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

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

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

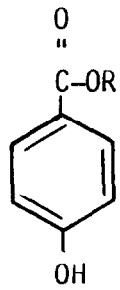
Numerous in vitro mutagenicity studies indicate that the Parabens are nonmutagenic. Methylparaben was noncarcinogenic when injected in rodents or when administered intravaginally in rats. Cocarcinogenesis studies on Propyl- and Methylparaben were negative. Teratogenic studies on Methyl- and Ethylparaben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

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

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

INTRODUCTION

This report on the Parabens summarizes much of the available data published between 1920 and 1982 and all of the unpublished data submitted to the Cosmetic Ingredient Review.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



CHEMISTRY

Structure and Preparation

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

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

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

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

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

Properties

The Parabens form small colorless crystals or white crystalline powders with practically no odor or taste. Parabens are soluble in alcohol, ether, glycerine, and propylene glycol and slightly soluble or almost insoluble in water. As the alkyl chain length increases, water solubility decreases. Parabens are hygroscopic and have a high oil/water partition coefficient.[4,10-16] Table 1 summarizes other physicochemical properties of the Parabens.

Analytical Methods

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

TABLE 1. Physicochemical Properties of the Parabens.

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

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

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

Reactivity/Stability

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

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

Potential Interaction with Other Cosmetic Ingredients

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

TABLE 2. Analytical Methods for Paraben Determination.

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

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

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

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

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

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

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

USE

Cosmetic

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

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

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

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

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

TABLE 3. Product Formulation Data.[107]

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

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

TABLE 3. (Continued.)

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

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

TABLE 3. (Continued.)

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

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

TABLE 3. (Continued.)

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

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

TABLE 3. (Continued.)

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

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

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

Food

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

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

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

Pharmaceutical

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

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

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

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

Other

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

GENERAL BIOLOGY

Absorption, Metabolism, and Excretion

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

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

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

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

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

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

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

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

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

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

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

Antimicrobial Effects

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

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

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

TABLE 4. Antimicrobial Effectiveness of Parabens.

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

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

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

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

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

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

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

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

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

Biochemical Effects

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

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

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

Cytotoxicity

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

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

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

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

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

Tissue Effects

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

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

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

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

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

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

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

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

Physiological Effects

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

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

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

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

Animal Toxicology

Acute Toxicity

Oral

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

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

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

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

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

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

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

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

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

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

Dermal

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

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

Subcutaneous

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

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

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

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

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

Intravenous

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

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

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

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

Intraperitoneal

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

Subarachnoid

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

Subchronic Toxicity

Oral

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

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

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

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

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

Dermal

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

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

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

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

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

Chronic Toxicity

Oral

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

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

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

Subcutaneous

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

Primary Irritation

Skin

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

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

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

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

Eye

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

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

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

A number of rabbit eye irritation studies have been conducted on products containing Methylparaben, Ethylparaben, Propylparaben, and/or Butylparaben at concentrations of 0.1 to 0.8 percent. Most products produced no signs of eye irritation.,[207,210,242-248] Other products produced slight or minimal eye irritation, with scores of 1.0 to 3.3/110.[211,249-252]

Mucous Membrane

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

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

Subchronic Irritation

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

Sensitization

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

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

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

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

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

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

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

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

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

Special Studies

Mutagenesis

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

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

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

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

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

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

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

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

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

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

Chromosomal Aberration Studies

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

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

Carcinogenesis

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

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

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

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

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

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

Teratogenesis

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

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

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

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

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

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

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

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

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

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

Fisher[275] coined the term "Paraben Paradox." He observed that Paraben-sensitive patients who react with allergic contact dermatitis when Paraben-containing pharmaceuticals are applied to eczematous or ulcerated skin can tolerate Paraben-containing cosmetics applied to normal, unbroken skin. No sensitization is induced even when these cosmetics contact the thin, delicate membrane of the eyelid. He noted that cosmetics are usually applied to normal

TABLE 5. Paraben Sensitization Results.[254]

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

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

TABLE 6. Results of Paraben Patch Tests.

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

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

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

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

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

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

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

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

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

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

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

TABLE 8. (Continued.)

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

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

TABLE 8. (Continued.)

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

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

Case Reports

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

Eye Irritation

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

Toxicity

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

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

Photocontact Sensitization

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

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

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

Phototoxicity

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

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

Industry Complaint Experience

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

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

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

Parabens are practically nonirritating and nonsensitizing in the population with normal skin. Paraben sensitization has occurred, especially when Paraben-containing medicaments have been applied to damaged or broken skin. Even when applied to patients with chronic dermatitis, Parabens generally induce sensitization in less than 3 percent of such individuals. Of 27,230 patients with chronic skin problems, 2.2 percent were sensitized by preparations of parabens at concentrations of 1 to 30 percent. Many patients sensitized to Paraben-containing medications can wear cosmetics containing these ingredients with no adverse effects. Skin irritation and sensitization tests on product formulations containing from 0.1 to 0.8 percent of one or two of the Parabens showed no evidence of significant irritation or sensitization potential for these ingredients. A subchronic oral toxicity study in humans indicated that Methylparaben was practically nontoxic at doses up to 2 g/kg/day. A primary eye irritation study in humans showed Methylparaben to be nonirritating at concentrations up to 0.3 percent. Photocontact sensitization and phototoxicity tests on product formulations containing 0.1 to 0.8 percent Methyl-, Propyl-, and/or Butylparaben gave no evidence for significant photoreactivity. Industry complaint experience data showed low to moderate numbers of safety-related complaints with the incidence depending on the product.

DISCUSSION

It is important to note the concentrations at which the Parabens are used in cosmetic products. In only two instances are the Parabens reported to be used at concentrations greater than 5 percent. In fact, 99.7 percent of the products that contain Parabens have concentrations of less than or equal to 1 percent. This information can be used to evaluate the adequacy of the data contained in this report with respect to the concentrations tested versus the concentrations used in cosmetic products.

A number of acute, subchronic, and chronic toxicity tests have been performed on the Parabens using a wide variety of routes of administration. From these data, it is readily apparent that these ingredients exhibit a very low order of toxicity and must certainly be considered safe in this respect for cosmetic use in the usual quantities employed as a preservative.

When tested on human skin, each of the Parabens began producing evidence of irritation only when concentrations exceeded 5 to 12 percent. Considering the order of magnitude of these concentrations, it may be concluded that the Parabens are relatively nonirritating at the concentrations used in cosmetic products.

The Food and Drug Administration's Ophthalmic Drug Panel concluded that Methylparaben and Propylparaben are unsafe as antimicrobial agents in OTC ophthalmic products because they are irritating to the eyes if used at concentrations effective against microorganisms. Supportive data were not available in the references cited in the Ophthalmic Drug Panel's report. Data available to the Cosmetic Ingredient Review indicate that there is no evidence for significant

ocular irritation potential. Methylparaben and Ethylparaben, each at 100 percent concentration, and a number of product formulations containing Methyl-, Ethyl-, Propyl-, and/or Butylparaben at concentrations of 0.1 to 0.8 percent produced no more than minimal, transient ocular irritation in rabbits. Instillation of aqueous solutions of 0.1 to 0.3 percent Methylparaben several times daily into the eyes of more than 100 human subjects produced no irritation.

Sensitization to Parabens has been reported, especially in cases where Paraben-containing medicaments have been applied to damaged skin. However, in a total pool of over 27,000 subjects with chronic dermatitides, only 2.2 percent became sensitized to Paraben preparations of 1 to 30 percent concentration. The results of tests obtained using healthy human skin confirm the results obtained in animals, both indicating that the Parabens are free from allergenic behavior under these circumstances. Frequently, patients sensitized to Parabens on damaged skin can tolerate usage on intact skin. In light of these data, it is recommended that Parabens not be used on damaged skin due to the increased risk of sensitization.

CONCLUSION

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

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