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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 ± 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. 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× 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× less than estradiol. In one study, Benzylparaben produced an estrogenic response in the uterotrophic assay, but the potency was at least 330,000× 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 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).

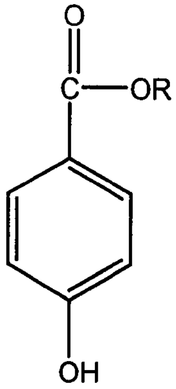

FIGURE 1
Paraben chemical structure. R = alkyl chains that are methyl (CH_3) for Methylparaben, ethyl (C_2H_5) for Ethylparaben, propyl (C_3H_7) for Propylparaben, isopropyl (C_3H_7) for Isopropylparaben, butyl (C_4H_9) for Butylparaben, and isobutyl (C_4H_9) for Isobutylparaben.

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.

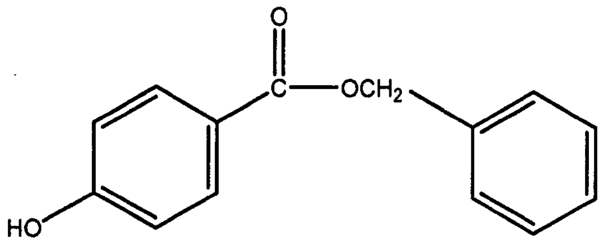

FIGURE 2
Benzylparaben chemical structure.

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*)

		Contains:						
Trade name mixture	Supplier	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 Pemulen® 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

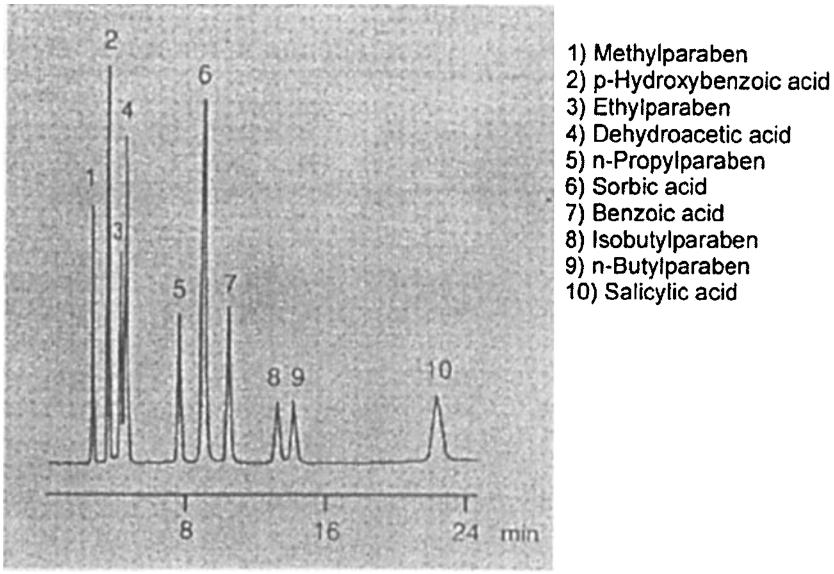

FIGURE 3
Separation of parabens obtainable using high-performance liquid chromatography. (Grom Chromatography GmbH 2004).

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.

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 $\times 10^{3)}$ = flux (μg/cm^{2}h) divided by saturation concentration (mg/ml).

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).

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%

(Continued on next page)

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 $\leq$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).

	Maximum parabens concentration						
Route/dosage form	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)*

	Maximum parabens concentration						
Route/dosage form	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 (μg/cm^{2}h)					
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

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

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 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 μl/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).

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

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).

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 (InfoGenes, 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 essentially 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 μW/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 μW/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 $\geq$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 infants. Certain parabens are also used as preservatives in foods, pharmaceuticals, and other products.

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[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.

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:

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

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

For infants, the relevant calculations are:

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

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

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.

REFERENCES

Aalto, T. R., M. C. Firman, and N. E. Rigler. 1953. *p*-Hydroxybenzoic acid ester as a preservative. 1. Utilization, bactericidal and fungicidal investigations, properties, and determination. *J. Am. Pharm. Assoc. Sci. Ed.* 42:449–457.

Adams, H. J., A. R. Mastri, and D. Charron. 1977. Morphological effects of subarachnoid methylparaben on rabbit spinal cord. *Pharmacol. Res. Commun.* 9:547–551.

Adams, R. M., and H. I. Maibach. 1985. A five-year study of cosmetic reactions. *J. Am. Acad. Dermatol.* 13:1062–1069.

Adler-Hradecky, C., and B. Kelentey. 1960. On the toxicity and local analgetic effect of *p*-hydroxybenzoic acid esters. *Arch. Int. Pharmacodyn. Ther.* 128:135–142.

Aeling, J. L., and D. D. Nuss. 1974. Systemic eczematous "contact-type" dermatitis medicamentosa caused by parabens. *Arch. Dermatol.* 110:640.

Aldrete, J. A., and D. K. Klug. 1970. Alteration of skin reactivity of local anesthetic drugs in guinea pigs. *Int. J. Dermatol.* 9:142–146.

Allwood, M.C 1973. Inhibition of Staphylococcus aureus by combinations of nonionic surface-active agents and antibacterial substances. *Microbios* 7:209–214.

Andersen, F. A., ed. 1995. Final report on the safety assessment of Isobutylparaben and Isopropylparaben. *J. Am. Coll. Toxicol.* 14:364–372.

Andersen, F. A., ed. 2001. Final report on the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. *Int. J. Toxicol.* 20:23–50.

Andersen, F. A., ed. 2005. Annual review of cosmetic ingredient safety assessments—2002/2003. *Int. J. Toxicol.* 24(Supl 1):34–42.

Ansel, H. C., and D. E. Cadwallader. 1964. Hemolysis of erythrocytes by antibacterial preservatives. *J. Pharm. Sci.* 53:169–172.

Applied Research Laboratories. 1939. Special Research Report (Jan. 30, 1939). Unpublished data.[4]

Applied Research Laboratories. 1942. Study of the chronic toxicity of a mixture of 60 parts of propyl and 40 parts of ethyl esters of sodium *para*-hydroxybenzoate. Unpublished data submitted by Heyden Chemical Corp. 10 pages.[4]

Ashby, J., H. Tinwell, P. A. Lefevre, R. Joiner, and J. Haseman. 2003. The effect on sperm production in adult Sprague-Dawley Rats exposed by gavage to bisphenol A between postnatal days 91–97. *Toxicol. Sci.* 74:129–138.

Atkins, F. 1950. Some aspects of creams in cosmetics. *Manuf. Chem.* 21:51–54.

Attebery, J. A. 1975. Use of micro-particles for preparative liquid chromatography. *Chromatographia* 8:121–123.

Austin, K. L., and L. E. Mather. 1978. Simultaneous quantitation of morphine and paraben preservatives in morphine injectables. *J. Pharm. Sci.* 67:1510–1511.

Bandman, H. J., C. D. Calnan, E. Cronin et al. 1972. Dermatitis from applied medicaments. *Arch. Dermatol.* 106:335–337.

Bando, H., S. Mohri, F. Yamashita, Y. Takakura, and M. Hashida. 1997. Effects of skin metabolism on percutaneous penetration of lipophilic drugs. *J. Pharm. Sci.* 86:759–761.

Batchelder, M., H. I. Tarlin, and G. Williamson. 1972. Column chromatographic separation and determination of methyl *p*-hydroxybenzoate. *J. Pharm. Sci.* 61:252–253.

Benmaman, J. D., and D. L. Sorby. 1965. Characterization of the degradation products of methyl and ethyl p-hydroxybenzoate by thin-layer chromatography. *J. Chromatogr.* 20:607–610.

Bijlsma, U. G. 1928. Solbrol-*p*-hydroxybenzoic acid methyl ester. *Arch. Int. Pharmacodyn. Ther.* 34:173–179.

Boehm, E. E. and D. N. Maddox. 1972. Recent applications of preservatives for pharmaceuticals. *Manuf. Chem. Aerosol News.* 43:21–23.

Bolle, A., and J. Mirimanoff. 1950. Antagonism between non-ionic detergents and antiseptics. *J. Pharm. Pharmacol.* 2:685–692.

Brodersen, R. 1974. Competitive binding of bilirubin and drugs to human serum albumin studied by enzymic oxidation. *J. Clin. Lab. Invest.* 54:1353–1364.

Bronswijk, J. E. V., and H. H. Koekkoek. 1971. Nipagin (*p*-methyl hydroxybenzoate) as a pesticide against a house dust mite: *Dermatophagoides pteronyssinus*. *J. Med. Entymol.* 8:748.

Brown, N. D., L. L. Hall, H. K. Sleeman, and B. P. Doctor. 1978a. Ion-pair high-performance liquid chromatographic separation of a multicomponent anticholinergic drug formulation. *J. Chromatogr.* 148:453–458.

Brown, I. M., M. M. Kaplan, and J. M. Szabocsik. 1978b. An improved in vitro screening method for cytotoxicity. *Dev. Ind. Microbiol.* 19:299–307.

Brulos, M. F., J. P. Guillot, M. C. Martini, and J. Cotte. 1977. Influence of perfumes on the sensitizing potential of cosmetic bases. 1. A technique for evaluating sensitizing potential. *J. Soc. Cosmet. Chem.* 28:357–365.

Bubnoff, M. V., D. Schnell, and J. Vogt-Moykoff. 1957. Concerning the pharmacology of benzoic acid, *p*-chlorobenzoic acid, as well as *p*-hydroxybenzoic acid and its esters. *Arzneim.-Forsch.* 7:340–344.

Budavari, S., ed. 1989. *The Merck Index*, 11th ed. Rahway, NJ: Merck & Co.

Byford, J. R., L. E. Shaw, M. G. Drew, G. S. Pope, M. J. Sauer, and P. D. Darbre. 2002. Oestrogenic activity of parabens in MCF7 human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 80:49–60.

Cantox Health Sciences International. 2004. Assessment of the endocrine distrupting/oestrogenic potential of parabens. Unpublished data submitted by CTFA. 90 pages.[4]

Cantwell, F. F. 1976. Pre-column reactions to eliminate interferents in the liquid chromatographic analysis of p-hydroxybenzoates in complex pharmaceuticals. *Anal. Chem.* 48:1854–1859.

Carradori, S., A. M. Peluso, and M. Faccioli. 1990. Systemic contact dermatitis due to parabens. *Contact Dermatitis* 22:238–239.

Caude, M., and X. P. Le. 1976. Analysis of pharmaceuticals associated in various formulations by high-speed liquid chromatography. *Chromatographia* 9:20–29.

Cavill, G. W. K., and J. M. Vincent. 1948. The fungistatic properties of *p*-aminobenzoic acid and related compounds. I. Growth curves obtained with *Aspergillus niger, Penicillium roqueforti, and Byssochlamys fulva. J. Soc. Chem. Ind.* 67:25–33.

Charles River Discovery and Development Services. 2005a. Oral (diet) reproduction toxicity study of methylparaben in male rats. Protocol 1203–008. Final report date: 4 May 2005. Unpublished data submitted by CTFA.[4]

Charles River Discovery and Development Services. 2005b. Oral (diet) reproduction toxicity study of butylparaben in male rats. Protocol 1203–006. Final report date: March 17, 2005. Unpublished data submitted by CTFA.[4]

Chiang, H. C. 1969. Polyamide-silica gel thin-layer chromatography of food preservatives. *J. Chromatogr.* 44:201–203.

Chichester, D. R., and F. W. Tanner, Jr. 1968. Antimicrobial food additives. In: *Handbook of food additives*, ed. T. E. Furia. Cleveland: Chemical Rubber Company.

Clarke, G., and I. A. Rashid. 1977. Quantitative determination of methyl and propyl *p*-hydroxybenzoates by high-performance liquid chromatography. *Analyst (Lond.)* 102:685–687.

Clemens, W. 1969. Paper chromatographic identification of food preservatives. *Fette Seifen Einshl. Anstrichm.* 57:109–111.

Close, J. A., and P. A. Nellsen. 1976. Resistance of a strain of Pseudomonas cepacia to esters of *p*-hydroxybenzoic acid. *Appl. Environ. Microbiol.* 31:718–722.

Conner M. W., B. H. Conner, J. G. Fox, and A. E. Rogers. 1983. Spontaneous amyloidosis in outbred CD-1 mice. *Surv Synth Path Res* 1:67–78.

Cooper, S. M. and S. Shaw. 1998. Allergic contact dermatitis from parabens in a tar shampoo. *Contact Dermatitis* 39:140.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1976a. Safety data test summary: Acute oral toxicity, Methylparaben. Unpublished data submitted by CTFA (Dec. 15, 1976; CTFA Code No. 2-7-87).[4]

CTFA. 1976b. Safety data test summary: Acute oral toxicity, eyeliner containing Butylparaben. Unpublished data submitted by CTFA (Feb. 10, 1976; CTFA Code No. 2-7-19).[4]

---

[4] Available upon request: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

CTFA. 1976c. Safety data test summary: Acute primary skin irritation test, methylparaben. Unpublished data submitted by CTFA (Dec. 15, 1976; CTFA Code No. 2-7-86).[4]

CTFA. 1976d. Safety data test summary: Three-day skin irritation test of eyeliner containing Butylparaben. Unpublished data submitted by CTFA (Feb. 5, 1976; CTFA Code No. 2-7-18).[4]

CTFA. 1976e. Safety data test summary: Rabbit eye irritation test, Methylparaben. Unpublished data submitted by CTFA (Dec. 15, 1976; CTFA Code No. 2-7-88).[4]

CTFA. 1976f. Safety data test summary: Allergic contact sensitization test of moisturizing facial mask containing Propylparaben. Unpublished data submitted by CTFA (Nov. 1, 1976; CTFA Code No. 2-7-127).[4]

CTFA. 1976g. Safety data test summary: Repeat insult patch test of eyeliner containing Butylparaben. Unpublished data submitted by CTFA (March 26, 1976; CTFA Code No. 2-7-20).[4]

CTFA. 1977a. Safety data test summary: Acute oral toxicity, product containing Propylparaben. Unpublished data submitted by CTFA (Aug. 11, 1977; CTFA Code No. 2-7-120).[4]

CTFA. 1977b. Safety data test summary: Four-day skin irritation test of product containing Propylparaben. Unpublished data submitted by CTFA (Aug. 11, 1977; CTFA Code No. 2-7-119).[4]

CTFA. 1977c. Safety data test summary: Single insult clinical patch test of product containing Propylparaben. Unpublished data submitted by CTFA (June 16, 1977; CTFA Code No. 2-7-122).[4]

CTFA. 1977d. Safety data test summary: Cumulative irritancy patch test of facial mask containing Propylparaben. Unpublished data submitted by CTFA (May 23, 1977; CTFA Code No. 2-7-126).[4]

CTFA. 1978a. Safety data test summary: Single insult clinical patch test of product containing Methylparaben. Unpublished data submitted by CTFA (March 16, 1978; CTFA Code No. 2-7-109).[4]

CTFA. 1978b. Safety data test summary: Single insult clinical patch test of product containing Methylparaben. Unpublished data submitted by CTFA (June 27, 1978; CTFA Code No. 2-7-94).[4]

CTFA. 1978c. Safety data test summary: Prophetic patch test and repeat insult patch test of eye makeup containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-16).[4]

CTFA. 1979a. Safety data test summary: Acute oral toxicity, product containing Methylparaben. Unpublished data submitted by CTFA (April 11, 1979; CTFA Code No. 2-7-92).[4]

CTFA. 1979b. Safety data test summary: Acute oral toxicity, product containing Methylparaben. (April 20, 1979; CTFA Code No. 2-7-106).[4]

CTFA. 1979c. Safety data test summary: Oral, dermal, and ocular testing of product containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-15).[4]

CTFA. 1979d. CIR safety data test summary: Primary skin irritation test of product containing Methylparaben. Unpublished data submitted by CTFA (April 11, 1979; CTFA Code No. 2-7-117).[4]

CTFA. 1979e. Safety data test summary: Primary skin irritation test of product containing Methylparaben. Unpublished data submitted by CTFA (April 20, 1979; CTFA Code No. 2-7-108).[4]

CTFA. 1979f. Safety data test summary: Rabbit eye irritation test of product containing Methylparaben. Unpublished data submitted by CTFA (April 11, 1979; CTFA Code No. 2-7-93).[4]

CTFA. 1979g. Safety data test summary: Rabbit eye irritation test of product containing Methylparaben. Unpublished data submitted by CTFA (April 20, 1979; CTFA Code No. 2-7-107).[4]

CTFA. 1979h. Safety data test summary: Controlled use study on eye makeup containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-17).[4]

CTFA. 1979i. Safety data test summary: Repeat insult patch test of hand cream containing Methylparaben. Unpublished data submitted by CTFA (June 4, 1979; CTFA Code No. 2-7-29).[4]

CTFA. 1979j. Safety data test summary: Repeat insult patch testing of product contining Methylparaben. (April, 1979; CTFA Code No. 2-7-32).[4]

CTFA. 1979k. Safety data test summary: Repeated insult patch testing of product containing Methylparaben. Unpublished data submitted by CTFA (April, 1979; CTFA Code No. 2-7-74).[4]

CTFA. 1980a. Safety data test summary: Acute oral toxicity, Ethylparaben. Unpublished data submitted by CTFA (Jan. 25, 1980; CTFA Code No. 2-7-89).[4]

CTFA. 1980b. Safety data test summary: Oral, dermal, and ocular testing of product containing Butylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-12).[4]

CTFA. 1980c. Acute oral, dermal, and mucous membrane toxicity testing of product containing Butylparaben and Propylparaben. Unpublished data Submitted by CTFA (June 16, 1980; CTFA Code No. 2-7-52).[4]

CTFA. 1980d. One-month oral toxicity evaluation of product CN 0031/9 containing Methylparaben and Propylparaben in the rat, study R04579. Unpublished data submitted by CTFA (Dec. 1980; CTFA Code No. 2-7-40).[4]

CTFA. 1980e. One-month oral toxicity evaluation of product BV-0011 containing Butylparaben and Propylparaben in the rat, study R05780. Unpublished data submitted by CTFA (Nov. 1980; CTFA Code No. 2-7-47).[4]

CTFA. 1980f.. Subchronic (three-month) dermal toxicity study in rabbits with product AI-0024 containing Methylparaben, study B-7049. Unpublished data submitted by CTFA (Sept. 1980; CTFA Code No. 2-7-34).[4]

CTFA. 1980g.. Subchronic (three-month) dermal toxicity study in rabbits with product AI-0025 containing Methylparaben, study B-7049. Unpublished data submitted by CTFA (Oct. 1980; CTFA Code No. 2-7-75).[4]

CTFA. 1980h.. Safety data test summary: Primary skin irritation test, Ethylyparaben. Unpublished data submitted by CTFA (Jan. 25, 1980; CTFA Code No. 2-7-91).[4]

CTFA. 1980i. Safety data test summary: Rabbit eye irritation test, Ethylparaben. Unpublished data submitted by CTFA (Jan. 25, 1980; CTFA Code No. 2-7-90). [4]

CTFA. 1980j. Safety data test summary: Controlled use study on eye makeup containing Butylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-14).[4]

CTFA. 1980k. Safety data test summary: Prophetic patch test and insult patch test of eye makeup containing Butylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-13).[4]

CTFA. 1980l. Repeated insult patch test of product BV-0011 containing Butylparaben and Propylparaben. Unpublished data submitted by CTFA (Sept. 1980; CTFA Code No. 2-7-46).[4]

CTFA. 1980m. Repeated insult patch test of product BV-0011 containing Butylparaben and Propylparaben. Unpublished data submitted by CTFA (April 1980; CTFA code No. 2-7-50).[4]

CTFA. 1981a. Safety data test summary: Acute oral toxicity, hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-81).[4]

CTFA. 1981b. Safety data test summary: Acute oral toxicity, product containing Ethylparaben. Unpublished data Submitted by CTFA (Feb. 17, 1981; CTFA Code No. 2-7-129).[4]

CTFA. 1981c. Safety data test summary: Acute oral toxicity, product containing Ethylparaben. Unpublished data submitted by CTFA (Feb. 17, 1981; CTFA Code No. 2-7-132).[4]

CTFA. 1981d. Safety data test summary: Acute dermal toxicity test of hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-80).[4]

CTFA. 1981e. Subchronic (three-month) dermal toxicity study in rabbits with product CN-0028 containing Methylparaben, study B-7398. Unpublished data submitted by CTFA (April 1981; CTFA Code No. 2-7-37).[4]

CTFA. 1981f. Thirteen-week subchronic dermal toxicity study in albino rats with medicated cream containing Methylparaben and medicated lotion containing Propylparaben, study project code AT0165. Unpublished data submitted by CTFA (April 24, 1981; CTFA Code No. 2-7-113).[4]

CTFA. 1981g. Safety data test summary: Primary skin irritation test of hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-79).[4]

CTFA. 1981h. Safety data test summary: Primary skin irritation test of product containing Ethylparaben. Unpublished data submitted by CTFA (Feb. 17, 1981; CTFA Code No. 2-7-131).[4]

CTFA. 1981i. CIR safety data test summary: Primary skin irritation test of product containing Ethylparaben. Unpublished data submitted by CTFA (Feb. 17, 1981; CTFA Code No. 2-7-135).[4]

CTFA. 1981j. CIR safety data test summary: Rabbit eye irritation test of product containing Ethylparaben. Unpublished data submitted by CTFA Feb. 17, 1981; CTFA Code No. 2-7-130).[4]

CTFA. 1981k. Safety data test summary: Rabbit eye irritation test of hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-78).[4]

CTFA. 1981l. Safety data test summary: Rabbit eye irritation test of product containing Ethylparaben. Unpublished data submitted by CTFA (Feb. 17, 1981; CTFA Code No. 2-7-134).[4]

CTFA. 1981m. Safety data test summary: Rabbit eye irritation test of product containing Propylparaben. Unpublished data submitted by CTFA (Aug. 28, 1981; CTFA Code No. 2-7-121).[4]

CTFA. 1981n. Safety data test summary: Rabbit eye irritation test of eyeliner containing Butylparaben. Unpublished data submitted by CTFA (Sept. 8, 1981; CTFA Code No. 2-7-17).[4]

CTFA. 1981o. Safety data test summary: 21-Day skin irritation test of hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-77).[4]

CTFA. 1981p. Modified Magnusson-Kligman guinea pig maximization test for contact sensitization potential of Ethylparaben and Methylparaben, Study Project GPA-05-81. Unpublished data submitted by CTFA (Sept. 28, 1981; CTFA Code No. 2-7-137).[4]

CTFA. 1981q. Safety data test summary: Guinea pig sensitization test of hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-76).[4]

CTFA. 1981r. Safety data test summary: Five-day clinical patch test of hairdressing containing Methylparaben. Unpublished data submitted by CTFA (Sept. 22, 1981; CTFA Code No. 2-7-82).[4]

CTFA. 1981s. Cosmetic complaint experience: Body scrub, bubble bath, hand lotion, and suntan lotions containing Methylparaben. Unpublished data submitted by CTFA (CTFA Code Nos. 2-7-58; 2-7-59; 2-7-60; 2-7-61; and 2-7-85).[4]

CTFA. 1981t. Cosmetic complaint experience: Protective face cream containing Propylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-57).[4]

CTFA. 1981u. Cosmetic complaint experience: Mascara 1237 containing Butylparaben and Propylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-22).[4]

CTFA. 1981v. Cosmetic complaint experience: Aftershave lotion containing Butylparaben and Propylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-28).[4]

CTFA. 1981w. Cosmetic complaint experience: Mascara 1438 containing Butylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-21).[4]

CTFA. 1981x. Cosmetic ingredient chemical description of Benzylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-7).[4]

CTFA. 1985. Benzylparaben: Acute oral toxicity, acute dermal irritation/corrosion, acute eye irritation/corrosion, and COLIPA summary. Unpublished data submitted by CTFA.[4]

CTFA. 2003. Concentration of use of paraben ingredients. Unpublished data submitted by CTFA.[4]

CTFA. 2004a. Concentration of use of benzyl, isopropyl, and isobutyl paraben ingredients. Unpublished data submitted by CTFA.[4]

CTFA. 2004b. Concentration of use of paraben ingredients in baby products. Unpublished data submitted by CTFA.[4]

CTFA. 2005. Calculation of margin of safety. Adult and baby exposures. Unpublished data submitted by CTFA.[4]

Cox, G. B., C. R. Loscombe, and K. Sugden. 1977. Some applications of bonded-phase high-performance liquid chromatography to the analysis of pharmaceutical formulations. *Anal. Chim. Acta* 92:345–352.

Cramer, H. J., and H. D. Unrein. 1963. Uber kontaktekzeme durch salbenkonservierungsmittel bei kosmetischen hautkremes. *Allerg. Asthma* 9:141–144.

Cronin, E. 1972. Clinical prediction of patch test results. *Trans. St. Johns Hosp. Dermatol. Soc.* 58:153–162.

Cross, S. E., and M. S. Roberts. 2000. The effect of occlusion on epidermal penetration of parabens from a commercial allergy test ointment, acetone and ethanol vehicles. *J. Invest. Dermatol.* 115:914–918.

Daenens, P., and L. Laruelle. 1973. Column chromatographic cleanup and gas-liquid chromatographic determination of hydroxybenzoic esters (parabens) in food. *J. Assoc. Off. Anal. Chem.* 56:1515–1517.

Dal Pozzo, A., and N. Pastori. 1996. Percutaneous absorption of parabens from cosmetic formulations. *Int. J. Cosmetic Sci.* 18:57–66.

Darbre, P. D., J. R. Byford, L. E. Shaw, R. A. Horton, G. S. Pope, and M. J. Sauer. 2002. Oestrogenic Activity of Isobutylparaben *In vitro* and *In vivo*. *J. Appl. Toxicol.* 22:219–226.

Darbre, P. D., J. R. Byford, L. E. Shaw, S. Hall, N. G. Coldham, G. S. Pope, and M. J. Sauer. 2003. Oestrogenic activity of Benzylparaben. *J. Appl. Toxicol.* 23:43–51.

Daston, G. P. 2004. Developmental toxicity evaluation of Butylparaben in Sprague-Dawley Rats. *Birth Defects Res. (Part B)* 71:296–302.

De Navarre, M. G. 1956. Interference between some nonionics and certain preservatives. *Congr. Mondial Detergence et Prods. Tensioactifs. Ier Congr.* 2:741–742.

De Navarre, M. G. 1957. The interference of nonionic emulsifiers with preservatives. III. *J. Soc. Cosmet. Chem.* 8:68–75.

Derache, R., and J. Gourdon. 1963. Metabolism of a food preservative: *p*-hydroxybenzoic acid and its esters. *Food Cosmet. Toxicol.* 1:189–195.

Deshusses, J. 1945. Identification of *p*-hydroxybenzoic acid esters. *Pharm. Acta. Helv.* 20:200–201.

Donato, S. J. 1965. Separation and estimation of methyl and propyl esters of *p*-hydroxybenzoic acid by gas chromatography. *J. Pharm. Sci.* 54:917–918.

Dymicky, M., and C. N. Huhtanen. 1979. Inhibition of *Clostridium botulinum* by *p*-hydroxybenzoic acid n-alkyl esters. *Antimicrob. Agents Chemother.* 15:798–801.

Echeverria, P., J. Loria, and A. L. Smith. 1975. Displacement of bilirubin from albumin by antibiotics and preservatives. *Pediatr. Res.* 9:283.

Edwards, R. W., H. R. Nanji, and M. K. Hassan. 1936. The detection and determination of *p*-hydroxybenzoic acid and its derivatives, with special reference to their distinction from salicylic and benzoic acids. *Analyst* 62:178–185.

Elder, R. L., ed. 1984. Final report on the safety assessment of Benzylparaben. *J. Am. Coll. Toxicol.* 5:301–307.

Elder, R. L., ed. 1984. Final report on the safety assessment of Methylparaben, Ethylparaben, Propylparaben, and Butylparaben. *J. Am. Coll. Toxicol.* 3:147–209.

Engst, R., L. Prahl, and E. Jarmatz. 1969. Analysis of preservatives. II. Colorimetric methods for the determination of hexamethylenetetramine (formaldehyde), benzoic acid, sorbic acid, and *p*-hydroxybenzoates. *Nahrung* 13:417–426.

Environmental Protection Agency (EPA). 2006a. Endocrine disrupter program development. http://www.epa.gov/scipoly/oscpendo/pubs/edspoverview/development.htm.

EPA. 2006. Endocrine disrupter methods validation—assay status table. http://www.epa.gov/scipoly/oscpendo/pubs/assayvalidation/status.htm.

Epstein, S. 1968. Paraben sensitivity: Subtle trouble. *Ann. Allergy* 26:185–189.

Esposito, E., F. Bortolotti, C. Nastruzzi, E. Menegatti, and R. Cortesi. 2003. Diffusion of preservatives from topical dosage forms: A comparative study. *J. Cosmet. Sci.* 54:239–250.

European Commission. 2005a. Scientific Committee on Consumer Products: Opinion on the safety evaluation of parabens. 28 January 2005. SCCP/0873/05. Brussels: EC.

European Commission. 2005b. Scientific Committee on Consumer Products: Extended opinion on the safety evaluation of parabens coverin in addition Isopropylparaben. 28 January 2005. SCCP/0873/05. Brussels: EC.

European Commission. 2005c. Scientific Committee on Consumer Products: Opinion on parabens, underarm cosmetics and breast cancer. 28 January 2005. SCCP/0874/05. Brussels: EC.

European Food Safety Authority. 2004. Opinion adopted by the AFC Panel related to para hydroxybenzoates (E 214–219), adopted 13 July, 2004. http://www.efsa.eu.int/science/afc/afc_opinions/630_en.html.

Evans, S. 1970. Epidermal sensitivity to lanolin and parabens: Occurrence in pharmaceutical and cosmetic products. *Br. J. Dermatol.* 82:625.

Fang, H., W. Tong, W. S. Branham et al. 2003. Study of 202 natural, synthetic, and environmental chemicals for binding to the androgen receptor. *Chem. Res. Toxicol.* 16:1338–1358.

Fasano, W. J. 2004a. Methylparaben and Butylparaben: In vitro dermal penetration and metabolism in rat and human skin. E. I. du Pont de Nemours and Company, Haskell Laboratory (DuPont-13966). Unpublished data submitted by CTFA. (175 pages.)[4]

Fasano, W. J. 2004b. Butylparaben: In vitro dermal penetration and metabolism using full thickness human skin. E. I. du Pont de Nemours and Company, Haskell Laboratory (DuPont-15565). Unpublished data submitted by CTFA. (54 pages.)[4]

Fasano, W. J. 2005. Butylparaben: In vitro kinetics and metabolism using full thickness human skin. E. I. du Pont de Nemours and Company, Haskell Laboratory (DuPont-14795). Unpublished data submitted by CTFA. (55 pages.)[4]

Fine, P. G., and D. L. Dingman. 1988. Hypersensitivity dermatitis following suction-assisted lipectomy: A complication of local anesthetic. *Ann. Plast. Surg.* 20:573–575.

Fellegiova, M. 1963. Paper-chromatographic determination of ethyl *p*-hydroxybenzoate. *Z. Lebensm.-Unters.-Forsch.* 120:17–20.

Ficicchia, F., and S. Del Mastro. 1977. Identification and assay of *p*-hydroxybenzoates in two complex formulations. *Boll. Chim. Farm.* 116:553–557.

Fischer, R. 1934. Identification of organic preservatives and commercial sweetening substances in foodstuffs. *Z. Unters. Lebensm.* 67:161–172.

Fischmeister, I., L. Hellgren, and J. Vincent. 1975. Infrared spectroscopy for tracing of topically applied ointment vehicles and active substances on healthy skin. *Arch. Dermatol. Res.* 253:63–69.

Fisher, A. A. 1971. The role of topical medication in the management of stasis ulcers. *Angiology* 22:206–210.

Fisher, A. A. 1975. Letter: Paraben-induced dermatitis. *Arch. Dermatol.* 111:657–658.

Fisher, A. A. 1979. Paraben dermatitis due to a new medicated bandage: The "paraben paradox." *Contact Dermatitis* 5:273–274.

Fisher, A. A. 1982. Cortaid cream dermatitis and the "paraben paradox." *J. Am. Acad. Dermatol.* 6:116–117.

Fisher, A. A. 1993. The Parabens: Paradoxical preservatives. *Cutis* 51:405–406.

Fisher, A. A. 1996. Esoteric contact dermatitis. Part I: The Paraben paradox. *Cutis* 57:65–66.

Fisher, J. S., K. J. Turner, D. Brown, and R. M. Sharpe. 1999. Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. *Environ. Health Perspect.* 107:397–405.

Fitzpatrick, F. A., A. F. Summa, and A. D. Copper. 1975. Quantitative analysis of methyl and propylparaben by high performance liquid chromatography. *J. Soc. Cosmet. Chem.* 26:377–387.

Food and Drug Administration (FDA). 1984. Frequency and concentration of use of cosmetic ingredients. *FDA database.* Washington, DC: FDA.

FDA. 2008. National Center for Toxicological Research. Estrogen Receptor Binding Dataset. http://www.fda.gov/nctr/science/centers/toxicoinformatics/edkb/documents/erNCTR232.xls.

FDA. 2004. Inactive Ingredient Search for Approved Drug Products. Center for Drug Evaluation and Research, FDA. http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.

FDA. 2006. Frequency of use of cosmetic ingredients. *FDA database.* Washington, DC: FDA.

Food and Drug Research Labs (FDRL). 1972. Teratologic evaluation of FDA 71–38 (methyl paraben). National Technical Information Service (NTIS) Report PB-221 785.

Food and Drug Research Labs. 1973. Teratologic evaluation of FDA 71–38 (methyl paraben). NTIS Report PB-223 817.

Food and Drug Research Labs. 1978a. Clinical safety evaluation of product CN-0028 containing Methylparaben and Propylparaben, photoallergy series. Unpublished data submitted by CTFA (Nov. 27, 1978 CTFA Code No. 2-7-35).[4]

Food and Drug Research Labs. 1978b. Clinical safety evaluation of two products, photoallergy series: Protective face cream containing Propylparaben. Unpublished data submitted by CTFA (Nov. 14, 1978; CTFA Code No. 2-7-68).[4]

Food and Drug Research Labs. 1979. Clinical safety evaluation of two products, photoallergy series: Product CN-0031/9 containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (June 22, 1979); (CTFA Code No. 2-7-38).[4]

Food and Drug Research Labs. 1984. Clinical safety evaluation of two products, photoallergy series: Suntan lotion pF-6 containing Methylparaben. Unpublished data submitted by CTFA (CTFA Code No. 2-7-84).[4]

Foster, P. M. D. 2004. What is the evidence of risks to human reproduction from exposure to endocrine active chemicals? Presentation to the CIR Expert Panel, June 28, 2004.[4]

Fraki, J. E., L. Peltonen., and V. K. Hopsu-Havu. 1979. Allergy to various components of topical preparations in stasis dermatitis and leg ulcer. *Contact Dermatitis* 5:95–100.

Freese, E., C. W. Sheu, and E. Galliers. 1973. Function of lipophilic acids as antimicrobial food additives. *Nature* 241:321–325.

Fukuda, T., K. Mimura, and K. Mikami. 1969. Identification of preservatives, sterilizers, and artificial sweeteners by paper electrophoresis. *Eisei Kagaku* 15:317–319.

Fujiwara, M., L. Matsumura, and K. Fujiwara. 1971. Determination of preservatives by ion-exchange chromatography. I. Separation by Dowex 2XB-Cl-form. *Shokuhin Eiseigaku Zasshi* 12:40–46.

Furr, J. R., and A. D. Russell. 1972a. Uptake of esters of *p*-hydroxybenzoic acid by *Serratia marcescens* and by fattened and non-fattened cells of *Bacillus subtilis*. *Microbios* 5:237–246.

Furr, J. R., and A. D. Russell. 1972b. Factors influencing the activity of esters of *p*-hydroxybenzoic acid against *Serratia marcescens*. *Microbios* 5:189–198.

Furr, J. R., and A. D. Russell. 1972c. Effect of esters of *p*-hydroxybenzoic acid on spheroplasts of *Serratia marcescens* and protoplasts of *Bacillus megaterium*. *Microbios* 6:47–54.

Geddes, B. A., and N. M. Lefcoe. 1973. Respiratory smooth muscle relaxing effect of commercial steroid preparations. *Am. Rev. Respir. Dis.* 107: 395–399.

Gelb, A. W., E. Gignac, P. H. Manninen, J. K. Farrar, and D. H. Lee. 1992. Methylparaben and propylparaben do not alter cerebral blood flow in humans. *Can. J. Anaesth.* 39:691–694.

Ghirardi, E. 1940. Contributo sperimentale alia conoscenza farmacologica degli esteri superiori dell' acido paraossibenzoica (translated from the original Italian). *Arch. Ital. Sci. Farmacol.* 9:298–302.

Goh, C. L., and R. Yuen. 1994. A study of occupational skin disease in the metal industry 1098–1990. *Annals Acad. of Med. Singapore* 23:639–644.

Golden, R., J. Gandy, and G. Vollmer. 2005. A review of the endocrine activity of parabens and implications for potential risks to human health. *Crit. Rev. Toxicol.* 35: 435–458.

Gomez, E., A. Pillon, H. Fenet, D. Rosain, M. J. Duchesne, J. C. Nicolas, P. Balaguer, and C. Casellas. 2005. Estrogenic activities of cosmetic components in reporter cell lines: Parabens, UV screens, and musks. *J. Toxicol. Environ. Health* 68: 239–251.

Goodwin, M., K. M. Gooding, and F. Regnier. 1979. Sex pheromone in the dog. *Science* 203:559–561.

Gossele, J. A. W. 1971. Modified thin-layer chromatographic separation of preservatives. *J. Chromatogr.* 63:433–437.

Goto, A., and F. Endo. 1979. Effect of alkylparabens on the solution state of sodium lauryl sulfate micelle. IV. Gel filtration of solubilized systems. *J. Colloid Interface Sci.* 68:163–172.

Gottfried, N. S. 1962. Alkyl *p*-hydroxybenzoate esters as pharmaceutical preservatives: A review of the parabens. *Am. J. Hosp. Pharm.* 19:310–314.

Gottschalck, T. E., and G. N. McEwen, Jr., eds. 2004. *International cosmetic ingredient dictionary and handbook*, 10th ed. Washington, DC: CTFA.

Greenberg, L. A., D. Lester, and H. Haggard, eds. 1954. *Handbook of cosmetic materials*. New York: Interscience.

Grom Chromatography GmbH. 2004. Analysis of Preservatives. http://www.grom.de/App2Abfrage_11.asp?Suchbegriff=01110. Accessed August 2, 2004.

Gucklhorn, I. R. 1969. Antimicrobials in cosmetics. Part 3. *Manuf. Chem. Aerosol News*. 40:71–75.

Gupta, R. C., and G. D. Lundberg. 1977. Application of gas chromatography to street drug analysis. *Clin. Toxicol.* 11:437–442.

Guthenberg, H., and I. Beckman. 1963. Identification of preservatives by ultraviolet irradiation of paper chromatograms. *Z. Lebensm.-Unters.-Forsch.* 120:461–464.

Hamilton, J. T., Y. Zhou, and A. W. Gelb. 1990. Paraben preservatives but not succinylcholine are vasodilators in vitro. *Anesthesiology* 73:1252–1257.

Handa, O., S. Kokura, S. Adachi, T. Takagi, Y. Naito, T. Tanigawa, N. Yoshida, and T. Yoshikawa. 2005. Detrimental potential of methyl paraben on UVB-induced damage of skin keratinocyte. Unpublished data submitted by CTFA. 24 pages.[4]

Hannuksela, M., M. Kousa, and V. Pirila. 1976. Allergy to ingredients of vehicles. *Contact Dermatitis* 2:105–110.

Hansen, J., and B. Möllgaard. 1990. Penetration and transformation of the parabens in human skin in vitro. *Contact Dermatitis* 23:294.

Harvey, B. H., M. E. Carstens, and J. J. Taljaard. 1992. Central effects of the preservative, methylparaben. In vivo activation of cAMP-specific phosphodiesterase and reduction of cortical cAMP. *Biochem. Pharmacol.* 44:1053–1057.

Harvey, P. W., and I. Johnson. 2002. Approaches to the assessment of toxicity data with endpoints related to endocrine disruption. *J. Appl. Toxicol.* 22:241–247.

Hassler, W. H. 1954. Oral fat emulsions. *Am. Prof. Pharm.* 20:427–467.

Hegyi, E. 1979. Development tendencies of contact allergy. *Allerg. Immunol. (Leipz)* 25:104–115.

Heim, F., F. Leuschner, and G. Wunderlich. 1957. Metabolism of *p*-hydroxybenzoic acid ethyl ester. *Klin. Wochenschr.* 35:823–825.

Henry, J. C., E. H. Tschen, and L. E. Becker. 1979. Contact urticaria to parabens. *Arch. Dermatol.* 115:1231–1232.

Hill Top Research. 1976. Repeated insult patch test of ten samples: Jelly containing Propylparaben. Unpublished data submitted by CTFA (Nov. 3, 1976; CTFA Code No. 2-7-124).[4]

Hill Top Research. 1977. Repeated insult patch test of ten samples: Cream containing Propylparaben. Unpublished data submitted by CTFA (Dec. 16, 1977 CTFA Code No. 2-7-123).[4]

Hill Top Research. 1978a. The study of cumulative irritant properties of a series of test materials: Lotion (MP-6-98) containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (April 27, 1978; CTFA Code No. 2-7-25).[4]

Hill Top Research. 1978b. The study of cumulative irritant properties of a series of test materials: Cream containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (Oct. 6, 1978; CTFA Code No. 2-7-36).[4]

Hill Top Research. 1979a. The study of cumulative irritant properties of a series of test materials: Cream containing Methylparaben. Unpublished data submitted by CTFA (March 13, 1979; CTFA Code No. 2-7-33).[4]

Hill Top Research. 1979b. The study of cumulative irritant properties of a series of test materials: Cream containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (July 16, 1979; CTFA Code No. 2-7-39).[4]

Hill Top Research. 1980. The study of cumulative irritant properties of a series of test materials: wax product containing Butylparaben and Propylparaben. Unpublished data submitted by CTFA. (June 13, 1980; CTFA Code No. 2-7-51).[4]

Hill Top Research. 1981. Report of a human skin test of cumulative irritation: Cream containing Methylparaben. Unpublished data submitted by CTFA (April 20, 1981; CTFA Code No. 2-7-31).[4]

Hirose, M., T. Inoue, M. Asamoto, Y. Tagawa, and N. Ito. 1986. Comparison of the effects of 13 phenolic compounds in induction of proliferative lesions of the forestomach and increase in the lavelling indicies of the glandular stomach and urinary bladder epithelium of Syrian golden hamsters. *Carcinogenesis* 7:1285-1289.

Hjorth, N., and C. Trolle-Lassen. 1962. Skin reactions caused by preservatives, especially paraben esters and sorbic acid. *Arch. Pharm. Chemi.* 69:9–16.

Hjorth, N., and C. Trolle-Lassen. 1963. Skin reactions to ointment bases. *Trans. St. Johns Hosp. Dermatol. Soc.* 49:127–140.

Homburger, F. 1968. Carcinogenicity of several compounds. NTIS Report PB No. 183 027. 26 pages.

Hopp, E. 1978. Assay of parabens in aqueous solution by gas liquid chromatography. *Medd. Nor. Farm. Selsk.* 40:153–157.

Hossaini, A., J. J. Larsen, and J. C. Larsen. 2000. Lack of oestrogenic effects of food preservatives (parabens) in uterotrophic assays. *Food Chem. Toxicol.* 38:319–323.

Hoyem, T. 1962. Separation, identification, and estimation of aromatic food preservatives and sorbic acid by paper chromatography and ultraviolet spectrophotometry. *J. Assoc. Off. Agric. Chem.* 45:902–905.

Husain, S. L. 1977. Contact dermatitis in the west of Scotland. *Contact Dermatitis* 3:327–332.

Inai, K., Y. Aoki, H. Akamizu, R. Eto, T. Nishida, and S. Tokuoka. 1985. Tumorigenicity study of Butyl and Isobutyl *p*-hydroxybenzoates administered orally to mice. *Food Chem. Toxicol.* 23:575–578.

Iguchi, S., M. Yamamoto, and T. Aoyama. 1963. Medicinal preparations by gas chromatography. *Yakugaku Zasshi* 83:721–723.

Informatics. 1972. *Monograph on methyl paraben and propyl paraben*. Rockville, MD: Informatics.

Ishidate, M., M. Hayashi, M. Sawada, et al. 1978. Cytotoxicity test on medical drugs. Chromosome aberration tests with Chinese hamster cells in vitro. *Eisei Shikensho Hokoku* 96:55–61.

Ishidate, M., Jr., and S. Odashima. 1977. Chromosome tests with 134 compounds on Chinese hamster cells in vitro: a screening for chemical carcinogens. *Mutat. Res* 48:337–354.

Ishidate, M., Jr., T. Sofuni, K. Yoshickawa, et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem Toxicol* 22:623–636.

Ishiwatari, S., T. Suzuki, T. Hitomi, T. Yoshino, S. Matsukuma, T. Tsuji, O. Handa, S. Kokura, and T. Yoshikawa. 2005. Effect of methyl paraben on skin keratinocytes. Unpublished data submitted by CTFA. 19 pages.[4]

Ishizaki, M., S. Ueno, N. Oyamada, K. Katsumura. 1978. Studies on degradation of food additives by irradiation (IV): Reaction of butyl p-hydroxybenzoate with potassium nitrate or sodium nitrite by irradiation of ultraviolet ray and mutagenicity of its reaction products. *J. Food Hyg. Soc. Japan* 19:305–310.

Ivy, R. S. 1983. Anesthetics and methylparaben. *J Am Dent Assoc* 106:302.

Ivy Research Laboratories. 1978. The appraisal of contact-sensitizing potential of four materials by means of the maximization test: Product CMP-06-98 containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (April 10, 1978; CTFA Code No. 2-7-26).[4]

Jian, L., and L. W. Po. 1993. Kinetic evaluation of the ciliotoxicity of methyl- and propyl-*p*-hydroxybenzoate using factorial experiments. *J. Pharm. Pharmacol.* 45:98–101.

Javors, B. R., Y. Applbaum, and P. Gerard. 1984. Severe allergic reaction: an unusual complication of barium enema. *Gastrointest. Radiol.* 9:357–358.

Jenni, C., and L. Zala. 1980. Eczema of the lower leg—clinical, allergological and differential diagnostic aspects. *Schweiz. Med. Wochenschr.* 110:124–128.

Jensen, F. 1977. Gas chromatographic method for the quantitative determination of glyceryl guaiacolate, methyl-*p*-hydroxybenzoate and propyl-*p*-hydroxybenzoate in cough mixture. *Medd. Nor. Farm. Selsk.* 39:38–48.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1998. Methyl *p*-Hydroxybenzoate. http://apps3.fai.org/jecfa/additive_specs/docs/6/additive-0720.htm.

Jones, P. S., D. Thigpen., J. L. Morrison, and A. P. Richardson. 1956. *p*-Hydroxybenzoic acid esters as preservatives. III. The physiological disposition of p-hydroxybenzoic acid and its esters. *J. Am. Pharm. Assoc. Sci. Ed.* 45:265–273.

Jones, T. R., J. T. Hamilton, and N. M. Lefcoe. 1975. The effect of methyl paraben on tracheal smooth muscle in vitro. *Arch. Int. Pharmacodyn. Ther.* 214:271–284.

Jun, H. W., R. T. Mayer, C. M. Himel, and L. A. Luzzi. 1971. Binding study of *p*-hydroxybenzoic acid esters to bovine serum albumin by fluorescent probe technique. *J. Pharm. Sci.* 60:1821–1825.

Kang, K.-S., J.-Y. Che, D.-Y. Ryu, T.-W. Kim, G.-X. Li, and Y.-S. Lee. 2002. Decreased Sperm number and motile activity on the F1 offspring maternally exposed to butyl *p*-hydroxybenzoic acid (Butyl Paraben). *J. Vet. Med. Sci.* 64:227–235.

Karasek, F., and J. Slavicek. 1967. Effect of heparin on the sensitivity of frog rectus abdominis muscle to acetylcholine. *Physiol. Bohemoslov.* 16:251–255.

Kassem, A. A., A. Abd El-Bary, M. T. Abdel Aziz, and S. A. Nour. 1976. Evaluation of certain spermicidal formulations. *Bull. Fac. Pharm., Cairo Univ.* 14:155–167.

King, W. P., K. T. Joseph, and P. T. Kissinger. 1980. Liquid chromatography with amperometric detection for determining phenolic preservatives. *J. Assoc. Off. Anal. Chem.* 63:137–142.

Kitada, Y., K. Tamase, M. Saski, and K. Tanigawa. 1980. Determination of saccharin, benzoic acid and p-hydroxybenzoate esters in soy sauce by high speed liquid chromatography. *J Food Hyg Soc Jpn* 21:480–484.

Kitagawa, S., H. Li, and S. Sato. 1997. Skin permeation of parabens in excised guinea pig dorsal skin, its modification by penetration enhancers and their relationship with n-octanol/water partition coefficients. *Chem. Pharm. Bull. (Tokyo)* 45:1354–1357.

Kitamura, Y. 1979. Effects of local anesthetics on the peripheral nerve and the spinal cord. *Osaka City Med. J.* 25:7–24.

Kiwada, H., S. Awazu, and M. Hanano. 1979. The study on the biological fate of paraben at the dose of practical usage in rat. I. The metabolism and excretion of ethyl *p*-hydroxybenzoate (ethyl paraben) and p-hydroxybenzoic acid. *J. Pharmacobio-Dyn.* 2:356–364.

Koda, T., T. Umezu, R. Kamata, K. Morohoshi, T. Ohta, and M. Morita. 2005. Uterotrophic effect of benzophenone derivatives and a *p*-hydroxybenzoate used in ultraviolet screens. *Environ. Res.* 98:40–45.

Kohn, R. 1933. Experimentelle Untersuchungen uber einige Ester der *p*-oxybenzoesaure (translated from the original German). *Med. Klin.* 29:983–984.

Kojima, M. 1992. A case of contact urticaria to methylparaben. *Skin Res.* 34:578–582.

Komatsu, H., and M. Suzuki. 1979. Percutaneous absorption of butylparaben through guinea pig skin in vitro. *J. Pharm. Sci.* 68:596–598.

Krauze, S., and B. Fitak. 1971. Influence on preservatives on the biosynthesis of nucleic acids and on the protein content of animal cells in tissue culture. *Mitt. Geb. Lebensmittelunters. Hyg.* 62:359–367.

Lach, J. L., and J.S. Sawardeker. 1965. Rapid method for the determination of mixtures of *p*-hydroxybenzoate esters by gas chromatography. *J. Pharm. Sci.* 54:424–426.

Lambion, R., E. Jacqmain, and J. Bessemans. 1968. Study on some antiseptics in foods by thin layer chromatography. *Qual. Plant. Mater. Veg.* 16:270–291.

Lang, M., and R. M. Rye. 1972. Uptake by Escherichia coli and growth-inhibitory properties of methyl, ethyl, and propyl *p*-hydroxybenzoates. *J. Pharm. Pharmacol.* 24(Supl.):160P–161P.

Lang, M., and R. M. Rye. 1973. Correlation between the antibacterial activity of some *p*-hydroxybenzoate esters and their cellular uptake. *Microbios* 7:199–207.

Larson, C. E. 1977. Methylparaben—an overlooked cause of local anesthetic hypersensitivity. *Anesth. Prog.* 24:72–74.

Latronica, R. J., A. F. Golberg, and J. R. Wightman. 1969. Local anesthetic sensitivity. Report of a case. *Oral Surg.* 28:439–441.

Laurent, A., and R. Bourdon. 1975. Assay of pharmaceutical products by low pressure chromatography using anion or cation exchangers. *Ann. Pharm. Fr.* 33:171–181.

Leberco Laboratories. 1978a. Acute oral toxicity of suntan lotion pF-8 containing Methylparaben. Unpublished data submitted by CTFA (Sept. 12, 1978; CTFA Code No. 2-7-63).[4]

Leberco Laboratories. 1978b. Acute oral toxicity of protective face cream containing Propylparaben. Unpublished data submitted by CTFA (Sept. 5, 1978; CTFA Code No. 2-7-65).[4]

Leberco Laboratories. 1978c. Primary skin irritation test of suntan lotion pF-8 containing Methylparaben. Unpublished data submitted by CTFA (Sept. 8, 1978; CTFA Code No. 2-7-62).[4]

Leberco Laboratories. 1978d. Primary skin irritation test of protective face cream containing Propylparaben. Unpublished data submitted by CTFA (Sept. 8, 1978; CTFA Code No. 2-7-66).[4]

Leberco Laboratories. 1978e. Rabbit eye irritation test of suntan lotion pF-8 containing Methylparaben. Unpublished data submitted by CTFA (Sept. 12, 1978; CTFA Code No. 2-7-64).[4]

Leberco Laboratories. 1978f. Rabbit eye irritation test of protective face cream containing Propylparaben. Unpublished data submitted by CTFA (Sept. 12, 1978; CTFA Code No. 2-7-67).[4]

Leberco Laboratories. 1979a. Acute oral toxicity of body scrub containing Methylparaben. Unpublished data submitted by CTFA (July 16, 1979; CTFA Code No. 71).[4]

Leberco Laboratories. 1979b. Primary skin irritation test of body scrub containing Methylparaben. Unpublished data submitted by CTFA (July 23, 1979; CTFA Code No. 2-7-69).[4]

Leberco Laboratories. 1979c. Rabbit eye irritation test of body scrub containing Methylparaben. Unpublished data submitted by CTFA. (July 23, 1979; CTFA Code No. 2-7-72).[4]

Lee, M. S. 1979. Detection of food preservatives by food fluorescence. *Han'guk Sikp'um Kwahakhoe Chi* 11:166–170.

Lemieszek-Chodorowska, K., and A. Snycerski. 1971. Identification of food preservatives by thin layer chromatography. *Rocz. Panstw. Zakl. Hig.* 22:421–426.

Lemini, C., G. Silva, C. Timossi, D. Luque, A. Valverde, M. González-Martinez, A. Hernández, C. Rubio-Póo, B. Chávez Lara, and F. Valenzuela. 1997. Estrogenic effects of *p*-hydroxybenzoic acid in CD1 Mice. *Environ. Res.* 75:130–134.

Lemini, C., R. Jaimez, M. E. Ávila, Y. Franco, F. Larrea, and A. E. Lemus. 2003. In vivo and in vitro estrogen bioactivities of alkyl parabens. *Toxicol. Indust. Health* 19:69–79.

Lemini, C., A. Hernández, R. Jaimez, Y. Franco, M. E. Avila, and A. Castell. 2004. Morpometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben. *Toxicol. Indust. Health.* 20:123–132.

Lestringant, G. G., A. Bener, M. Sawaya, I. H. Galadari, and P. M. Frossard. 1999. Allergic contact dermatitis in the United Arab Emirates. *Int. J. Dermatol.* 38:181–186.

Leuenberger, U., R. Gauch, and E. Baumgartner. 1979. Determination of food preservatives and saccharin by high-performance liquid chromatography. *J. Chromatogr.* 173:343–348.

Lide, D. R., ed. 1993. *CRC handbook of chemistry and physics*, 74th ed. Boca Raton: CRC Press.

Lindner, K., H. J. Cramer, and R. Köhler. 1989. Do Varicosan bandages really rarely cause contact eczema? Case report of allergic contact dermatitis caused by propyl hydroxybenzoate following the use of Varicosan bandages. *Dermatol Monatsshr* 175:655–657.

Litton Bionetics. 1974. Mutagenic evaluation of Compound 71-38, Methylparaben. NTIS Report PB-245 459.

Litton Bionetics. 1975. Mutagenic Evaluation of Compound FDA 73-68, Propyl Paraben (USP). NTIS Report PB-245 498.

Lobemeier, C. C. Tschoetschel, S. Westie, and E. Heymann. 1996. Hydrolysis of parabens by extracts from differing layers of human skin. *Biol. Chem.* 377:647–651.

Loos, H. O. 1935. Zur bekampfung der epidermorphytie der fube und hande mit benzoesaureprepara-ben (translated from the original German). *Arch. Dermat. Syph.* 173:109–116.

Loria, C. J., P. Echeverria, and A. L. Smith. 1976. Effect of antibiotic formulations in serum protein: bilirubin interaction of newborn infants. *J. Pediatr.* 89:479–482.

LSRO/FASEB. 1972. Evaluation of the health aspects of methyl paraben and propyl paraben as food ingredients. NTIS Report PB-22 950.

Ludwig, E., and U. Freimuth. 1965. Thin-layer chromatography in food chemistry. V. Identification of some organic preservatives by means of polyamide layers. *Nahrung* 9:751–754.

Macioci, F., and C. Fiotek. 1975. Determination of the components of an ointment using fluorescence quenching densitometry and UV spectrophotometry after chromatographic separation. *Boll. Chim. Farm.* 114:468–477.

Maeda, Y., M. Yamamoto, K. Owada, et al. 1987. High-performance liquid chromatographic determination of six p-hydroxybenzoic acid esters in cosmetics using Sep-Pak Florisil cartridges for sample pre-treatment. *J Chromatogr* 410:413–418.

Marks, J. G., Jr., D. V. Belsito, V. A. DeLeo, et al. 1995. North American Contact Dermatitis Group standard tray patch-test results (1992 to 1994). *Am. J. Contact Dermatitis.* 6:160–165.

Marks, J. G., Jr., D. V. Belsito, V. A. DeLeo, et al. 1998. North American Contact Dermatitis Group patch-test results for the detection of delayed-type hypersensitivity to topical allergans. *J. Am. Acad. Dermatol.* 38:911–918.

Marks, J. G., Jr., D. V. Belsito, V. A. DeLeo, et al. 2000. North American Contact Dermatitis Group patch-test results, 1996 to 1998. *Arch. Dermatol.* 136:272–273.

Marks, J. G., Jr., D. V. Belsito, V. A. DeLeo, et al. 2003. North American Contact Dermatitis Group patch-test results, 1998 to 2000. *Am. J. Contact Dermatitis.* 14:59–62.

Marzulli, F. N., T. R. Carson, and H. I. Maibach. 1968. Delayed contact hypersensitivity studies in man and animals. *Proc. Joint Conf. Cosmet. Sci.* 107–122.

Marzulli, F. N., and H. I. Maibach. 1976. Contact allergy: predictive testing in man. *Contact Dermatitis* 2:1–17.

Mason, M. M., C. C. Cate, and J. Baker. 1971. Toxicology and carcinogenesis of various chemicals used in the preparation of vaccines. *Clin. Toxicol.* 4:185–204.

Matsuoka, A., M. Hayashi, and M. Ishidate. 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. *Mutat. Res.* 66:277–290.

Matthews, C., J. Davidson, E. Bauer, et al. 1956. *p*-hydroxybenzoic acid esters as preservatives. II. Acute and chronic toxicity in dogs, rats, and mice. *J. Am. Pharm. Assoc. Sci. Ed.* 45:260–267.

Maucher, O. M. 1974. Cross and coupled allergy due to parahydroxybenzoic acid ester. *Berufsdermatosen* 22:183–187.

Maurer, T., E. G. Weirich, and R. Hess. 1980. The optimization test in the guinea pig in relation to other predictive sensitization methods. *Toxicology* 15:63–71.

McCann, J., E. Choi., E. Yamasaki, and B. N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test; Assay of 300 chemicals. *Proc. Natl. Acad. Sci. U. S. A.* 72:5135–5139.

McDavid, J. E. 1974. Characteristics of the parabens as preservatives. *Dev. Biol. Stand.* 24:49–55.

Menne, T., and N. Hjorth. 1988. Routine patch testing with parabens esters. *Contact Dermatitis* 19:189–191.

Menne, T., P. J. Frosch, N. K. Veien, et al. 1991. Contact sensitization to 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (MCI/MI). *Contact Dermatitis* 24:334–341.

Metzger, W. I., L. T. Wright, and J. C. Dilorenzo. 1954. Effect of esters of parahydroxybenzoic acid on Candida and yeast-like fungi. *JAMA* 155:352–358.

Meynadier, J. M., J. Meynadier, A. Colmas, P. Y. Castelain, G. Ducombs, G. Chabeau, M. Lacroix, P. Martin, and Z. Ngangn. 1982. Allergie aux conservateurs (translated from the original French). *Ann. Dermatol. Venereol.* 109:1017–1023.

Mirick, D. A., S. Davis, and D. B. Thomas. 2002. Antiperspirant use and the risk of breast cancer. *J. Nat. Cancer Inst.* 94:1578–1580.

Montes, A. L. 1956. A determination method for Nipagin and other *p*-hydroxybenzoic acid esters in medicine and food. *An. Asoc. Quim. Argent.* 44:82–89.

Moore, K. E., and R. J. Stretton. 1978. Detection of preservatives in pharmaceutical and cosmetic products using agar gel electrophoresis. *J. Chromatogr.* 156:211–217.

Moriyama, I., K. Hiraoka., and R. Yamaguchi. 1975. Teratogenic effects of food additive ethyl-*p*-hydroxybenzoate studied in pregnant rats. *Acta Obstet. Gynaecol. Japan* 22:94–106.

Mostow, S. R., R. B. Dreisin, B. R. Manawadu, and F. M. La Force. 1979. Adverse effects of lidocaine and methylparabens on tracheal ciliary activity. *Laryngoscope* 89:1697–1701.

Mowad, C. 2000. Allergic contact dermatitis caused by Parabens: 2 case reports and a review. *Am. J. Contact Dermatitis* 11:53–56.

Murrell, W. G., and J. M. Vincent. 1950. The 4-hydroxybenzoic acid esters and related compounds. 4. The bacteriostatic action of 4 hydroxybenzoic acid-n-alkyl esters. *J. Soc. Chem. Ind.* 69:109–113.

Nagasawa, K., H. Yoshidome, and R. Takeshita. 1969. Chromatography of food preservatives on polyamide layers and columns. *J. Chromatogr.* 43:473–479.

Nagel, J. E., J. T. Fuscaldo, and P. Fireman. 1977. Paraben allergy. *JAMA* 237:1594–1595.

Nakagawa, Y., and P. Moldéus. 1998. Mechanism of *p*-hydroxybenzoate ester-induced mitochondrial dysfunction and cytotoxicity in isolated rat hepatocytes. *Biochem. Pharmacol.* 55:1907–1914.

Narafu, T., J. Hayakawa, H. Takahashi, and Y. Ishida. 1969. Determination of food additives by gas chromatography. II. Simultaneous determination of synthetic preservatives by programmed temperature gas chromatography. *Shokuhin Eiseigaku Zasshi* 10:186–189.

Nathan, P. W., and T. A. Sears. 1961. Action of methyl hydroxybenzoate on nervous conduction. *Nature* 192:668–669.

National Academy of Sciences. 1996. *Food Chemicals Codex*, 4th ed. Washington, DC: National Academy Press.

Neidig, C. P., and H. Burrell. 1944. The esters of p-hydroxybenzene acid as preservatives. *Drug Cosmet. Ind.* 54:408–410, 481–489.

Nera, E. A., E. Lok, F. Iverson, E. Ormsby, K. F. Karpinski, and D. B. Clayton. 1984. Short-term pathological and proliferative effects of butylated hydroxyanisole and other phenolic anti-oxidants in the forestomach of Fischer 344 rats. *Toxicology* 32:197–213.

Nes, I. F., and T. Eklund. 1983. The effect of parabens on DNA, RNA, and protein synthesis in *Escherichia coli* and *Bacilus subtilis*. *J. Appl. Bacteriol.* 54:237–242.

Nikitakis, J. M., and G. N. McEwen, Jr. 1990. *CTFA compendium of cosmetic ingredient composition—Descriptions I and II.* Washington, DC: CTFA.

Nishimoto, K., and M. Uyeta. 1965. Gas-chromatographic analysis of food additives. II. Simultaneous quantitative determination of sorbic acid, dehydroacetic acid, benzoic acid, and butyl *p*-hydroxybenzoate in food. *Shokuhin Eiseigaku Zasshi* 6:231–234.

North American Contact Dermatitis Group. 1972. Epidemiology of contact dermatitis in North America, 1972. *Arch. Dermatol.* 108:537–540.

North American Contact Dermatitis Group. 1980. Epidemiology of contact dermatitis in North America, 1979-1980. Unpublished data submitted by NACDG.[4]

Odashima, S. 1976. The cooperative development in Japan of methods for screening chemicals for carcinogenicity. *I. A. R. C. Sci. Publ.* 12:61–79.

Odashima, S. 1980. Cooperative program on long-term assays for carcinogenicity in Japan. *I. A. R. C. Sci. Publ.* 27:315–322.

Oishi, S. 2001. Effects of butylparaben on the male reproductive system in rats. *Toxicol. Indust. Health* 17:31–39.

Oishi, S. 2002a. Effects of butylparaben on the male reproductive system in mice. *Arch. Toxicol.* 76:423–429.

Oishi, S. 2002b. Effects of propyl paraben on the male reproductive system. *Food Chem. Toxicol.* 40:1807–1813.

Oishi, S. 2004. Lack of spermatotoxic effects of methyl and ethyl esters of *p*-hydroxybenzoic acid in rats. *Food Chem. Toxicol.* 42:1845–1849.

Okubo, T., Y. Yokoyama, K. Kano, and I. Kano. 2001. ER-dependent estrogenic activity of parabens assessed by proliferation of human breast cancer MCF-7 cells and expression of ERα and PR. *Food Chem. Toxicol.* 39:1225–1232.

O'Neill, J. J., and C. A. Mead. 1982. The parabens: Bacterial adaptation and preservative capacity. *J. Soc. Cosmet. Chem.* 33:75–84.

Otagiri, M., and J. H. Perrin. 1977. Circular dichroic investigations of the binding of salicylate and related compounds to human serum albumin. *Biochem. Pharmacol.* 26:283–288.

Patel, N. K. 1968. Experiments in physical pharmacy part 1 binding and in vitro biological activity. *Am. J. Pharm. Educ.* 32:547–550.

Pedersen, K. L., S. N. Pedersen, L. B. Christiansen, B. Korsgaard, and P. Bjerregaard. 2000. The Preservatives Ethyl-, Propyl-, and Butylparaben are Oestrogenic in an *in vivo* Fish Assay. *Pharmacol. Toxicol.* 86:110–113.

Peereboom, J. W. C., and H. W. Beekes. 1964. Thin-layer chromatography of preserving agents. *J. Chromatogr.* 14:417–23.

Person, J. R. 1985. Mounting evidence of paraben sensitivity in dogs. *Arch. Dermatol.* 121:1107.

Pevny, I., and G. Glassl. 1971. Nipaester allergy. A new test method for the oral mucosa. *Allerg. Immunol.* 17:306–312.

Phillips, J. C., C. S. Topp, and S. D. Gangolli. 1978. The metabolism of ethyl and n- propyl-*p*-hydroxybenzoate ("parabens") in male cats. *Toxicol. Lett.* 2:137–142.

Pomerat, M., and C. D. Leake. 1954. Short-term cultures for drug assays. *Ann. N. Y. Acad. Sci.* 58:1110–1128.

Pompy, L., A. Karlin, C. M. Capuano, J. E. Cottrell, and J. Hartung. 1991. Paraben preservatives do not increase intracranial pressure in cats. *Anesthesiology* 75:669–672.

Pratt, M. D., D. V. Belsito, V. A. DeLeo, et al. 2004. North American Contact Dermatitis Group patch-test results, 2001–2001 Study Period. *Dermatitis* 15:176–183.

Pugazhendhi, D., G. S. Pope, and P. D. Darbre. 2005. Oestrogenic activity of *p*-hydroxybenzoic acid (common metabolite of paraben esters) and methylparaben in human breast cancer cell lines. *J. Appl. Toxicol.* 25:301–309.

Rangone, R., and C. Ambrosio. 1970. Identification and quantitative analysis of *p*-hydroxybenzoic acid and its esters using reversed-phase thin-layer chromatography. *J. Chromatogr.* 50:436–441.

Rasmussen, L. F., E. E. Ahlfors, and R. P. Wennberg. 1976. Effect of paraben preservatives on albumin binding of bilirubin. *J. Pediatr.* 89:475–478.

Rastogi, S. C., A. Schouten, N. de Kruijf, and J. W. Weijland. 1995. Contents of methyl-, ethyl-, propyl-, butyl-, and benzylparaben in cosmetic products. *Contact Dermatitis* 32:28–30.

Raval, N. N., and E.L Parrott. 1967. Hydrolysis of methylparaben. *J. Pharm. Sci.* 56:274–275.

Reed, W. B. 1969. Paraben allergy, a case of intractable dermatitis. *Arch. Dermatol.* 100:503–504.

Registry of Toxic Effects of Chemical Substances (RTECS). 1993. Isobutylparaben and Isopropylparaben entries. *RTECS database.* National Library of Medicine Toxicology Data Network (TOXNET).

Reimers, F. 1938. Titration of *p*-hydroxybenzoic acid ester. *Dan. Tidsskr. Farm.* 12:203–210.

Reimers, F. 1940. Investigations of microchemical methods. II. The microdetermination of melting point. Studies with barbituric acid derivatives. *Dan. Tidsskr. Farm.* 14:145–168.

Reimers, F. 1941. Analytical chemistry of *p*-hydroxybenzoic acid esters. *Z. Anal. Chem.* 122:414–418.

Report of the European Workshop on Endocrine Disrupters. 2001. Workshop on 18-20 June 2001 organized by the Environment Directorate General of the European Commission. Report dated October 2001. 58 pages.[4]

Research Testing Laboratories. 1978. Human subject patch study 570.0478: Lotions CMP-6-98 and CMP 6-100 containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (May 15, 1978; CTFA Code No. 2-7-27).[4]

Research Testing Laboratories. 1979a. Patch study 698.0479: Foundation containing Methylparaben. Unpublished data submitted by CTFA (May 25, 1979; CTFA Code No. 2-7-95).[4]

Research Testing Laboratories. 1979b. Patch study 671.0179: Liquid blush and foundation containing Methylparaben. Unpublished data submitted by CTFA (June 15, 1979; CTFA Code No. 2-7-96 and 2-7-110).[4]

Rigdon, R. H., and T. Schadewald. 1972. Amyloid in the gastrointestinal tract of mice. *Tex Rep Biol Med* 30:85–95.

Ritzau, M. and K. Swangsilpa. 1977. The prophylactic use of propylic ester of *p*-hydroxybenzoic acid on alveolitis sicca dolorosa. A preliminary report. *Oral Surg.* 43:32–37.

Rodrigues, C., E. Lok, E. Nera, F. Iverson, D. Page, K. Karpinski, and D. B. Clayson. 1986. Short-term effects of various phenols and acids on the Fischer 344 male rat forestomach epithelium. *Toxicology* 38:103–117.

Romaguera, C., and F. Grimalt. 1980. Statistical and comparative study of 4,600 patients tested in Barcelona. *Contact Dermatitis* 6:309–315.

Rook, A., D. S. Wilkinson, and F. J. G. Ebling. 1968. *Textbook of dermatology.* Oxford, UK: Blackwell Scientific Publication.

Rosen, W. E., and P. A. Berke. 1973. Modern concepts of cosmetic preservation. *J. Soc. Cos. Chem.* 24:663–675.

Routledge, E. J., J. Parker, J. Odum, J. Ashby, and J. P. Sumpter. 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Appl. Pharmacol.* 153:12–19.

Rubach, K., C. Breyer, and E. Kirchhoff. 1980. Isotashophoretic determination of preservatives. *Z. Lebensm.-Unters.-Forsch.* 170:99–102.

Rudner, E. J. 1978. Patch test results of the North American Contact Dermatitis Group. *Cosmet. Toilet.* 93:53–54.

Sabalitschka, T. 1930. Application of ethyl *p*-hydroxybenzoate in maintenance of sterility, in sterilization and in disinfection. *Arch. Pharm.* 268:653–673.

Sabalitschka, T., and R. Neufeld-Crzellitzer. 1954. Zum Verhalten der *p*-oxybenzoesaureester im menschlichen Korper (translated from the original German). *Arzneimittelforsch.* 4:575–579.

Sado, I. 1973. Synergistic toxicity of officially permitted food preservatives. *Nippon Eiseigaku Zasshi* 28:463–476.

Saiki, J. H., S. Thompson, F. Smith, and R. Atkinson. 1972. Paraplegia following intrathecal chemotherapy. *Cancer* 29:370–374.

Sarkany, I. 1960. Contact dermatitis from parabens. *Br. J. Dermatol.* 72:345–347.

Sarsunova, M. 1973. Quality of some ointments which are mass-produced and prepared for reserve. II. Detection of ointment ingredients by thin-layer chromatography. *Cesk. Farm.* 22:259–265.

Sasseville, D. 2004. Hypersensitivity to preservatives. *Dermatol. Ther.* 17:251–263.

Satoh, K., F. Nagai, N. Aoki, and M. Nishijima. 2000. Competitive binding of some alkyl *p*-hydroxybenzoates to human estrogen receptor α and β. *Yakugaku Zasshi* 120:1429–1433.

Sauermann, G., W. Hofeditz, and W. Engel. 1978. Method for the determination of the distribution of preservatives in emulsions. *J. Soc. Cosmet. Chem.* 29:767–776.

Seko, N., H. Bando, C. W. Lim, F. Yamashita, and M. Hashida. 1999. Theoretical analysis of the effect of cutaneous metabolism on skin permeation of parabens based on a two-layer skin diffusion/metabolism model. *Biol. Pharm. Bull.* 22:281–287.

Schamberg, I. L. 1967. Allergic contact dermatitis to methyl and propyl paraben. *Arch. Dermatol.* 95:626–628.

Schneider, R. 1957. *p*-Hydroxybenzosaureester. *Kosmetik*, 30:155–157; 337–339; 459–461; 535–538; 580–582; 626–629.

Schnuch, A., J. Geier, W. Uter, and P. J. Frosch. 1998. Patch testing results with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. *Br. J. Dermatol.* 138:467–476.

Schoorl, N. 1941. Determination of esters by saponification in the cold. *Pharm. Weekblad* 78:413–420.

Schorr, W. F., and A. H. Mohajerin. 1966. Paraben sensitivity. *Arch. Dermatol.* 93:721–723.

Schorr, W. F. 1968. Paraben allergy: A cause of intractable dermatitis. *JAMA* 204:859–862.

Schriftman, H. 1968. Analysis of pharmaceuticals by ultraviolet densitometry on thin-layer chromatograms, I. Parabens in gels and creams. *J. Pharm. Sci.* 57:1760–1763.

Schuebel, K. 1930. Toxicology of new preservatives: *p*-Chlorobenzoic acid and *p*-hydroxybenzoic acid esters. *Muench. Med. Wochenschr.* 77:13–14.

Schwartz, E. E, S. N. Glick, M. B. Foggs, and G. S. Silverstein. 1984. Hypersensitivity reactions after barium enema examination. *Am J Roentgenol* 143:103–104.

Shaffer, M. P., P. M. Williford, and E. F. Sherertz. 2000. An old reaction in a new setting: The paraben paradox. *Am. J. Contact Dermatitis* 11:189.

Sheppard, E. P. and C. H. Wilson. 1975. Preservatives in cosmetics. Partition chromatography and ultraviolet spectrophotometry. *J. Assoc. Off. Anal. Chem.* 58:937–940.

Sheu, C. W., D. Salomon, J. L. Simmons, et al. 1975. Inhibitory effects of lipophilic acids and related compounds on bacteria and mammalian cells. *Antimicrob. Agents Chemother.* 7:349–363.

Shibah, F., W. Sheffield, J. Sprowls, and J. Nematoliahi. 1970. MR analysis of some alkyl *p*-hydroxybenzoates. *J. Pharm. Sci.* 59:1182–1183.

Shibata, M. A., M. Yamada, M. Hirose, E. Asakawa, M. Tatematsu, and N. Ito. 1990. Early proliferative responses of forestomach and glandular stomach of rats treated with five different phenolic antioxidants. *Carcinogenesis* 11:425–429.

Shiralkar, N. D., S. P. Manjrekar, and D. V. Rege. 1976. Some antimicrobial properties of *p*-hydroxybenzoates (parabens). *Indian Food Packer* 30:22–27.

Shiralkar, N. D., D. V. Rege, and S. P. Manjrekar. 1977. Distribution studies of propylparabens in an Aerobacter strain. *Indian J. Microbiol.* 17:190–193.

Shiroma, H., and Z. Oshiro. 1986. Simultaneous determination of seven preservatives in foods by high-performance liquid chromatography. *Okinawa-ken Kogai Eisei Kenkyushoho* 20:73–76.

Shiseido Co., Ltd. 2005. Comments on the study of the detrimental potential of methyl paraben on UVB-induced damage of skin keratinocyte. Unpublished data submitted by Shiseido Co., Ltd.[4]

Siegel, M. 1953. Semiquantitative assay of neomycin-fungistat (propylparaben *p*-hydroxybenzoic acid derivative) ointment. *J. Am. Pharm. Assoc. (Sci. Ed.)* 42:408–410.

Simonelli, M., and R. Marri. 1939. Toxicity and possible therapeutic application of the methyl ester of *p*-hydroxybenzoic acid. *Boll. Soc. Ital. Biol. Sper.* 14:289–290.

Simpson, J. R. 1978. Dermatitis due to parabens in cosmetic creams. *Contact Dermatitis* 4:311–312.

Soehring, K., O. Klingmuller, and F. Neuwald. 1959. Studies on the irritant action of some substances used as preservatives in ophthalmic solutions. *Arzneim.-Forsch.* 9:349–351.

Sokol, H. 1952. Recent developments in the preservation of pharmaceuticals. *Drug Standards* 20:89–106.

Song, B.-L., H.-Y. Li, and D.-R. Peng. 1989. In vitro spermicidal activity of parabens against human spermatozoa. *Contraception* 39:331–335.

Soni, M. G., G. A. Burdock, S. L. Taylor, and N. A. Greenberg. 2001. Safety assessment of propyl paraben: a review of the published literature. *Food Chem. Toxicol.* 39:513–532.

Soni, M. G., S. L. Taylor, N. A. Greenberg, and G. A. Burdock. 2002. Evaluation of the health aspects of methyl paraben: A review of the published literature. *Food Chem. Toxicol.* 40:1335–1373.

Soret, M. G., T. Peterson, B. Wyse, E. M. Block, and W. E. Dulin. 1977. Renal amyloidosis in KK mice that may be misinterpreted as diabetic glomerulosclerosis. *Arch. Pathol. Lab. Med.* 101:464–468.

Stevenson, S. G., B. Pharm, and J. C. L. Resuggan. 1938. A calorimetric test for the detection of parahydroxybenzoic acid in the presence of salicylic acid. *Analyst* 63:152–155.

Stillmeadow. 1978a. Rat acute oral toxicity, lotion CMP-6-100 containing Methylparaben and Propylparaben. Unpublished data Submitted by CTFA (March 22, 1978; CTFA Code No. 2-7-23).[4]

Stillmeadow. 1978b. Rabbit eye irritation test of lotion CMP-6-100 containing Methylparaben and Propylparaben. Unpublished data submitted by CTFA (Feb. 17, 1978; CTFA Code No. 2-7-24).[4]

Storrs, F. J., L. E. Rosenthal, R. A. Adams, et al. 1989. Prevalence and relevance of allergic reactions in patients patch-tested in North America—1984 to 1985. *J. Am. Acad. Dermatol.* 20:1038–1045.

Sugimura, T., S. Sato, M. Nagao, et al. 1976. Overlapping of carcinogens and mutagens. In: Fundamentals in Cancer Prevention (Magee, P. N., T. Mutsushima, T. Sugimura, and S. Takayama, eds.). University of Tokyo Press, Tokyo, and University Park Press, Baltimore, Maryland, pp. 191–215.

Talukdar, P. B., and A. K. Datta. 1969. Quantitive determination of paraben in sulphacetamide sodium solution. *Res. Ind.* 14:79–81.

Tammilehto, S., and S. Buchi, Jr. 1969. Investigation on p-hydroxybenzoate esters (Nipagine). Part 3. Thin layer and gas chromatographic determination of *p*-hydroxybenzoic acid esters in pharmaceutical preparations. *Pharm. Acta Helv.* 44:290–300.

Tatematsu, A., T. Nadai, H. Yoshizumi, et al. 1970. Analysis of mixed drugs by mass spectrometry. XIII. Food additives. 3. Quantitative determination of ethyl *p*-hydroxybenzoate. *Shitsuryo Bunseki* 18:948-955.

Techni-Med Consultants. 1980. Human repeated insult patch test: moisture milk lotion and night cream containing Ethylparaben. Unpublished data submitted by CTFA (Nov. 28, 1980; CTFA Code No. 2-7-133).[4]

Terada, H., and Y. Sakabe. 1985. Studies on the analysis of food additives by high-performance liquid chromatography. V. Simultaneous determination of preservatives and saccharin in foods by ion-pair chromatography. *J Chromatogr* 346:333–340.

Terasaka, S., A. Inoue, M. Tanji, and R. Kiyama. 2006. Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity. *Toxicol. Lett.* 163:130–141.

Testkit Laboratories. 1978. Repeated insult patch test of hand lotion containing Methylparaben. Unpublished data submitted by CTFA (May 16, 1978; CTFA Code No. 2-7-73).[4]

Testkit Laboratories. 1979. Repeated insult patch test of body scrub containing Methylparaben. Unpublished data submitted by CTFA (Oct. 11, 1979; CTFA Code No. 2-7-70).[4]

Thielemann, H. 1975. Thin-layer chromatographic detection limits (semiquantitative determination) of methyl and propyl ester derivatives of *p*-hydroxybenzoic acid on prepared sheets using different spray reagents. *Pharmazie* 30:337.

Thielemann, H. 1977. Paper and thin-layer chromatographic separation and detection limits (semiquantitative determination) of Nipagin (methyl *p*-hydroxybenzoate) and Nipasol (propyl *p*-hydroxybenzoate). *Z. Chem.* 17:231–232.

Thune, P., and A. Granholt. 1975. Provocation tests with antiphlogistica and food additives in recurrent urticaria. *Dermatolotica (Basel)* 151:360–367.

Tiscornia E. and A. Stacchini. 1964. Identification and determination of *p*-hydroxybenzoic esters in semi-preserved fish. *Atti Accad. Ligure Sci. Lett. (Genoa)* 21:339–352.

Tosti, A., P. A. Fanti, and S. Pileri. 1989. Dermal contact dermatitis from benzylparaben. *Contact Dermatitis* 21:49–51.

Toyoda, M., T. Kanamori, Y. Ito, and M. Iwaida. 1977. Determination of sorbate, dehydroacetate, benzoate, salicylate and esters of *p*-hydroxybenzoic acids in several foods. *Eisei Kagaku* 23:100–105.

Trifiro, E. 1960. The detection of sublimable preservatives. *Ind. Conserve (Parma)*. 279–286.

Tsukamoto, H., and S. Terada. 1960. Metabolism of drugs. XXIII. Metabolic fate of *p*-hydroxybenzoic acid and its derivatives in rabbits. *Chem. Pharm. Bull. (Tokyo)* 8:1066–1070.

Tsukamoto, H., and S. Terada. 1962. Metabolism of drugs. XXVI. Metabolic fate of *p*-hydroxybenzoic acid and its derivatives in rabbit. *Chem. Pharm. Bull. (Tokyo)* 10:86–90.

Tsukamoto, H., and S. Terada. 1964. Metabolism of drugs. XLVII. Metabolic fate of *p*-hydroxybenzoic acid and its derivatives in rabbit. *Chem. Pharm. Bull. (Tokyo)* 12:765–769.

Twoney, K. 2000a. 4-Hydroxybenzoic Acid: dose setting study in immature mice. Central Toxicology Laboratory (CTL) study CTL/ZM1589/SUM/REPT. Unpublished data provided by CTFA 04/28/05. 8 pages.[4]

Twomey, K. 2000b. 4-Hydroxybenzoic Acid: uterotrophic assay in immature mice. CTL study CTL/ZM1590/REG/REPT. Unpublished data provided by CTFA. 40 pages.[4]

Tymes, N. W. 1977. The determination of corticoids and related steroid analogs by high-performance liquid chromatography. *J. Chromatogr. Sci.* 15:151–155.

Tzortzatou, F. and F. G. J. Hayhoe. 1974. The effect of folate antagonists on dihydrofolate reductase activity demonstrated cytochemically. *Br. J. Haematol.* 28:209–216.

United States Pharmacopeial Convention. 1995. *United States Pharmacopeia*, 23rd ed. Rockville, MD: United States Pharmacopeial Convention.

Valdehita, M. T., A. Carballido, and M. C. Garcia-Moreno Del Rio. 1979. Preservatives in foods. I. Extraction, separation and identification by thin-layer chromatography. *An. Bromatol.* 31:31–37.

Valdez, C., E. I. Isaacson, and F. P. Cosgrove. 1968. Interaction of methyl and propyl parabens with selected sucrose esters. *J. Pharm. Sci.* 57:2093–2096.

Valencien, C., and J. Deshusses. 1939. The identification and the bromometric determination of the esters of salicylic acid. *Mitt. Lebensm. Hyg.* 30:85–86.

Veien, N. K., T. Hattel, and G. Laurberg. 1996. Oral challenge with parabens in paraben-sensitive patients. *Contact Dermatitis* 34:433.

Verhaeghe, I., and A. Dooms-Goossens. 1997. Multiple sources of allergic contact dermatitis from parabens. *Contact Dermatitis* 36:269–270.

Vilaplana, J., and C. Romaguera. 2000. Contact dermatitis from parabens used as preservatives in eyedrops. *Contact Dermatitis* 43:248.

Vogel, J., and J. Deshusses. 1965. Identification of dehydroacetic acid, sorbic acid, benzoic acid, *p*-chlorobenzoic acid, salicylic acid, and of methyl, ethyl, propyl, and butyl esters of *p*-hydroxybenzoic acid by gas chromatography. *Mitt. Lebensm. Hyg.* 56:35–37.

Wahbi, A.-Z. M., H. Abdine, and S. M. Blaih. 1977. Spectrophotometric determination of some preservatives in milk. *J. Assoc. Off. Anal. Chem.* 60:1175–1179.

Wahl, G. 1983. Local anesthesia and para group allergy. *Zahnarztl Prax* 34:314–317.

Wang, R. T., and S. S. Chou. 1970. Simple and rapid separation of preservatives and artificial sweeteners by polyamide layer chromatography. *J. Chin. Chem. Soc. (Taipei)* 17:188–191.

Weinreb, R. N., I. Wood, L. Tomazzoli, and J. Alvarado. 1986. Subconjunctival injections: preservative-related changes in the corneal endothelium. *Invest. Ophthalmol. Vis. Sci.* 27:525–531.

Weisenberg, E., B. Gershon, and J. Schoenberg. 1977. Microdetermination of *p*-hydroxybenzoic esters in pharmaceuticals and cosmetics. *J. Assoc. Off. Anal. Chem.* 60:56–59.

White, A. A. 1967. Stimulation of the growth of organ cultures by methylparabens and propyl paraben. *Proc. Soc. Exp. Biol. Med.* 126:588–591.

Whitworth, C. W., and H. W. Jun. 1973. Influence of polysorbate 20 and sodium cholate on uptake of *p*-hydroxybenzoates by the frog, Rana pipiens. *J. Pharm. Sci.* 62:1890–1891.

Wilkinson, J. D., S. Shaw, K. E. Andersen, et al. 2002. Monitoring levels of preservative sensitivity in Europe—A 10-year overview (1991–2000). *Contact Dermatitis* 46:207–210.

Wilson, C. H. 1972. Determination of the esters of *p*-hydroxybenzoic acid by gas chromatography. *Am. Cosmet. Perfum.* 87:43–44.

Wilson, C. H. 1975. Identification of preservatives in cosmetic products by thin-layer chromatography. *J. Soc. Cosmet. Chem.* 26:75–81.

World Health Organization/Food Agriculture Organization (WHO/FAO). 1998. Methyl *p*-Hydroxybenzoate specifications established by the Joint WHO/FAO Expert Committee on Food Additives (JECFA). http://apps3.fao.org/jecfa/additive_specs/docs/6/additive-0720.htm. Accessed Feb. 7, 2005.

Wuepper, K. D. 1967. Paraben contact dermatitis. *JAMA* 202:579–581.

Wulf, K., and A. R. Memmesheimer. 1969. Harmful effects of preservatives in ointments. *Ger. Med. Mon.* 14:482–483.

Würbach, G., H. Schubert, and I. Phillipp. 1993. Contact allergy to benzyl alcohol and benzyl paraben. *Contact Dermatitis* 28:187–188.

Yost, R., J. Stoveken, and W. MacLean. 1977. Positive peak identification in liquid chromatography using absorbance ratioing with a variable-wavelength spectrophotometric detector. *J. Chromatogr.* 134:73–82.

Zacharias, L. F., and C. W. Fisgus. 1971. An aerosol for relief of episiotomy discomfort. *Clin. Med.* 78:24–25.