<0.2		43.9
Butylparaben	Frederiksen et al. 2011 (total); male	<LOD		0.87
	Sandanger et al. 2011; female	<LOD		<LOD
Benzylparaben	Frederiksen et al. 2011 (total); male	<LOD		0.29
Seminal plasma				
Methylparaben	Frederiksen et al. 2011 (total); male	0.99		180
Ethylparaben		0.14		5.65
Propylparaben		0.68		35.5
Butylparaben		0.06		1.73
Benzylparaben		<LOD		1.48
Urine				
Methylparaben	Ye et al. 2006 (free/total); adults	0.8/43.9 (2%)	27.8/680 (4%)	
	Calafat et al. 2009 (free/total); premature neonates	23/243 (9%)		515/4010 (13%)
	Calafat et al. 2010 (total); 6-11 year	25	125	
	Calafat et al. 2010 (total); female	137	1110	
	Calafat et al. 2010 (total); male	23.7	491	
	Meeker et al. 2011 (total); male	32.6	340	1037
	Frederiksen et al 2011 (total); male	17.7		2002
Ethylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/1.0	1.5/47.5 (3%)	
	Calafat et al. 2010 (total); 6-11 year	<LOD	9.90	
	Calafat et al. 2010 (total); female	1.30 (<LOD-2.20)	98.7	
	Calafat et al. 2010 (total); male	<LOD	25.2	
	Frederiksen et al 2011 (total); male	1.98		564

Propylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/9.1	3.4/279 (1%)	
	Calafat et al. 2009 (free/total); premature infants	1.7/17.0 (10%)		171/1360 (13%)
	Calafat et al. 2010 (total); 6-11 year	2.50	125	
	Calafat et al. 2010 (total); female	29.1	357	
	Calafat et al. 2010 (total); male	2.30	306	
	Meeker et al. 2011 (total); male	4.45	107	229
	Frederiksen et al 2011. (total); male	3.60		256
Butylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/0.5	0.3/29.5 (1%)	
	Calafat et al. 2010 (total); 6-11 year	<LOD	7.50	
	Calafat et al. 2010 (total); female	0.50	34.9	
	Calafat et al. 2010 (total); male	<LOD	3.20	
	Meeker et al. 2011 (total); male	<LOD	3.73	32
	Frederiksen et al. 2011 (total); male	0.19		67.6
Benzylparaben				
	Ye et al. 2006 (free/total); adults	<LOD/<LOD	<LOD/0.5	
	Frederiksen et al. 2011 (total); male	<LOD		2.06

*Percentage of free paraben in relation to the total paraben concentration
**Limit of detection

Discussion

Parabens are used as antimicrobial preservatives in cosmetics and food. The estrogenic activities of parabens have been associated with the free parabens and it is unlikely the conjugated parabens have estrogenic activity (see chapter 3.3.1.). Most of the biomonitoring studies discussed above have only measured the total concentration (free plus conjugated) of the paraben and only a few studies have measured both free and total parabens.

In one study on serum (Ye et al. 2008) and two studies on urine (Ye et al. 2006, Calafat et al. 2009) both free and total parabens were measured. Both in serum and urine from adults the amount of free parabens was in the range 1% to 4% of total paraben measured

(Ye et al. 2006, 2008). In one study of urine from premature infants (Calafat et al. 2009) the amount of free parabens was between 9% and 13%. Thus, the relative amounts of free parabens in relation to conjugated parabens may be higher in premature infants than in adults.

In all studies methylparaben was present in the highest concentration followed by propylparaben. Ethylparaben, butylparaben, and benzylparaben were generally present in lower concentrations. Generally, the levels of parabens were higher in women than in men. The levels of parabens in the urine of children (6 – 11 years old) were similar to those in males. The medium and maximum levels of methylparaben and propylparaben in urine were higher in premature infants than in any of the other groups (Calafat et al. 2009).

Frederiksen et al. (2011) have studied the levels of parabens from the same persons in serum, seminal plasma and urine. Their results indicate that the concentrations of parabens were similar in serum and seminal plasma, but more than 10 times higher in urine than in serum. When all results are considered together it can be concluded that the concentration of parabens are generally much higher in urine than in serum.

Possible relationships between the parabens levels and adverse health effects were only considered by Meeker et al. (2011) in their studies of males attending an infertility clinic. Urinary BP concentration were not associated with hormone levels or conventional semen quality parameters, but they were positively associated with sperm DNA damage (measured as DNA tail% in a comet assay) (p for trend = 0.03).

Free and total MP and PP in urine from premature infants were studied by Calafat et al. (2009). The urinary concentrations of MP and PP were surprisingly high compared to that measured in urine from adults. The finding that the levels of MP and PP were highly correlated (Spearman $r = 0.73$, $p < 0.0001$), indicate that exposures to these parabens most likely share common pathways. As discussed above, the relative amounts of free parabens compared to the total amounts of parabens were significantly higher in the premature infants than in adults. The authors point out that their findings suggest that infants may be exposed during critical periods of their development to several potential reproductive and developmental toxicants at levels higher than those reported for the general population. Their study focused on biomarkers of exposure and they did not explore whether such exposures were associated with adverse health effects in the infants. No information was given about the possible source of the parabens especially whether the source(s) of exposure for this subpopulation is representative for "normal full-term babies" outside the hospital or whether the premature infants were exposed from medical or other specialised products not used otherwise.

Janjua et al. (2007) studied the systemic uptake of butylparaben (800 mg/person; 10 mg/kg bw) after whole-body topical application. 3 hours after application, 0.1% of the applied dose was found in the blood circulation. Under the assumption that the authors measured free BP, the SCCS has calculated the half life of free BP in serum to be about 7 hours. It is noted that the amount applied under estimated worst case conditions of PP and BP (with 0.19% PP in all cosmetic product is about ([17.400 mg x 0.0019] / 60 kg) 0.55 mg/kg bw). The amount applied in the study by Janjua et al. (2007) was thus nearly 20 times higher than the worst case exposure dose. The study by Janjua et al. (2007) clearly shows that the parabens do not accumulate in the body.

Since the parabens do not accumulate it is possible to calculate the systemic exposure dose (SED) on the basis of their urinary excretion. It should be noted, however, that the calculations do not take into account the amount of parabens hydrolyzed to p-hydroxybenzoic acid (PHBA) after reaching the systemic circulation. This may lead to an underestimation of the internal exposure of humans to free parabens. The systemic circulation of p-hydroxybenzoic acid is unknown and yet remains to be determined.

Moreover, the proportion of parabens (and PHBA in food) taken up by the oral route is unknown. Thus, the calculation below will represent the sum of dermal and oral exposure.

Table A4-2: Excretion of parabens in urine calculated as µg/kg bw/day.

Paraben	**Study**	**Median ng/ml**	**95 percentile / Maximum ng/ml**	**Medium excretion µg/kg bw/d***	**95 percentile / Maximum excretion µg/kg bw/d**
Methylparaben	Ye et al. 2006; adults	43.9	680 (95)	1.46	22.7 (95)
	Calafat et al. 2010; 6-11 year	25	125 (95)	1.67	8.33 (95)
	Calafat et al. 2010; female	137	1110 (95)	4.56	37.0 (95)
	Calafat et al. 2010; male	23.7	491 (95)	0.79	16.4 (95)
	Meeker et al. 2011; male	32.6	340 (95); 1037	1.09	11.3(95); 34.6
	Frederiksen et al 2011; male	17.7	2002	0.59	66.7
Ethylparaben					
	Ye et al. 2006; adults	1.0	47.5 (95)	0.03	1.58 (95)
	Calafat et al. 2010; 6-11 year	<LOD**	9.90 (95)	--	0.66 (95)
	Calafat et al. 2010; female	1.30	98.7 (95)	0.04	3.29 (95)
	Calafat et al. 2010; male	<LOD	25.2 (95)	--	0.84 (95)
	Frederiksen et al. 2011;	1.98	564	0.07	18.8

	male				
Propylparaben					
	Ye et al. 2006; adults	9.1	279 (95)	0.30	9.3 (95)
	Calafat et al. 2010; 6-11 year	2.50	125 (95)	0.17	8.3 (95)
	Calafat et al. 2010; female	29.1	357 (95)	0.97	11.9 (95)
	Calafat et al. 2010; male	2.30	306 (95)	0.08	10.2 (95)
	Meeker et al. 2011; male	4.45	107 (095); 226	0.15	3.57 (95); 7.53
	Frederiksen et al 2011; male	3.60	256	0.12	8.53
Butylparaben					
	Ye et al. 2006	0.5	29.5 (95)	0.02	0.98 (95)
	Calafat et al. 2010; 6-11 year	<LOD	7.50 (95)	--	0.50 (95)
	Calafat et al. 2010; female	0.50	34.9 (95)	0.02	1.16 (95)
	Calafat et al. 2010; male	<LOD	3.20 (95)	--	0.11 (95)
	Meeker et al. 2011; male	<LOD	3.73(95); 32	--	0.12(95); 1.07
	Frederiksen et al 2011; male	0.19	67.6	0.01	2.25

Benzylparaben					
	Ye et al. 2006; adults	<LOD	0.5 (95)		0.02 (95)
	Frederiksen et al 2011; male	<LOD	2.06		0.07

* For adults an average body weight of 60 kg was assumed. For the age group of children 6-11 years, an average body weight of 30 kg was assumed. For all groups, a daily urine volume of 2 liter was assumed

** Limit of detection

All the calculations in Table **A4-2** are based on the concentrations of total parabens. The highest values for exposure for parabens were found for females in the study of Calafat et al. (2010). For methylparaben the SEDs were calculated to 4.56 µg/kg bw/d and 37.0 µg/kg bw/d for medium and 95 percentile, respectively. The corresponding values for propylparaben were 0.97 µg/kg bw/d and 11.9 µg/kg bw/d for medium and 95 percentile, respectively and in the case of butylparaben the values were 0.02 and 1.16 µg/kg bw/d, respectively. The results of the biomonitoring studies thus support that the exposure calculation made in opinion SCCS/1348/10 overestimates consumer exposure. It also has to be noted, that the use levels of parabens in the USA are not regulated and might be higher than in Europe.

References Annex 4

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Personal Care Products Council
Committed to Safety, Quality & Innovation

To: Alan Andersen
Director, CIR

From: Halyna Breslawec
EVP Science

Date: December 15, 2011

Subject: Request for CIR Expert Panel to Consider Parabens Re-review

The European Union's Scientific Committee on Consumer Safety(SCCS) adopted an opinion (SCCS/1348/10) on the use of parabens in cosmetics and personal care products in December, 2010 (revised in March, 2011)[1]. The opinion concluded that, consistent with previous opinions of the SCCS and its predecessor, the Scientific Committee on Consumer Products (SCCP), the use of methylparaben and ethylparaben at concentrations of 0.4% (individual) or 0.8% (total mixture of parabens) can be considered safe. However, the opinion concluded that the allowed levels for propylparaben and butylparaben should be reduced such that the sum of their individual concentrations should not exceed 0.19%. In arriving at this conclusion, the SCCS used values for dermal absorption and reproductive toxicity that they identified as conservative, based on their view that the available dermal absorption and reproductive toxicity studies had significant limitations. The SCCS further concluded that the available data for evaluation of isopropylparaben and isobutylparaben were too limited to allow for the evaluation of human risk.

SCCS recently clarified its opinion in response to action by Denmark to ban the use of parabens in children under the age of 3. The clarification was adopted in October 2011, and is also attached[2]. In summary, SCCS concluded that for general cosmetic products containing parabens, excluding specific products for the nappy area, there is no safety concern in children (any age group). 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.

In light of these developments, the Personal Care Products Council respectfully requests that the CIR Expert Panel re-examine its recent review of Parabens, and, if needed, re-review the use of Parabens as ingredients for use in cosmetics and personal care products.

Attachments
[1] SCCS Opinion on Parabens SCCS/1348/10
[2] Clarification on SCCS Opinion on Parabens SCCS/1348/11

cc: Jay Ansell
Carol Eisenmann
Linda Loretz