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Final Report on the Safety Assessment of Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85

The Polysorbates are a series of polyoxyethylenated sorbitan esters that are used as hydrophilic, nonionic surfactants in a variety of cosmetic products. Polysorbates are hydrolyzed by pancreatic and blood lipases; the fatty acid moiety is released to be absorbed and metabolized, whereas the polyoxyethylene sorbitan moiety is very poorly absorbed and is excreted unchanged. Acute and long-term oral toxicity in animals indicates a low order of toxicity with oral ingestion of the Polysorbates.

Polysorbate 80 was shown to be nonmutagenic in the Ames and micronucleus tests. The Polysorbates were noncarcinogenic in laboratory animals. Multiple studies have shown that the Polysorbates enhance the activity of known chemical carcinogens while not actually being carcinogenic themselves.

Extensive clinical skin testing showed Polysorbates to have little potential for human skin irritation or evidence of skin sensitization or phototoxicity. The available data indicate that these ingredients are used in numerous preparations without clinical reports of significant adverse effects. It is concluded that they are safe for use in cosmetics at present concentrations of use.

CHEMICAL AND PHYSICAL PROPERTIES COMPOSITION

The Polysorbates are a series of general purpose, hydrophilic, nonionic surfactants. They are obtained by reaction of sorbitol and its anhydrides with ethylene oxide (C_2H_4O) under conditions that cause splitting of water from the sorbitol, leaving sorbitan. A specified molar ratio of ethylene oxide to sorbitol and its mono- and dianhydrides is used in the condensation to effect an oxyethylene copolymerization at the free hydroxyl groups of sorbitan. The resulting polyoxyethylene sorbitans are esterified with 1 or 3 moles of a fatty acid (lauric, palmitic, stearic, oleic) to produce the Polysorbates. Therefore, in summary the Polysorbates are polyoxyethylene (W + X + Y + Z) sorbitan mono- or triesters, where the sum of w + x + y + z is the average number of moles of ethylene oxide per mole of sorbitol, and where R denotes 1 or 3 moles of an esterified fatty acid. Specific data for these variables are listed in Table 1 for each of the Polysorbates.[1-19]

TABLE 1. Compositions of the Polysorbates.

Ingredient	*Chemical Name*	*Average Moles of Ethylene Oxide (w+x+y+z)*	*Major Fatty Acid Moieties*	
			R	*No. of Moles*
Polysorbate 21	Polyoxyethylene(21) sorbitan monolaurate	21	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_{11}CH_3$	1
Polysorbate 21	Polyoxyethylene(4) sorbitan monolaurate	4	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_{11}CH_3$	1
Polysorbate 41	Polyoxyethylene(21) sorbitan monopalmitate	21	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_{14}CH_3$	1
Polysorbate 61	Polyoxyethylene(21) sorbitan monostearate	21	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_{16}CH_3$	1
Polysorbate 61	Polyoxyethylene(4) sorbitan monostearate	4	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_{16}CH_3$	1
Polysorbate 65	Polyoxyethylene(21) sorbitan tristearate	21	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_{16}CH_3$	3
Polysorbate 81	Polyoxyethylene(21) sorbitan monooleate	21	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_7CH=CH(CH_2)_7CH_3$	1
Polysorbate 81	Polyoxyethylene(5) sorbitan monooleate	5	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_7CH=CH(CH_2)_7CH_3$	1
Polysorbate 85	Polyoxyethylene(21) sorbitan trioleate	21	$-O-\overset{O}{\overset{\|}{C}}(CH_2)_7CH=CH(CH_2)_7CH_3$	3

Properties

The Polysorbates are, for the most part, viscous liquids that range in color from yellow to orange to tan. They possess a faint, characteristic odor and a warm, somewhat bitter taste.[11,13-16,18,19]

The solubility characteristics, physical properties, and chemical properties of the Polysorbates are listed in Tables 2, 3, and 4, respectively.

Polyoxyethylene esters are characterized by a very strong infrared absorption peak at 9 μm.[20] Detailed discussions of other physical properties, especially regarding the surface active properties of the Polysorbates, are available in the scientific literature. These include studies on oil/water interfacial tension,[21] emulsion stability,[22] suspension stability,[23,24] rheological characteristics of emulsions,[25,26] hydrophile:hydrophobe proton ratio,[27] and critical micelle concentration.[28-32]

Chemical Reactivity

Aqueous solutions of Polysorbate 20 undergo autoxidation on storage at room temperature, with changes in the peroxide number, pH, surface tension, and cloud point. Autoxidation is accelerated by light, elevated temperature, and copper sulfate. Hydrolysis of Polysorbate 20 also occurs at room temperature, whereas the oxyethylene moieties undergo chain shortening at temperatures above 40°C. Evidence of such degradation has been detected in unopened commercial samples of Polysorbates 20, 40, and 60.[33] Degradation of Polysorbates might also occur in cosmetic formulations because of microorganisms. Bacteria

TABLE 2. Solubilities of the Polysorbates.[11,13-15,18,19]

	Polysorbate								
Solvent	*20*	*21*	*40*	*60*	*61*	*65*	*80*	*81*	*85*
Water	+		+/−	+/−	+	+	+	+	+
Mineral oil	−		−	−		+/−	−	+	+
Fixed oils							+		
Vegetable oils				−		+			
Cottonseed oil							+		
Corn oil							+		
Alcohol	+		+	+/−	+	+	+	+	+
Methanol	+					+	+		
Polyols	−			−			−		
Etheralcohols				+/−					
Acetone				+/−		+			
Toluene				+			+		
Carbon tetrachloride				+		+			
Petroleum solvents				+/−					
Mineral spirits	−					+			
Ethyl acetate	+			+			+		
Dioxane	+			+		+			
Aniline				+					
Aromatic hydrocarbons				+					

+ = Soluble or dispersible.
− = Insoluble.
+/− = Slightly soluble, depending on temperature; or conflicting reports.
Blank spaces indicate no data.

TABLE 3. Physical Properties of the Polysorbates.

Ingredient	*Density*	*Physical State*	*Setting Point*	*Viscosity*	*Refr. Index*	*pH (5 percent aq. sol.)*	*HLB*
Polysorbate 20		Viscous, oily liquid[11,13,15,16,42]	14°–16°C[15]	4000–6000 centipoise at 25°C[15]	1.472[15]		16.7[42]
Polysorbate 21		Liquid[42]					13.3[42]
Polysorbate 40	1.05[15]	Oily liquid or Vaseline-like[11,15,16,42]					15.6[42]
Polysorbate 60		Oily liquid[42] or semigel,[13] wax,[15] Vaseline-like[16]	45°–55°C[15]				14.9[42]
Polysorbate 61		Waxy solid[11,42]					6.9[42]
Polysorbate 65		Waxy solid[11,13,42]	31°C[11]				10.5[42]
Polysorbate 80	1.08[14] 1.06–1.10[19] 1.07–1.09[18]	Viscous, oily liquid[13-16,18,19,42]	5°–6°C[15]	345–445 centistokes at 25°C and 150–210 centistokes at 38°C[18] 270–430 centistokes[19]		6.0–8.0[15,18] 5–7[19]	15.0[42]
Polysorbate 81		Liquid,[42] may gel at room temperature[11]		350–550 centipoise at 25°C[11]			10.0[42]
Polysorbate 85		Liquid,[16,42] may gel at room temperature[11]					11.0[42]

TABLE 4. Chemical Properties of the Polysorbates.

Ingredient	*Fatty Acid Moiety*	*Oxyethylene Content (%)*	*Fatty Acid Content (g/100g-sample)*	*Acid Value*	*Hydroxyl Value**	*Saponification Value*
Polysorbate 20	Monolaurate	70.0–74.0[13]	15–17[13]	2.0 max[11,13] 7.0 max[15] 4.0 max[16]	95–115[11] 96–108[13]	50–51[11] 40–50[13] 43–57[16]
Polysorbate 21	Monolaurate			3.0 max[6]	225–255[6]	100–115[6]
Polysorbate 40	Monopalmitate			2.0 max[11] 4.0 max[16]	89–105[11]	43–49[11] 41–55[16]
Polysorbate 60	Monostearate	65.0–69.5[13]	24–26[13]	2.0 max[7,13] 5.0 max[15] 4.0 max[16]	81–96[7,13]	45–55[7,13] 43–55[16]
Polysorbate 61	Monostearate			2.0 max[8,11]	170–200[11] 180–190[8]	95–114[11] 45–55[8]
Polysorbate 65	Tristearate	46.0–50.0[13]	42–44[13]	2.0 max[9,11,13]	44–60[9,11,13]	88–98[9,11,13]
Polysorbate 80	Monooleate	65.0–69.5[13]	22–24[13]	2.0 max[13] 10.0 max[15] 4.0 max[16]	65–80[13]	45–55[13] 135–140[15] 40–55[16]
Polysorbate 81	Monooleate			2.0 max[11]	136–152[11]	95–105[11]
Polysorbate 85	Trioleate			2.0 max[11] 4.0 max[16]	39–52[11]	82–95[11] 83–105[16]

*Number of milligrams potassium hydroxide equivalent to one gram of sample.

in the deionized water used to manufacture cosmetic products were found to enzymatically decompose Polysorbate 20.[34]

The kinetics of the hydrolysis of Polysorbate 80 in aqueous buffers was studied over the pH range of 1.10 to 10.28. The hydrolysis was specific acid-catalyzed at pH values below 3 and specific base-catalyzed at pH values greater than 7.6.[35]

Analytical Methods

Positive identification of the Polysorbates can be made through close matching to standard infrared spectra[11] or through any of several physical or chemical assays.[13,16,18,20] Quantitative and/or qualitative determinations of the Polysorbates have been made using gas chromatography,[36] thin-layer chromatography,[37] paper chromatography,[38,39] acidic complex precipitation tests,[40] and solubility assays.[41] The fatty acid moieties are determined as methyl esters after saponification of the Polysorbates and esterification of the resulting fatty acids.[43] Methods adapted for the quantitative and qualitative analysis of Polysorbate 80 in pharmaceutical preparations have been reviewed.[44]

The surface active properties of the Polysorbates have been evaluated with such methods as proton magnetic resonance,[27] interference refractometry,[29] membrane osmometry,[45] surface tension determination,[31,32] and density determination of aqueous solutions of the Polysorbates.[30]

Chemical properties of the Polysorbates for which assay methods have been described include hydroxyl value, saponification value, acid value, oxyethylene content, free fatty acid content, arsenic content, heavy metal content, water content, and residue on ignition.[13]

Impurities

Since the fatty acids used in the production of cosmetic ingredients frequently contain fatty acids other than the principal acid named, each of the Polysorbates may contain a complex fatty acid moiety. In a study on Polysorbates 20, 40, 60, and 80, 15 different fatty acids were detected. The main constituents are listed in Table 5. The fatty acid compositions of the minor Polysorbates (21, 61, 65, 81, and 85) can be expected to reflect those of their major counterparts listed in Table 5.[46]

Peroxides, the ethoxylates of isosorbide, and unreacted free fatty acids may be present at unspecified concentrations.[2-10] A method for the removal of per-

TABLE 5. Fatty Acid Moiety Compositions of the Polysorbates.[46]

	Fatty Acid (%)					
Ingredient	*Lauric*	*Palmitic*	*Stearic*	*Oleic*	*Myristic*	*Palmitoleic*
Polysorbate 20 (monolaurate)	36.9	15.3		13.7	22.8	
Polysorbate 40 (monopalmitate)		86.4	10.2			
Polysorbate 60 (monostearate)		44.4	45.0			
Polysorbate 80 (monooleate)		6.4		76.9		6.4

oxides has been reported.[47] Birkel et al.[48] detected 1,4-dioxane at levels of 5.5 to 378 ppm in samples of Polysorbates 60 and 80. One manufacturer who followed this work has reported that dioxane is no longer detectable in their product.[49] During the manufacturing process, the Polysorbates are steam stripped to remove such unwanted water-soluble byproducts as 1,4-dioxane.[2-10] Since 1,4-dioxane is reported to induce carcinoma of the nasal turbinates in rats and hepatocellular carcinoma in mice when given in the drinking water at 0.5 to 1.0 percent,[50] the presence of traces of 1,4-dioxane is undesirable. More specific data on impurities are listed in Table 6.

USE

Purpose in Cosmetics

The Polysorbates are a series of general purpose, hydrophilic, nonionic surfactants supplied by the manufacturers at 100 percent concentration.[42] Various terms used to describe their roles in cosmetics include oil/water emulsifier, detergent, dispersing agent, solubilizer, and stabilizer.[1,13,15,51]

The Polysorbates exhibit high hydrophile–lipophile balance (HLB) values, indicating they will function to disperse oil in water as opposed to water in oil. HLB values for most emulsifiers fall in the range of 1.8 to 18.6, with the Polysorbates in the range 9.6 to 16.7 (Table 3). Because of their nonionic nature, the Polysorbates are comparatively insensitive to hard water and electrolytes and may be used in both acidic and basic formulations.[52]

Scope and Extent of Use in Cosmetics

The Polysorbates are used in a wide variety of cosmetic products. Table 7 lists product types and the number of product formulations containing the Polysorbates as reported by the Food and Drug Administration (FDA) in 1981. The 1979 totals for all product categories are listed in Table 7 for comparison to the 1981 figures.

TABLE 6. Impurities.

Ingredient	*Arsenic Content (as As)*	*Heavy Metal Content (as Pb)*	*Water Content*	*Residue on Ignition*
Polysorbate 20	< 3 ppm[13]	< 10 ppm[13]	< 3.0%[11,13,16]	< 0.15%[13]
				< 1.0%[16]
Polysorbate 21			< 3.0%[6]	
Polysorbate 40			< 3.0%[11,16]	< 1.0%[16]
Polysorbate 60	< 3 ppm[13]	< 10 ppm[13]	< 3.0%[7,13,16]	< 0.25%[13]
				< 1.0%[16]
Polysorbate 61			< 3.0%[11]	
			< 1.0%[8]	
Polysorbate 65	< 3 ppm[13]	< 10 ppm[13]	< 3.0%[9,11,13]	< 0.25%[13]
Polysorbate 80	< 3 ppm[13,18]	< 10 ppm[13,18]	< 3.0%[13,16]	< 0.15%[13,18]
				< 1.0%[16]
Polysorbate 81			< 3.0%[11]	
Polysorbate 85			< 5.0%[11]	< 1.0%[16]
			< 3.0%[16]	

TABLE 7. Product Formulation Data.[53]

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25-50	>10-25	>5-10	>1-5	>0.1-1	≤0.1
Polysorbate 20										
Baby shampoos	35	6	—	—	—	2	—	2	2	—
Other baby products	15	1	—	—	—	—	—	—	1	—
Bath oils, tablets, and salts	237	7	—	—	2	2	—	1	2	—
Bubble baths	475	7	—	—	—	—	3	2	2	—
Bath capsules	3	10	—	—	—	1	—	1	8	—
Eyeliner	369	11	—	—	—	—	1	4	6	—
Eye shadow	2582	1	—	—	—	—	—	—	1	—
Eye makeup remover	81	2	—	—	—	—	—	2	—	—
Mascara	397	2	—	—	—	—	—	—	2	—
Other eye makeup preparations	230	3	—	—	—	—	—	1	2	—
Colognes and toilet waters	1120	13	—	3	—	1	—	9	—	—
Perfumes	657	7	—	1	—	5	—	—	—	—
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	28	—	—	—	—	—	—	28	—
Other fragrance preparations	191	7	—	—	—	1	—	3	2	1
Hair conditioners	478	33	—	—	—	—	1	10	21	1
Hair sprays (aerosol fixatives)	265	2	—	—	—	—	—	—	1	1
Permanent waves	474	54	—	—	—	—	2	6	38	8
Hair rinses (noncoloring)	158	14	—	—	—	—	1	2	10	1
Hair shampoos (noncoloring)	909	47	—	—	—	2	2	8	33	2
Tonics, dressings, and other hair grooming aids	290	14	—	—	—	—	—	6	7	1
Wave sets	180	31	—	—	—	—	—	1	19	11
Other hair preparations (noncoloring)	177	7	—	—	—	—	—	1	5	1
Hair dyes and colors (all types requiring caution statement and patch test)	811	4	—	—	—	—	—	4	—	—
Hair tints	15	14	—	—	—	—	—	13	1	—
Hair shampoos (coloring)	16	7	—	—	—	—	—	—	7	—

Other hair coloring preparations	49	3	—	—	—	—	—	2	1	—
Blushers (all types)	819	10	—	—	—	—	—	5	4	1
Makeup foundations	740	20	—	—	—	—	—	1	16	3
Lipstick	3319	1	—	—	—	—	—	1	—	—
Makeup bases	831	60	—	—	—	—	1	6	36	17
Rouges	211	4	—	—	—	—	—	—	3	1
Makeup fixatives	22	1	—	—	—	—	—	—	1	—
Other makeup preparations (not eye)	530	5	—	—	—	—	—	2	3	—
Cuticle softeners	32	4	—	—	—	—	—	4	—	—
Nail creams and lotions	25	3	—	—	—	—	—	1	1	1
Nail polish and enamel remover	41	1	—	—	—	—	—	—	1	—
Other manicuring preparations	50	1	—	—	—	—	—	—	1	—
Mouthwashes and breath fresheners (liquids and sprays)	53	4	—	—	—	—	—	—	3	1
Bath soaps and detergents	148	4	—	—	—	—	—	4	—	—
Deodorants (underarm)	239	5	—	—	—	—	—	2	3	—
Douches	26	5	—	—	—	—	—	3	2	—
Other personal cleanliness products	227	14	—	—	—	—	—	—	14	—
Aftershave lotions	282	6	—	—	—	—	1	3	2	—
Preshave lotions (all types)	29	1	—	—	—	—	—	—	1	—
Shaving cream (aerosol, brushless, and lather)	114	7	—	—	—	—	—	4	3	—
Other shaving preparation products	29	1	—	—	—	—	—	—	—	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	51	—	—	—	—	5	21	18	7
Face, body, and hand skin care preparations (excluding shaving preparations)	823	35	—	—	—	—	—	22	11	2
Moisturizing skin care preparations	747	23	—	—	—	—	—	5	13	5
Night skin care preparations	219	7	—	—	—	—	—	3	2	2
Paste masks (mud packs)	171	12	—	—	—	—	1	5	6	—
Skin lighteners	44	1	—	—	—	—	—	1	—	—
Skin fresheners	260	44	—	—	—	—	—	22	20	2
Wrinkle smoothers (removers)	38	2	—	—	—	—	—	1	1	—

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Polysorbate 20—Cont.										
Skin lighteners	44	1	—	—	—	—	—	1	—	—
Skin fresheners	260	44	—	—	—	—	—	22	20	2
Wrinkle smoothers (removers)	38	2	—	—	—	—	—	1	1	—
Other skin care preparations	349	26	—	—	—	—	—	10	14	2
Suntan gels, creams, and liquids	164	5	—	—	—	—	—	3	2	—
Indoor tanning preparations	15	2	—	—	—	—	—	1	1	—
Other suntan preparations	28	2	—	—	—	—	—	—	2	—
1981 TOTALS		702	—	4	2	14	18	209	383	72
1979 TOTALS[54]		719	52	6	2	14	17	205	363	60
Polysorbate 21										
Night skin care preparations	219	1	—	—	—	—	—	—	1	—
1981 TOTALS	—	1	—	—	—	—	—	—	1	—
1979 TOTALS[54]	—	1	—	—	—	—	—	—	1	—
Polysorbate 40										
Eye lotion	13	2	—	—	—	—	—	2	—	—
Other eye makeup preparations	230	3	—	—	—	—	—	3	—	—
Colognes and toilet waters	1120	3	—	—	—	—	3	—	—	—
Hair conditioners	478	3	—	—	—	—	—	1	1	1
Hair straighteners	64	1	—	—	—	—	—	1	—	—
Permanent waves	474	3	—	—	—	—	—	—	—	3
Hair shampoos (noncoloring)	909	1	—	—	—	—	—	—	1	—
Tonics, dressings, and other hair grooming aids	290	3	—	—	—	—	—	3	—	—
Makeup foundations	740	1	—	—	—	—	—	—	—	1
Nail creams and lotions	25	1	—	—	—	—	—	1	—	—
Other manicuring preparations	50	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	21	—	—	—	—	—	17	4	—

Face, body, and hand skin care preparations (excluding shaving preparations)	823	8	—	—	—	—	—	6	2	—
Moisturizing skin care preparations	747	8	—	—	—	—	—	4	4	—
Night skin care preparations	219	2	—	—	—	—	—	2	—	—
Skin fresheners	260	2	—	—	—	—	—	—	2	—
Other skin care preparations	349	2	—	—	—	—	—	—	2	—
Suntan gels, creams, and liquids	164	3	—	—	—	—	—	3	—	—
Indoor tanning preparations	15	1	—	—	—	—	—	1	—	—
1981 TOTALS		69	—	—	—	—	3	44	17	5
1979 TOTALS[54]		59	11	—	—	—	2	31	12	3
Polysorbate 60										
Bubble baths	475	1	—	—	—	—	1	—	—	—
Other bath preparations	132	2	—	—	—	—	—	1	1	—
Eyebrow pencil	145	3	—	—	—	—	—	—	3	—
Eyeliner	369	2	—	—	—	—	—	—	2	—
Eye shadow	2582	116	—	—	—	—	—	2	—	114
Mascara	397	10	—	—	—	—	2	1	7	—
Other eye makeup preparations	230	7	—	—	—	—	1	4	2	—
Colognes and toilet waters	1120	1	—	—	—	—	—	1	—	—
Perfumes	657	1	—	—	—	—	—	—	1	—
Sachets	119	7	—	—	—	—	—	7	—	—
Other fragrance preparations	191	5	—	—	—	—	—	—	5	—
Hair conditioners	478	2	—	—	—	—	—	—	2	—
Hair straighteners	64	3	—	—	—	—	—	3	—	—
Hair rinses (noncoloring)	158	1	—	—	—	—	—	1	—	—
Tonics, dressings, and other hair grooming aids	290	3	—	—	—	—	1	1	1	—
Other hair preparations (noncoloring)	177	2	—	—	—	1	—	1	—	—
Other hair coloring preparations	49	1	—	—	—	—	—	1	—	—
Blushers (all types)	819	24	—	—	—	—	1	—	1	22
Face powders	555	26	—	—	—	—	—	1	—	25
Makeup foundations	740	22	—	—	—	—	1	12	8	1
Lipstick	3319	1	—	—	—	—	—	—	—	1
Makeup bases	831	12	—	—	—	—	—	3	4	5
Rouges	211	8	—	—	—	—	—	1	—	7

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Polysorbate 60—Cont.										
Nail creams and lotions	25	2	—	—	—	—	—	1	1	—
Mouthwashes and breath fresheners (liquids and sprays)	53	2	—	—	—	—	—	1	1	—
Other personal cleanliness products	227	1	—	—	—	—	—	—	1	—
Shaving cream (aerosol, brushless, and lather)	114	19	—	—	—	—	—	9	10	—
Other shaving preparation products	29	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	67	—	—	—	—	—	48	19	—
Face, body, and hand skin care preparations (excluding shaving preparations)	823	46	—	—	—	—	—	27	17	2
Moisturizing skin care preparations	747	64	—	—	—	—	1	37	24	2
Night skin care preparations	219	23	—	—	—	—	—	20	3	—
Paste masks (mud packs)	171	7	—	—	—	—	—	4	3	—
Skin lighteners	44	2	—	—	—	—	—	—	2	—
Skin fresheners	260	2	—	—	—	—	—	—	2	—
Other skin care preparations	349	8	—	—	—	—	—	7	1	—
Suntan gels, creams, and liquids	164	12	—	—	—	—	1	5	6	—
Indoor tanning preparations	15	5	—	—	—	—	—	5	—	—
Other suntan preparations	28	5	—	—	—	—	—	3	2	—
1981 TOTALS		526	—	—	—	1	9	207	130	179
1979 TOTALS[54]		512	44	—	—	—	4	181	103	180
Polysorbate 61										
Baby lotions, oils, powders, and creams	56	1	—	—	—	—	—	1	—	—

Makeup bases	831	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	—	—	—	—	—	1	1	—
Face, body, and hand skin care preparations (excluding shaving preparations)	823	1	—	—	—	—	—	—	1	—
Moisturizing skin care preparations	747	1	—	—	—	—	—	1	—	—
Night skin care preparations	219	1	—	—	—	—	—	1	—	—
Skin lighteners	44	1	—	—	—	—	—	1	—	—
Other skin care preparations	349	2	—	—	—	—	—	2	—	—
1981 TOTALS		10	—	—	—	—	—	7	3	—
1979 TOTALS[54]		7	—	—	—	—	—	4	3	—
Polysorbate 65										
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	—	—	—	—	—	2	—	—
Hormone skin care preparations	747	2	—	—	—	—	—	—	2	—
1981 TOTALS		4	—	—	—	—	—	2	2	—
1979 TOTALS[54]		2	—	—	—	—	—	2	—	—
Polysorbate 80										
Baby shampoos	35	1	—	—	—	—	1	—	—	—
Baby lotions, oils, powders, and creams	56	1	—	—	—	—	—	—	1	—
Bath oils, tablets, and salts	237	4	—	—	—	2	—	1	1	—
Bubble baths	475	2	—	—	—	—	—	1	1	—
Eyeliner	369	4	—	—	—	—	—	1	—	3
Eye shadow	2582	18	—	—	—	—	—	1	8	9
Other eye makeup preparations	230	1	—	—	—	—	—	—	1	—
Colognes and toilet waters	1120	3	—	—	—	—	—	1	2	—
Other fragrance preparations	191	1	—	—	—	—	—	1	—	—
Hair conditioners	478	8	—	—	—	—	—	—	7	1
Hair sprays (aerosol fixatives)	265	1	—	—	—	—	—	—	1	—

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Polysorbate 80–Cont.										
Permanent waves	474	3	—	—	—	—	—	—	—	3
Wave sets	180	5	—	—	—	—	—	—	4	1
Other hair preparations (noncoloring)	177	1	—	—	—	—	—	—	—	1
Blushers (all types)	819	9	—	—	—	—	—	1	2	6
Face powders	555	2	—	—	—	—	1	1	—	—
Makeup foundations	740	7	—	—	—	—	1	1	4	1
Makeup bases	831	17	—	—	—	—	—	—	2	15
Rouges	211	2	—	—	—	—	—	1	1	—
Other makeup preparations (not eye)	530	1	—	—	—	—	—	—	1	—
Cuticle softeners	32	1	—	—	—	—	—	—	1	—
Other manicuring preparations	50	3	—	—	—	—	—	—	3	—
Mouthwashes and breath fresheners (liquids and sprays)	53	4	—	—	—	—	—	—	3	1
Bath soaps and detergents	148	1	—	—	—	—	—	—	1	—
Deodorants (underarm)	239	2	—	—	—	—	—	—	1	1
Douches	26	1	—	—	—	—	—	—	1	—
Other personal cleanliness products	227	1	—	—	—	—	—	—	1	—
Shaving cream (aerosol, brushless, and lather)	114	3	—	—	—	—	—	3	—	—
Other shaving preparation products	29	1	—	—	—	—	—	1	—	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	23	—	—	—	—	—	12	9	2
Face, body, and hand skin care preparations (excluding shaving preparations)	823	18	—	—	—	—	1	4	11	2
Moisturizing skin care preparations	747	25	—	—	—	—	2	8	11	4

Night skin care preparations	219	2	—	—	—	—	—	2	—	—
Paste masks (mud packs)	171	4	—	—	—	—	—	2	1	1
Skin fresheners	260	16	—	—	—	—	—	8	7	1
Other skin care preparations	349	3	—	—	—	—	1	—	2	—
Suntan gels, creams, and liquids	164	4	—	—	—	—	—	1	3	—
1981 TOTALS		203	—	—	—	2	7	51	91	52
1979 TOTALS[54]		166	18	—	—	2	4	42	57	43
Polysorbate 81										
Hair conditioners	478	2	—	—	—	—	—	1	1	—
Tonics, dressings, and other hair grooming aids	290	2	—	—	—	—	—	1	1	—
Blushers (all types)	819	2	—	—	—	—	—	—	2	—
Makeup foundations	740	5	—	—	—	—	—	—	5	—
Other makeup preparations (not eye)	530	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	1	—	—	—	—	—	—	1	—
Face, body, and hand skin care preparations (excluding shaving preparations)	823	1	—	—	—	—	—	—	1	—
Moisturizing skin care preparations	747	1	—	—	—	—	—	—	1	—
1981 TOTALS		15	—	—	—	—	—	2	13	—
1979 TOTALS[54]		12	—	—	—	—	—	1	11	—
Polysorbate 85										
Eye shadow	2582	3	—	—	—	—	—	—	—	3
Eye makeup remover	81	1	—	—	—	—	—	1	—	—
Hair conditioners	478	4	—	—	—	—	—	4	—	—
Tonics, dressings, and other hair grooming aids	290	4	—	—	—	—	—	3	1	—
Hair lighteners with color	2	1	—	1	—	—	—	—	—	—
Hair bleaches	111	1	—	1	—	—	—	—	—	—
Blushers (all types)	819	4	—	—	—	—	—	—	—	4

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Polysorbate 85 – Cont.										
Other makeup preparations (not eye)	530	3	—	—	—	—	—	—	2	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	4	—	—	—	—	—	2	1	1
Face, body, and hand skin care preparations (excluding shaving preparations)	823	1	—	—	—	—	—	—	1	—
Moisturizing skin care preparations	747	2	—	—	—	—	1	—	1	—
Night skin care preparations	219	1	—	—	—	—	—	1	—	—
Paste masks (mud packs)	171	1	—	—	—	—	—	—	1	—
1981 TOTALS		30	—	2	—	—	1	11	7	9
1979 TOTALS[54]		43	28	2	—	—	1	8	4	—

*Preset product categories and concentration ranges in accordance with federal filing regulations (21 CFR 720.4); see Scope and Extent of Use in Cosmetics.

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. Ingredients are listed in prescribed concentration ranges under specific product type categories. Although the Polysorbates are thought to be supplied only in undiluted form, 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 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.

Table 7 lists the total number of reported formulations containing each of the Polysorbates as well as a summary analysis of the concentrations at which they are used; total figures for 1981 and 1979 data were compiled separately for each of the Polysorbates. These figures can be found in the columns labeled 1981 TOTALS and 1979 TOTALS at the end of the product category listings for each ingredient.

Surfaces to Which Commonly Applied

Products containing the Polysorbates are applied to all areas of the skin, hair, nails, and mucous membranes (Table 7).

Frequency and Duration of Application

Formulations containing the Polysorbates are applied as many as several times a day and remain in contact with the skin for variable periods of time following each application. Daily or occasional use may extend over many years.

Potential Interactions with Other Ingredients

As surface active agents, the Polysorbates interact with many of the other ingredients used in cosmetic formulations; these interactions can go beyond the primary action of oil/water emulsification. As shown in biopharmaceutical and drug release studies, the Polysorbates can produce marked effects on suspensions of insoluble ingredients.[55,56] Numerous studies on the interactions of the Polysorbates with specific drugs can be found in the scientific literature.

The Polysorbates have been shown to inactivate preservatives in several studies. The micellar preservative concentration increased with surfactant concentration, causing a decrease in the amount of free, biologically active preservative. The preservatives were bound to two distinct loci within the Polysorbate micelle. A high-affinity site was assumed to be located near the junction of the hydrocarbon core, and a very low affinity site was located in the polyoxyethylene region. The cosmetic ingredients found to be influenced in these studies included *p*-hydroxybenzoic acid and its methyl, ethyl, propyl, and butyl esters; benzoic acid; benzyl alcohol; capric acid; chlorbutol; chlorocresol; chloroxylenol; glycerol monolaurate; *o*-phenylphenol; and sorbic acid.[57-65] In another study, the effect of Polysorbates on perservatives was not simple inactivation; decrease or increase in the activity of cationic germicidal agents depended upon the critical micelle concentration.[66]

Noncosmetic Use

The Polysorbates find numerous uses in industry, research, pharmacy, and food production. They are used in the textile industry as antistatic agents, fiber lubricants, and finish emulsifiers.[42,51] In biological research, they find uses in membrane protein extraction, virus deactivation, and growth culture preparation.

The Polysorbates are used in pharmaceuticals for various reasons, including the modification of an active ingredient's absorption.[67] The FDA has approved Polysorbate 80 at up to 1.0 percent as an active demulcent in ophthalmic preparations; it is "recognized as safe and effective" at the recommended concentrations of 0.2 to 1.0 percent.[68] Polysorbates 20 and 80 are listed as wetting or clarifying agents in ophthalmic products and as cleaning, wetting, or solvent agents for contact lenses in concentrations not to exceed 1.0 percent. Polysorbate 20 is classified as an "inactive ingredient or pharmaceutical necessity" in topical analgesic, antirheumatic, otic, burn, and sunburn treatment/prevention products. Polysorbate 80 holds the same status in dentifrices and other dental care agents; it may also be used as an alcohol denaturant in mouthwashes.[69]

The Polysorbates find almost ubiquitous use in the food industry and have been approved by the FDA as direct and indirect food additives for human consumption with certain restrictions.[68,70] The details of these FDA regulations for the food use of Polysorbates are listed in Table 8. Polysorbates 20, 60, and 80 are approved for direct use in all food types as synthetic flavorings (21 CFR 172.515). Polysorbates 60, 65, and 80 are approved for direct use in a wide variety of specified food types as emulsifiers, solubilizers, dispersing agents, surfactants, wetting agents, opacifiers, defoaming agents, dough conditioners, and/or adjuvants. Usage limits range from 10 ppm to 4.5 percent of the finished product; limits for vitamin–mineral preparations range from 175 to 475 mg/day, based on the recommended daily dose (21 CFR 172.836, 172.383, 172.840 and as amended 9/5/80). Polysorbates 20, 40, 60, and 80 are approved for indirect addition to all food types as components of adhesives (21 CFR 175.105). Polysorbates 20, 40, 60, 65, 80, and 85 are approved for indirect addition to all food types as emulsifiers and/or surfactants (21 CFR 178.340). The FDA has also approved Polysorbates 60 and 80 for various uses in animal feeds (21 CFR 573.840–.860).

BIOLOGICAL PROPERTIES

Absorption, Metabolism, Storage, and Excretion

The metabolism of Polysorbates in rats has been studied in detail with ^{14}C-label tracer techniques. When administered orally, the ester link of the Polysorbate molecule is hydrolyzed by pancreatic lipase, and the fatty acid moiety is released to be absorbed and metabolized as any other dietary fatty acid. The efficiencies with which rats hydrolyzed and absorbed the labeled fatty acid portions of Polysorbates 80, 60, and 65 when fed at a dietary level of 10 percent were 100 percent, 98 percent, and 84 percent, respectively.[71] Treon et al.[72] found that the labeled lauric acid moiety of Polysorbate 20 was rapidly absorbed and oxidized by rats. After 24 hours, some 75 percent of the lauric acid was oxidized and expired as CO_2; 4 percent was not absorbed from the alimentary tract. Nelson et al.[73] fed Polysorbate 20 to rats and followed the distribution of labeled lauric acid to various tissues. The approximate distribution of radioactivity 24 hours after oral

TABLE 8. FDA Regulation Status of Polysorbates Found Safe for Human Consumption.[68,71]

Ingredient	*Category*	*Food Type*	*Purpose*	*Usage Limit*	*1979 21 CFR Code*
Polysorbate 21	DFA (Direct Food Additive)	All	Synthetic flavoring	Minimum required for intended effect	172.515
	IFA (Indirect Food Additive)	All	Component of adhesives	GMP (Good Manufacturing Practices)	175.115
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3411
Polysorbate 41	IFA	All	Component of adhesives	GMP	175.115
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3411
Polysorbate 61	DFA	All	Synthetic flavoring	Minimum required for intended effect	172.515
	DFA	Whipped edible oil topping	Emulsifier	≤1.4% (≤1.77% if with sorbitan monostearate)	172.836
	DFA	Cakes and cake mixes	Emulsifier	≤1.46%	172.836
	DFA	Nonstandardized confectionery coatings and cacao products	Emulsifier	≤1.5%	172.836
	DFA	Cake icings and cake fillings	Emulsifier	≤1.46%	172.836
	DFA	Sugar-type confection coating	Opacifier	≤1.2%	172.836
	DFA	Nonstandardized dressings	Emulsifier	≤1.3%	172.836

TABLE 8. (Continued.)

Ingredient	*Category*	*Food Type*	*Purpose*	*Usage Limit*	*1979 21 CFR Code*
Polysorbate 61 continued	DFA	Shortenings and edible oils	Emulsifier	≤1.1% (may be exceeded if properly labeled)	172.836
	DFA	Vegetable fat-water coffee creamers	Emulsifier	≤1.4%	172.836
	DFA	Alcoholic drink mixes	Foaming agent	≤4.5%	172.836
	DFA	Yeast-leavened bakery products	Dough conditioner	≤1.5%	172.836
	DFA	White mineral oil and/or petrolatum wax for protective coating on raw fruits and vegetables	Emulsifier	GMP	172.836
	DFA	Gelatin desserts and mixes	Dispersing agent	≤0.5%	172.836
	DFA	Chocolate flavored syrups	Emulsifier	≤0.5%	172.836
	DFA	Natural and artificial colors in soft drinks, gelatin desserts, and pudding mixes	Surfactant and wetting agent	GMP	172.836 (as amended 9/5/80)
	IFA	All	Component of adhesives	GMP	175.105
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400
Polysorbate 65	DFA	Frozen desserts	Emulsifier	≤0.1%	172.838
	DFA	Cakes and cake mixes	Emulsifier	≤0.32%	172.838
	DFA	Whipped edible oil topping	Emulsifier	≤0.4%	172.838

	DFA	Vegetable fat-water coffee creamers	Emulsifier	≤0.4%	172.838
	DFA	Cake icings and cake fillings	Emulsifier	≤0.32%	172.838
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400
Polysorbate 80	DFA	All	Synthetic flavoring	Minimum required for intended effect	172.515
	DFA	Frozen desserts	Emulsifier	≤0.1%	172.840
	DFA	Yeast-defoamer	Defoaming agent	≤0.4% (≤4 ppm in finished food)	172.840
	DFA	Pickles and pickle products	Solubilizing and dispersing agent	500 ppm	172.840
	DFA	Vitamin-mineral preparations containing calcium caseinate but not fat-soluble vitamins	Solubilizing and dispersing agent	≤175 mg/day (based on recommended daily dose)	172.840
	DFA	Vitamin-mineral preparations containing fat-soluble vitamins but not calcium caseinate	Solubilizing and dispersing agent	≤300 mg/day (based on recommended daily dose)	172.840
	DFA	Vitamin-mineral preparations containing both calcium caseinate and fat-soluble vitamins	Solubilizing and dispersing agent	≤475 mg/day (based on recommended daily dose)	172.840
	DFA	Sodium chloride crystals	Surfactant	≤10 ppm	172.840
	DFA	Special dietary foods	Emulsifier	≤360 mg/day	172.840
	DFA	Dill oil in canned spiced green beans	Solubilizing and dispersing agent	≤30 ppm	172.840

TABLE 8. (Continued.)

Ingredient	*Category*	*Food Type*	*Purpose*	*Usage Limit*	*1979 21 CFR Code*
Polysorbate 80 continued	DFA	Shortenings and edible oils	Emulsifier	≤1.0 percent (may be exceeded if properly labeled)	172.840
	DFA	Whipped edible oil topping	Emulsifier	≤0.4%	172.840
	DFA	Scald water for poultry defeathering	Wetting agent	≤0.0175%	172.840
	DFA	Gelatin desserts and mixes	Dispersing agent	≤0.082%	172.840
	DFA	Residues from herbicides and plant growth regulators	Adjuvant	No tolerance requirement	172.840
	DFA	Creaming mixture for cottage cheese	Defoaming agent	≤0.008%	172.840
	DFA	Natural and artificial colors used in barbecue sauce	Surfactant and wetting agent	GMP	172.840 (as amended 9/5/80)
	IFA	All	Component of adhesives	GMP	175.105
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400
Polysorbate 85	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400

administration was: expired CO_2, 80 percent; carcass, 12 percent; unabsorbed from the gastrointestinal tract, 4 percent; urine, 2.5 percent; and liver, 1.2 percent.

The polyoxyethylene sorbitan moiety left after hydrolysis of the ester is poorly absorbed from the rat's gastrointestinal tract. In one study with a radioactive carbon label in the polyoxyethylene portion of Polysorbate 20, 90 percent was excreted in the feces and 8 percent in the urine. No radioactivity was found in the liver, carcass, or expired CO_2.[73] When the sorbitol moiety of Polysorbate 80 was labeled, 91 percent of the radioactivity was recovered in the feces, 2.1 percent in the urine, 1.6 percent in the carcass, and none in expired CO_2, liver, kidney, spleen, adrenals, brain, gonads, or fat.[72]

After intravenous injection into rats, the ester bond is hydrolyzed by blood lipases. When Polysorbate 20 was injected into rats, the labeled lauric acid moiety was metabolized and appeared mostly as expired CO_2. The polyoxyethylene moiety was not catabolized, since no radioactivity was recovered as CO_2 when this portion of the molecule was labeled. Most of the labeled polyoxyethylene appeared in the urine, but some was present in the feces, indicating biliary excretion.[72] After intravenous injection of Polysorbate 20 into rats in another study, the distribution of the labeled lauric acid moiety was: expired CO_2, 68 percent; carcass, 22 percent; urine, 5 percent; feces and gastrointestinal contents, 2.5 percent; and liver 0.7 percent. The distribution of the labeled polyoxyethylene moiety was: urine, 83 percent; feces, 11 percent; carcass, 2 percent; liver, 0.15 percent; and expired CO_2 nil.[73]

Clinical tests have shown that essentially the same pattern of metabolism is followed as in the rat. The ethoxyl values of the urine and stools of four subjects fed 4.5 g of Polysorbate 80 per day were determined to ascertain the amount of the polyoxyethylene portion excreted. The results showed that the polyoxyethylene fraction was excreted quantitatively; approximately 95 percent was excreted in the feces and 5 percent in the urine. Since there were no polyoxyethylenated fatty acids detected in the urine, it was concluded that the polyoxyethylene moiety in the urine represented polyoxyethylene sorbitan and not the parent ester. The Polysorbate 80 was most likely hydrolyzed by pancreatic lipase, with the liberated oleic acid following the normal metabolic pathways of unsaturated fatty acids. The source of the polyoxyethylene in the urine was that portion absorbed from the upper intestinal tract following hydrolysis of the ester bonds. Since there was no carryover of the polyoxyethylene sorbitan in the urine during the postmedication control periods, there was no storage of this moiety in the body.[74] The possibility of oxalic acid poisoning from the polyoxyethylene component would seem negligible in light of its quantitative excretion. Urinary studies for oxalate content in patients taking oral Polysorbate 80 indicated no increase in oxaluria.[75]

General Effects

Biochemistry

The Polysorbates have been shown to activate or inhibit the numerous in vitro biochemical reactions listed in Table 9. The particular effect of Polysorbate 80 on one in vitro reaction listed in Table 9 was most likely due to its surface active properties alone.[76] The mechanisms of activation or inhibition include changes in substrate or enzyme dispersal and availability and are sometimes dependent

TABLE 9. Specific in Vitro Biochemical Effects of the Polysorbates.

Polysorbate	*Effect*	*Enzyme or Reaction*	*Determination Made in*	*Reference*
80	Inhibited	Dimethylnitrosamine demethylase, and ethylmorphine demethylase	Rat hepatic microsome	79
80	Inhibited	Dimethylnitrosamine demethylase	Hepatic extract	78
80	Inhibited (weakly)	Dichloro-*p*-nitro-anisole O-demethylase	Rat hepatic microsome	80
80	Inhibited	Biphenyl 2- and 4-hydroxylation	Hamster hepatic microsome	81
80	Inhibited	Biphenyl 4-hydroxylase	Rabbit hepatic microsome	82
80	Inhibited	Aniline 4-hydroxylase	Rat hepatic microsome	79
80	Activated	Aniline 4-hydroxylase	Hamster hepatic microsome	81
80	Inhibited	Glucose-6-phosphate phosphohydrolase	Rat hepatic microsome	83
80	Inhibited	Esterification of cholesterol by palmitoyl coenzyme A	Rabbit adrenal microsome	84
80	Concentration-dependent activation and inhibition	Palmityl coenzyme A carnitine O-palmityltransferase	Purified enzyme	85
80	Inhibited	Ribonuclease and deoxyribonuclease	Rat hepatic lysosome	86
80	Inhibited	Acid phosphatase	Rat hepatic lysosome	86
80	Activated	4-Hydroxybenzoate: polyprenyl transferase	Aged rat and guinea pig liver mitochondria	87
80	Activated	Acetylcholinesterase	Rabbit and ox caudate nuclei homogenates	76
80	Activated at low concentration and inhibited at high concentration of Polysorbate	Na/K ATPase and Mg ATPase	Isolated brush border membrane of rat intestinal epithelium	88
80	Accelerated	Photodegradation of flavin mononucleotide	Kinetic study on purified FMN	89
60	Inhibited	Serine dehydratase	Rat hepatic microsome	90
40	Inhibited	Serine dehydratase	Rat hepatic microsome	90
20	Inhibited	Biphenyl 4-hydroxylase	Rabbit hepatic microsome	82
20	Inhibited	Glucose-6-phosphate phosphohydrolase	Rat hepatic microsome	83

20	Inhibited	Esterification of cholesterol by palmitoyl coenzyme A	Rabbit adrenal microsome	84
20	Concentration-dependent activation and inhibition	Palmityl coenzyme A carnitine o-palmityl transferase	Purified enzyme	85
20	Activated low concentration and inhibited at high concentration of Polysorbate	Na/K ATPase and Mg ATPase	Isolated brush border of rat intestinal epithelium	88
20	Inhibited	Cholesterol oxidase	Purified enzyme	77
20	Inhibited	Diacylglycerol choline and ethanolamine phosphotransferases	Rat fat cell microsome	91
20	Activated	Alkaline phosphatase	Dissolved calf thymus plasma membranes	92
20	Activated	Alkaline phosphatase	Solubilized bovine milk fat globule membrane	93
20	Inhibited	Acid phosphatase	Solubilized bovine milk fat globule membrane	93
20	Activated	Phosphodiesterase I and gamma-glutamyl transpeptidase	Solubilized bovine milk fat globule membrane	93
20	Inhibited	Lipoxygenase	Human and rabbit washed platelets	94
20	Activated	5′ Adenylic acid deaminase	Particulate fraction from rat brain and liver	95
20	Inhibited	Potassium phosphatase and Na/K ATPase	Purified enzyme from dog kidney	96

on Polysorbate concentration.[77] However, these in vitro actions may not be necessarily indicative of the in vivo effects of the Polysorbates. Whereas Polysorbate 80 inhibited rat hepatic dimethylnitrosamine demethylase in vitro, it slightly induced the enzyme when tested in vivo.[78]

Cellular Metabolism

The Polysorbates have been shown to influence directly or indirectly the processes of DNA replication, transcription, and translation. Polysorbate 80 in the incubation medium of isolated nuclei from hamster kidney cells stimulated the synthesis of DNA to 150 percent of controls.[97] A single intraperitoneal injection of Polysorbate 40 at 600 to 800 mg/kg into albino mice increased short-term ^{32}P labeling of mRNA in the liver and increased the turnover rates of both rRNA and mRNA in this organ over a period of 24 hours. The activity of DNA-dependent RNA polymerases in the nuclei of treated animals was also stimulated up to 60 percent over that of control nuclei.[98] When tested in vitro on normal embryonic chicken, hamster, or murine or human renal cells, Polysorbate 80 stimulated the incorporation of labeled amino acids into the acid-soluble fraction of the cell suspensions. It was suggested that the Polysorbate might affect a system located in the cell membrane that controls intracellular protein synthesis.[99]

The Polysorbates affect the processes of cellular respiration in a dose-dependent manner. Inhibition of oxygen consumption in rat small intestine epithelial cells by the Polysorbates was in the order Polysorbate 20 > Polysorbate 80 > Polysorbate 60. Lactic acid formation was increased by low concentrations and decreased by high concentrations of Polysorbates 20 and 80.[100] At low concentrations and in the absence of exogenous adenosine diphosphate, Polysorbate 80 provoked a threefold to fourfold increase of in vitro mitochondrial respiration. At higher concentrations, Polysorbate 80 slightly inhibited mitochondrial oxidation; it progressively decreased phosphorylating capacity with increasing concentration.[101]

The effects of the Polysorbates on mitochondrial respiration seem to reflect a direct action on the ferrocytochrome C step in the electron transport chain of oxidative phosphorylation. The addition of Polysorbate 80 to suspensions of liver mitochondria from normal and tumor-bearing rats led to an increase in the activity of succinate-cytochrome C reductase in one study[102] and an increase in the activity of cytochrome C oxidase in another.[103] The activation of cytochrome C oxidase by the Polysorbates is well documented. They have been shown to reversibly convert cytochrome oxidase from an inactive to an active coupling state by providing a suitable environment for the most active conformational state.[104-106] This effect was pH independent when demonstrated with purified cytochrome oxidase.[103]

Biological Membranes

Due to the surface active properties of the Polysorbates and the physicochemical nature of cellular membrane bilayers, the Polysorbates can affect the structure and function of biological membranes. Extensive studies have been made on the action of nonionic surfactants using test systems ranging from artificial lipid monolayers to natural multilayer epithelia.

Whether the effect the Polysorbates have on membranes is solely a function of their hydrophile–lipophile balance or whether the specific structure of the

Polysorbate molecule may also determine its biological activity is unclear. In an effort to answer this question, the hemolytic action of Polysorbates 20, 40, 60, and 80 on human erythrocytes was measured and correlated with the physical properties of the surfactants. The hemolytic power depended on the mutual effect of the hydrophobic and hydrophilic fragments of the Polysorbate molecule and did not depend on the hydrophile–lipophile balance as such. It was suggested that the role of the polyoxyethylene moiety in the action of the Polysorbates on membranes lies with its effect on the relative lipophilicity of the compound. The polyoxyethylene fragment may have also interacted with surface components when the molecule was adsorbed onto the membrane. It was concluded that the lysis of erythrocytes by the Polysorbates was caused not by the destruction of the membrane but by some rearrangement of the membrane structure accompanying adsorption of the surfactant.[107]

Surfactants are well known to generally increase the permeability of skin; although the degree of permeability to different substances varies greatly, the rate of water desorption could provide an indication of the skin's overall barrier function.[67,108] An in vitro method showed that excised rabbit skin treated with petrolatum containing 10 percent Polysorbate 85 had a greater transepidermal water desorption rate than skin treated with petrolatum alone. It was concluded in this study that Polysorbate 85 affected membrane structure, thereby increasing permeability.[67] An in vivo method for monitoring water desorption from human forearms confirmed the increase in epidermal permeability caused by Polysorbate 85.[108] In another test of epithelial water permeability, it was observed that the injection of Polysorbate 80 in normal saline into the anterior chambers of rabbits' eyes promptly and regularly produced corneal edema, which was accompanied by marked corneal endothelial cytolysis and increased limbal vascular permeability.[109] Polysorbate 80 also alternatively increased and decreased the osmotic resistance of erythrocytes, the nature of the effect depending on surfactant concentration. The response to Polysorbate 80 was presumed to be due to its influence on the erythrocyte plasma membrane.[110]

Closely related to the maintenance of osmotic equilibria are the transmembrane diffusion and active transport of electrolytes. The normal function of all cell types is dependent on proper ionic permeability characteristics of the plasma membrane, but changes in the ability to control ion exchange are most prominently seen in such cell types as neurons, secretory cells, and others whose primary function depends directly on an electrical potential difference across the membrane. In a study on artificial membranes, Polysorbates 20 and 60 penetrated a lecithin monolayer and produced blockade of charge transfer through the interface.[111] When added to a bimolecular oxidized cholesterol membrane, these Polysorbates increased membrane resistance and decreased its stability. It was suggested that the Polysorbates (1) lowered the conductance of the membrane by making it less permeable to charged molecules and (2) decreased membrane stability by becoming incorporated into the membrane structure.[112]

The electrophysiologic effect of the Polysorbates on several natural tissues has also been studied. Addition of Polysorbate 80 to diluted rat blood, at concentrations having no hemolytic effect, produced an increase in the transmembrane electrical potential of the erythrocytes.[113] In the isolated rat jejunum, Polysorbate 80 increased the transmural potential differences by 20 to 34 percent and short-circuit currents by 66 to 112 percent. It decreased net tissue resistance by 19 to 30 percent.[114]

The Polysorbates also influence the transport of larger molecules across membranes and thus can affect drug activity and toxicity. Toxic synergy in golden hamsters was revealed by Polysorbate-type surface active substances when used as emulsifiers and stabilizers in foodstuffs and food colorants.[115] In contrast, Polysorbate 80 decreased the acute oral toxicity in mice of tetracycline, norsulfazole, theophylline, tubazid, procainamide, amidopyrine, and pentobarbital.[116]

Other studies support the concept that the influence of the Polysorbates on the permeability of some larger molecules is due to a change in the membrane. Polysorbate 80 increased the solubilized concentration of butylparaben, yet it decreased percutaneous penetration of the preservative through in vitro guinea pig skin.[117] Although Polysorbate 80 caused a general increase in the membrane permeability of in situ rat intestine, the absorption of *p*-aminobenzoic acid was significantly inhibited. The specific inhibitory effect in this study was attributed to the solubilization and then release of membrane proteins, which are responsible for the absorption of *p*-aminobenzoic acid.[118] Support for the theory that the Polysorbates influence the absorption of some drugs by interacting with membrane proteins also comes from the observation that Polysorbate 80 did not affect the permeability of rat small intestine to benzocaine and sulfisoxazole, which are thought to be absorbed by a passive diffusion mechanism independent of membrane proteins.[119]

The potential also exists for the major site of action for Polysorbate-induced changes in epithelial and tissue permeability to be the intercellular space instead of the cellular membrane. In two studies on the mechanism of the inhibitory effect of Polysorbate 80 on the intramuscular absorption of drugs, the inhibition of absorption could not be attributed to a direct or indirect effect on the capillary wall. It was concluded that the effect was mainly due to its influence on the extracellular space and the permeability of connective tissue.[120,121]

Neuromuscular Systems

The Polysorbates produce various, seemingly disparate effects in neuromuscular systems. Polysorbate 80 stimulated colonic motility in anesthetized rabbits.[122] In contrast, it depressed in vitro small intestinal smooth muscle cell contraction when this function was assessed by measuring the contractile activity of electrically stimulated guinea pig ileum.[123] Polysorbates 20, 40, 60, 80, 81, and 85 inhibited the spasmogenic effect of acetylcholine, barium chloride, and histamine when tested on isolated guinea pig duodenum. This spasmolytic activity of the Polysorbates was of both musculotropic and neurotropic origin; the musculotropic spasmolytic activity was similar to that of papaverine, an alkaloid with smooth muscle relaxant properties.[124] In another study, Polysorbates 20 and 80 caused 30 to 100 percent inhibition of the effect of 11 different spasmogenic compounds on the isolated guinea pig ileum. They also inhibited the spasmogenic effects of some of these compounds when tested on isolated rabbit jejunum, uteri of estrus-induced rats, and isolated guinea pig seminal vesicles. They did not reduce the inhibitory effect of epinephrine and isoproterenol on the rabbit jejunum.[125]

Lipid Metabolism

Polysorbates 20 and 80 markedly stimulated secretion of bile when injected intraduodenally at 1 ml/kg into rats.[126] Polysorbate 80 produced a weak stimulation of pancreatic enzyme secretion under the same conditions.[127] Surface ac-

tive agents are also thought to produce micellar solutions in the intestinal lumen in much the same way as bile salts, thus enhancing the uptake of fatty acids.[128] Polysorbate 80 acted synergistically with low concentrations of bile salts in the anesthetized rat, increasing the absorption and esterification of oleic acid.[129] When fed to rats for 1 week at 0.1 percent and 1 percent of the diet, Polysorbate 80 augmented the absorption of fats when they were present at 10 to 33 percent of the diet; this effect was not seen when fats comprised less than 7 percent of the diet.[130]

Polysorbate 80 and other surface active agents have been reported to reduce the severity of atherosclerosis in rabbits. It inhibited and reversed the in vitro insoluble complex formation between low-density lipoproteins from cholesterol-fed rabbits and such sulfated polysaccharides as heparitin sulfate and dextran sulfate. The binding of low-density lipoproteins to collagen and elastin was also inhibited, as were platelet adhesiveness and the production of aortic atheromas.[131,132] These effects were not observed in similar experiments with pigeons.[133]

Hemodynamics

There have been several studies on the hemodynamic effects of the Polysorbates. The effects of the Polysorbates vary from species to species, with a general trend toward a depression of cardiac output. When a 5 percent aqueous solution of Polysorbate 80 was injected intravenously in doses of 1 ml/kg into cats, rabbits, and rhesus monkeys, there was a slight and transient fall in blood pressure; dogs exhibited a prolonged depressor response. This effect was never elicited by oral administration of the Polysorbates, and the depressor reaction has not been obtained in man.[134]

Polysorbate 80 showed a coronary vasodilatory effect and increased the cardiac output in isolated guinea pig and rabbit hearts when present at 2.4 mg/L in the perfusion fluid. If the concentration of Polysorbate 80 was increased stepwise from 0.7 to 4.0 mg/L, there was a dose-related increase in the coronary output. At higher doses, a slight decrease in the amplitude of contraction and an increase in the heart rate were seen.[135] In another study, the cardiovascular effects of two concentrations of Polysorbate 80 were examined in dogs. A 10 percent dextran solution with 0.05 percent Polysorbate 80 injected into the left atrium caused systemic and/or cardiac alterations in all four dogs studied. Reactions consisted of a reduction in cardiac dimensions with or without hypotension and tachycardia. Administration of a lower concentration of Polysorbate 80 (0.01 percent) induced reactions in 6 of 14 dogs. Subsequent administration of this concentration on the same day rarely induced adverse reactions.[136] When injected intravenously into dogs, Polysorbate 20 initially increased cardiac output, coronary sinus flow, and heart rate, with maintenance of systemic pressure. After 3 to 4 minutes, this was followed by greatly decreased cardiac output, vascular pressure, and coronary flow, with a continuance of tachycardia.[137]

The intravenous infusion of 5 ml of Polysorbate 20 at a rate of 0.2 ml/15 sec into 14 intact and 8 splenectomized dogs evoked anaphylactic-like symptoms that may have been mediated by endogenous histamine release. Histamine release was indicated by skin changes, tachyphylaxis, and protection by antihistamines. Other changes included decreased arterial pressure, heart rate, and plasma volume and increased respiratory rate, lymph flow, and hematocrit.[138] The Polysorbates have been shown to be nonspecific histamine releasers,[139,140] and their

hemodynamic effects are entirely compatible with histamine release. The later response of decreased output, hypotension, and tachycardia is almost indistinguishable from that found in endotoxic or hemorrhagic shock.[137]

When 10 percent aqueous Polysorbate 85 was applied repeatedly to the skin of guinea pigs for 5 hours, it caused an increase in cutaneous bloodflow. Polysorbate 20 had no effect, regardless of the duration of its application.[141]

Immune System

Mice given 0.3 ml intraperitoneal injections of 25 percent Polysorbate 80 in saline solution prior to immunization with ovalbumin absorbed to $Al(OH)_3$ demonstrated no primary IgE response, indicating that Polysorbate 80 inhibited this response.[142] Prior intraperitoneal injection of 0.3 ml of 25 percent Polysorbate 80 in saline also caused a total suppression of the primary IgG response and a partial suppression of the passive hemagglutination response to ovalbumin in mice. Jerne plaque assays showed significant suppression of the primary antibody response. Contact sensitivity to oxazolone was not suppressed.[143]

Polysorbate 80 inactivated the rabbit serum antibody to crystalline bovine serum albumin in vitro when added to bentonite flocculation suspensions at concentrations used in immunologic studies (0.01 to 5.0 percent). It was thought either to inactivate irreversibly the antibody bound to the bentonite or to remain adsorbed to the bentonite in some manner so as to cause continued inhibition.[144]

Mutagenesis

Polysorbate 80 was tested in the micronucleus and Ames tests as part of an evaluation of the micronucleus test as a short-term mutagenicity assay for the identification of potential carcinogens. Polysorbate 80 was negative in both assays for mutagenicity. Metabolic activation was not specified in the Ames test; the micronucleus test is an in vivo method, which implies some degree of metabolism.[145]

Carcinogenesis

Table 10 summarizes several bioassays for carcinogenesis that have been performed on the Polysorbates. Oral studies showed no evidence for carcinogenicity by this route. Upon topical application to the skin, the Polysorbates did produce skin tumors in some studies, mostly benign dermal tumors with a tendency to regression. After reviewing many of these studies and conducting multiple experiments, Setala[146] concluded that the Polysorbates are not carcinogenic when applied to the skin. Several studies have also investigated the production of tumors after subcutaneous injection, with variable results. In one such study by Grasso et al.,[147] repeated subcutaneous injections of 2 ml of a 6 percent aqueous solution of Polysorbate 80 three times weekly for 40 weeks induced local sarcomas in 11/17 rats. As shown by concurrent tests with the food additives Blue VRS, Patent Blue V, calcium cyclamate, and sodium cyclamate, the induction of sarcomas and the tissue reaction at the site of repeated subcutaneous injections were predictable and dependent on the surface activity and calcium ion concentration of the injected solution. The authors concluded that such local sarcomas in the rat, produced by long-continued repeated injections into the same subcutaneous site, do not constitute a valid index of chemical carcinogenicity for purposes of safety evaluation.

TABLE 10. Bioassays for Carcinogenesis.

Ingredient	*Reference*	*Animal*	*Preparation and Dose*	*Route or Site*	*Tumors*	*Survival*	*Duration of Experiment*
Polysorbate 20	150	14 rats	25% of diet	PO	0	6/14	59 days
	151	36 hamsters	5% of diet	PO	0	22/36	68 days
	152	10 hamsters	5% in bread diet	PO	0	9/10	39 weeks
		10 hamsters	10% in bread diet	PO	0	2/10	39 weeks
		10 hamsters	15% in bread diet	PO	0	1/10	39 weeks
		10 rats	25% in synthetic diet	PO	0	9/10	21 weeks
	153	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor at 36 weeks	30/50 at 36 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks
	155	mice	0.18 mole. conc. twice daily, 6 days/week	Skin	0	100%	30 days
		mice	0.18 mole. conc. once daily, 6 days/week	Skin	0	100%	30 days
Polysorbate 40	153	50 mice	100% once daily, 6 days/week	Skin	2 with benign dermal tumors, first at 24 weeks	43/50 at 24 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor	43/50	24 weeks
Polysorbate 60	156	3 beagles	10% of diet	PO	0	3/3	1 year
		36 hamsters	5% of diet	PO	0	33/36	13 months
		36 hamsters	1% of diet	PO	0	36/36	13 months
		24 mice	2.5, 5, or 10% of diet	PO	0		4 months
	157	6 hamsters	0.05 ml of 1% aq. sol. once weekly	Intra-tracheal	0	3/6 at 9 months	15 months
	153	50 mice	100% once daily, 6 days/week	Skin	5 with 6 benign dermal tumors, first at 16 weeks. Liquid paraffin control gave no tumors	45/50 at 16 weeks	52 weeks
	158	3 rabbits	2.5% in olive oil once daily	Skin	0		116 days
	154	50 mice	100% once daily, 6 days/week	Skin	2 with benign dermal tumor	45/50	24 weeks
	159	30 mice	100% once daily, 6 days/week	Skin	1 papilloma		16 months

TABLE 10. (Continued.)

Ingredient	*Reference*	*Animal*	*Preparation and Dose*	*Route or Site*	*Tumors*	*Survival*	*Duration of Experiment*
Polysorbate 60 (cont'd.)	160	60 rats	60 mg undiluted 6 times/week	Skin	10 with 23 epithelial tumors	42/60	40 weeks
		60 mice	60 mg undiluted 6 times/week	Skin	12 with 67 epithelial tumors and 2 carcinomas	29/60	55 weeks
	155	Mice	0.18 mole. conc. in Carbowax 400, 6 times/week	Skin	0		30 days
		Mice	As above, 6 times/week	Skin	0	100%	30 days
		Mice	As above, 12 times/week	Skin	0	100%	30 days
		Mice	As above, 12 times/week	Skin	0	100%	30 days
		Mice	As above, 6 times/week	Skin	0	100%	30 days
	161	48 mice	100%, 60 mg once daily, 6 days/week	Skin	14 with 30 benign dermal tumors (20 regressed)	41/48 at 35 weeks, 14/48 at 60 weeks	70 weeks
		54 mice	As above	Skin	12 with 17 benign dermal tumors (11 regressed)	25/54 at 25 weeks, 9/54 at 60 weeks	70 weeks,
		60 mice	100%, 2 drops daily	Skin	22 with 202 benign dermal tumors, 5 squamous cell carcinomas, and 1 basal cell carcinoma	10/60	60–82 weeks
		60 mice (M + F)	100%, 60 mg twice weekly	Skin	19 with 91 benign dermal tumors, 1 squamous cell carcinoma, and 1 dermal fibrosarcoma	20/60 at 60 weeks	75–80 weeks
		30 mice (F)	100%, 30 mg twice weekly	Skin	2 with 2 benign dermal tumors	22/30 at 50 weeks	50 weeks
		30 mice (F)	100%, 30 mg once daily, 6 days/week	Skin	1 benign dermal tumor, 1 squamous cell carcinoma	15/30 at 50 weeks	50 weeks
	162	50 mice (tumor-susceptible strain)	0.18 mole. aq. sol.	Skin	Local skin tumors beginning at 7 weeks	1	7 weeks

163	30 mice	100% once daily	Skin	16 with 150 epidermal tumors (114 regressions) and 3 squamous cell carcinomas	10/30	60 weeks
	30 mice	As above	Skin	6 with 52 epidermal tumors (19 regressions) and 1 squamous cell carcinoma	7/30 at 40 weeks	60 weeks
	30 mice	100% twice weekly	Skin	9 with 46 epidermal tumors (32 regressions) and 1 squamous cell carcinoma	11/30	60 weeks
	30 mice	As above	Skin	10 with 44 epidermal tumors (32 regressions)	8/30	60 weeks
164	50 mice (F)	100% once daily, 6 days/week	Skin	1 skin tumor in neck region at 22 weeks	50/50	32 weeks
	40 mice (M)	As above	Skin	1 skin tumor in handling region at 16 weeks	40/40	32 weeks
	50 mice (F; tumor-susceptible CFW strain)	As above	Skin	4 with tumors in neck region and 9 animals with 13 tumors in handling region	49/50	32 weeks
	40 mice (M; tumor-susceptible CFW strain)	As above	Skin	11 with tumors in neck region and 15 animals with 25 tumors in handling region	40/40	32 weeks
	50 mice (F; tumor-susceptible CF-1 strain)	As above	Skin	2 with tumors in neck region and 14 animals with 24 tumors in handling region	50/50	32 weeks
	40 mice (M; tumor-susceptible CF-1 strain)	As above	Skin	1 skin tumor in neck region and 15 animals with 22 tumors in handling region	39/40	32 weeks
165	50 mice (F)	0.06 ml of 25% aq. sol. twice weekly	Skin	1 skin tumor with regression, 4 malignant lymphomas, 2 mammilary adenocarcinomas, 8 lung adenomas	31/50	60 weeks

TABLE 10. (Continued.)

Ingredient	*Reference*	*Animal*	*Preparation and Dose*	*Route or Site*	*Tumors*	*Survival*	*Duration of Experiment*
Polysorbate 60 (cont'd)		19 mice (M)	As above	Skin	3 skin tumors in 1 mouse with 1 regression and 6 lung adenomas	8/19	60 weeks
	166	20 mice	100%, 0.1 ml twice weekly	Skin	1 skin tumor with regression		19 weeks
	167	30 mice	100% twice weekly	Skin	0	25/30	20 weeks
	168	30 rats	1 ml of 6% aq. sol. weekly	SC	5 with injection site fibrosarcomas and 3 introabdominal lympho-sarcomas (probably spontaneous)		73 weeks
	169	24 rats	5 ml/kg body wt of a 6% aq. sol. once weekly × 28	SC	No subcutaneous tumors	All died or sacrificed	2 years
		24 rats	As above but × 16 weeks		No subcutaneous tumors	All died or sacrificed	2 years
	170	Mice	100% once weekly × 4	SC	1% with subcutaneous sarcoma, 5% with hepatoma	Sacrificed at 1 year	1 year
	171	49 mice	1.1 mg in 0.1 ml saline on days 1 and 7; 2.2 mg in 0.2 ml on days 14 and 21; total dose 6.6 mg	SC	2 malignant lymphomas and 6 pulmonary tumors at 49–53 weeks; 1 thyroid adenoma at 50 weeks. 5/73 untreated controls with pulmonary tumors and 2/73 with single hepatomas	48/49 at 49 weeks	53 weeks
	172	49 mice	1% on days 1, 7, 14, and 21 of life	SC	No significant increase in tumor incidence as compared to controls		1 year
		22 mice	As above but 10% concentration	SC	As above		1 year
		10 mice	As above but 100% concentration	SC	As above		1 year

Polysorbate 65	155	Mice	0.18 mole. conc. in Carbowax 400 6 times weekly	Skin	0		30 days
Polysorbate 80	173	28 mice	100 mg daily in diet for 10 weeks	PO	0	25/28 at 30 weeks, 20/28 at 50 weeks	51 weeks
	174	25 ducks	25 ml of 1% saline sol. × 1	Intra-tracheal	0		400 days
	175	1 dog	30 ml of 1% saline sol × 1	Intrabron-chial	0	Died at 177 days	177 days
	153	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor at 43 weeks	34/50 at 43 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks
	176	10 mice	0.1 ml of 0.5% in saline, once weekly × 15	SC	1 pulmonary adenoma		270 days
	147	20 rats	2 ml of 6% aq. sol., 3 weeks	SC	11 with injection site fibrosarcomas (with varying degrees of differentiation), first at 27 weeks; some invasive but 0 metastasis; 5 successful transplants	17/20 at 27 weeks	40 weeks
Polysorbate 81	153	50 mice	100% once daily, 6 days/week	Skin	0	18/50	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks
Polysorbate 85	153	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor at 27 weeks	40/50 at 27 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks

The National Toxicology Program has begun a long-term oral carcinogenesis bioassay on Polysorbate 80. About 64 months are required from inception of the study to publication of the final report; prechronic testing began in February, 1981.[148]

Mixed cultures of epidermal and dermal cells from term fetuses of Balb/cAn mice were exposed to a medium containing Polysorbate 80 as a control for a study on the neoplastic changes caused by 50 μg/ml of 7,12-dimethylbenz[a]anthracene (DMBA) in the Polysorbate 80 medium. The cultures began to exhibit accelerated growth in vitro and an epithelioid morphology at 15 weeks in culture; similar changes were shown by DMBA plus Polysorbate 80 at 5 weeks. Injection of cells beginning approximately 21 weeks after treatment into syngeneic hosts gave rise to undifferentiated, apparently malignant epidermoid tumors. Polysorbate 80 did not produce the degree of in vivo malignancy nor the same types of changes in morphology and cell differentiation in culture as DMBA.[149]

Tumor Enhancement*

Numerous reports are available on tumor promotion and cocarcinogenesis by the Polysorbates. Tumor promotion and cocarcinogenesis have been demonstrated with a number of known carcinogenic agents and are such that succedent or concurrent administration of a Polysorbate produces increased yields of tumors. These data are summarized in Table 11.

The terms "tumor promotion" and "cocarcinogenesis" are used because the Polysorbates are neither genotoxic nor carcinogenic. Tumor promoters, by definition, enhance the effects of complete carcinogens when given subsequently; examples of tumor promoters include phorbol esters, phenol, anthralin, bile acids, tryptophan metabolites, and saccharin. Cocarcinogens enhance the effects of complete carcinogens when given at the same time; examples include phorbol esters, pyrene, catechol, ethanol, *n*-dodecane, and SO_2.[185]

A comprehensive review and discussion of tumor promotion and cocarcinogenesis by the Polysorbates was published by Setala in 1960.[146] As in that report, the term "tumor enhancement" is used in this review to encompass both concepts. Setala concluded that "tumor enhancement in mouse skin is a fully benign, slow, and at least partly reversible process. It merely provides the conditions for the manifestations of the actual carcinogenic process started by pretreatment with carcinogen, or, in susceptible mouse strains, even without it. Accordingly, the tumor-enhancing process as brought about by dipole-type agents is not part of the carcinogenic process itself." The reader is referred to this paper by Setala for a detailed review of the data and knowledge available in 1960. Following is a summary of the literature on the subject published after 1960.

When applied to the skin of mice, Polysorbates 40 and 60 have been shown to increase the mitotic index, shorten the mitotic cycle, and accelerate cell differentiation in the basal layer of the epidermis. This action seems to involve the selection of cells with a shortened G1 phase, which may be associated with a weakened response to the factors regulating cell proliferation. These changes in the kinetics of epidermal growth are also found when carcinogens alone are applied to the skin.[186-189] It has been hypothesized that the transformation of normal cells into cancer cells is determined by two factors: a genotoxic factor and a cell

*The term "tumor enhancement" encompasses both tumor promotion and cocarcinogenesis.[146]

TABLE 11. Tumor Promotion and Cocarcinogenesis.

Ingredient	*Reference*	*Animal*	*Route or Site*	*Carcinogenic Agent*	*Preparation and Dose*	*Results*	*Duration of Experiment*
Polysorbate 20	177	ICR Swiss mice	Skin	7, 12-dimethyl-benz[a]anthracene (DMBA)	0.125 mg DMBA followed by repeated appl. of 0.2 ml 0.3–3% Polysorbate 20	Weak tumor promotion	
	178	Wistar rats	PO	N-methyl-N′-nitro-N-nitrosoquanidine (MNNG)	50 mg/L MNNG in drinking water containing 0.4% Polysorbate 20	Increased incidence of glandular stomach tumors as compared to MNNG controls	26–30 weeks
Polysorbate 40	179	Wistar rats	PO	MNNG	50 mg/L MNNG in drinking water containing 0.4% Polysorbate 40	Increased incidence of glandular stomach tumors as compared to MNNG controls	26–30 weeks
Polysorbate 60	164	50 CF-1 mice (skin tumor-susceptible strain)	Skin	DMBA	100% Polysorbate 60 containing 0.001% DMBA, 3/week	Tumors in handling region; cocarcinogenesis not demonstrated	13 weeks
		50 CF-1 mice (skin tumor-susceptible strain)	Skin	DMBA	As above, except 25% aqueous Polysorbate 60	As above	13 weeks
	166	Swiss mice	Skin	DMBA	0.1 ml 100% Polysorbate 60, 2 weeks, preceded 1 week by single application of 0.1 ml 1.5% DMBA in Nujol	Tumor promotion as compared to DMBA controls	19 weeks
	167	Swiss mice	Skin	DMBA	As above, except DMBA in mineral oil	As above	20 weeks
	180	Golden Syrian hamsters	Cheek pouch	DMBA	0.5% DMBA in undiluted Polysorbate 60, 3/week until tumor appearance	Shorter latency of cheek pouch tumors than with DMBA control	58 days
		Golden Syrian hamsters	Cheek pouch	DMBA	As above, except 3/week × 5	As above	139 days
		Golden Syrian hamsters	Cheek pouch	DMBA	As above, except 0.2% DMBA and 3/week until appearance of first tumor	As above	139 days

TABLE 11. (Continued.)

Ingredient	*Reference*	*Animal*	*Route or Site*	*Carcinogenic Agent*	*Preparation and Dose*	*Results*	*Duration of Experiment*
Polysorbate 60 (cont'd.)	181	SWR mice	Gastric intubation	3-methyl-chloanthrene (MCA)	0.1 ml undiluted Polysorbate 60 with 0.25% MCA, 2/week for life	Skin carcinomas in 14–34 weeks; MCA controls had no skin tumors	Life
		SWR mice	Gastric intubation	MCA	As above, except 1.0% MCA	As above	Life
		C57BL/6 mice	Gastric intubation	DMBA	0.15 ml undiluted Polysorbate 60 with 2% DMBA; 2/week for life	Benign skin tumors in 8–30 weeks; DMBA controls had no skin tumors	Life
		SWR mice	Gastric intubation	3,4-benz[a]-pyrene (BP)	0.1 ml undiluted Polysorbate 60 with 0.25% BP, 2/week for life	No skin tumors	Life
		SWR mice	Gastric intubation	BP	As above, except 1.0% BP	No skin tumors	Life
	181	C57BL/6 mice	Gastric intubation	BP	0.15 ml undiluted Polysorbate 60 with 2.0% BP, 2 weeks × 1 week	No skin tumors	Life

	182	Wistar rats	PO	MNNG	100 mg/L MNNG in drinking water containing 0.4% Polysorbate 60	Insignificant increase in incidence of glandular stomach tumors as compared to MNNG controls	18 weeks
	178	Wistar rats	PO	MNNG	50 mg/L MNNG in drinking water containing 0.4% Polysorbate 60	Increased incidence of glandular stomach tumors as compared to MNNG controls	26–30 weeks
	183	Wistar rats	PO	MNNG	100 mg/L MNNG in drinking water containing 0.4% Polysorbate 60	Insignificant increase in incidence of glandular stomach tumors as compared to MNNG controls	
	184	Rats, hamsters and dogs	PO	MNNG	50–83 mg/L MNNG in drinking water containing Polysorbate 60	Increased incidence of anaplastic stomach tumors as compared to MNNG controls	7–12 months
Polysorbate 80	177	ICR Swiss mice	Skin	DMBA	0.25 mg DMBA followed by repeated appl. of 0.2 ml 0.3–3% Polysorbate 80	Tumor promotion	—
	178	Wistar rats	PO	MNNG	50 mg/L MNNG in drinking water containing 0.4% Polysorbate 80	No increase in tumor incidences as compared to MNNG controls	26–30 weeks

proliferation/promotion factor. Both carcinogens and cocarcinogens produce epidermal hyperplasia, but only the carcinogens are genotoxic.[190] Tumor enhancement by the Polysorbates has been linked to epidermal hyperplasia in studies involving either skin painting with both the carcinogen and Polysorbate or gastric administration of the carcinogen followed by application of the Polysorbate to the skin. Other tumor promoters have also been tested under the same conditions in some studies, and it was found that the promoting activity of the particular agent is directly related to its ability to induce epidermal hyperplasia.[167,190-193] One study showed that Polysorbate 20 does not produce such epidermal hyperplasia.[194]

A second possible mechanism for tumor enhancement by the Polysorbates is inhibition of DNA repair. A variety of tumor enhancers has been examined for their effects on DNA excision repair by following the incorporation of tritiated thymidine into the DNA of cell suspensions damaged by ultraviolet, neutron, or gamma-ray irradiation. Hydroxyurea was included in these studies to produce concomitant suppression of ordinary semiconservative DNA replication. These in vitro assay systems used suspensions of mouse spleen cells, rat blood cells, or normal human lymphocytes. Polysorbate 80 produced a concentration-dependent suppression of DNA excision repair in every study, with 50 percent inhibition evident at concentrations as low as 0.002 percent.[195-200] Another study also demonstrated an inhibition of semiconservative DNA synthesis.[201]

A third possible mechanism of tumor enhancement by the Polysorbates is that of an effect on biological membranes. The important factor may be the effect the Polysorbates have on lipid integrity[177] and, more specifically, lysosomal membranes.[202] Alternatively, the increase in plasma membrane permeability caused by the Polysorbates may initiate RNA and protein synthesis in the G1 phase of the tumor cells and, in this way, cause the initiation of cell division.[203] This mechanism would coordinate well with that of cellular hyperplasia.

Other effects of the Polysorbates that have been linked to tumor enhancement include (1) facilitation of direct contact of a carcinogen with mucosal cell surfaces,[179] (2) induction of ornithine decarboxylase, a polyamine biosynthetic enzyme in mouse epidermis,[204] (3) inhibition of epidermal histidase activity, an enzyme found in normal epidermis but not in mouse squamous cell carcinoma,[205] and (4) enhancement of in vitro cell transformation and plaque formation by viruses.[206,207]

In addition, another effect of the Polysorbates on cell membranes manifested by a change in cellular adhesiveness and volume may have important consequences for tumor cell metastases. The incidence of experimental metastases after intravenous injection of Walker tumor cell suspensions into rats was increased by prior incubation with Polysorbate 80. This effect was attributed to an increase in cellular adhesiveness and volume.[208,209] In contrast, however, another study showed a decrease in human amniotic cell adhesion when incubated with Polysorbate 80.[210]

Sivak and Goyer[211] prepared an evaluation of the skin tumor enhancement potential of Polysorbates as used in cosmetic products for consideration in the safety review.

Tumor Growth Inhibition

Several studies have shown that the Polysorbates at higher concentrations also have tumor growth inhibition activity. In vitro tests with Polysorbates 20, 21,

40, 60, and/or 80 on mouse Ehrlich ascites carcinoma cells produced reversible alterations in cellular membranes,[212] inhibition of respiration,[213] increased sensitivity to hyperthermia,[214] and an unspecified cytotoxicity.[215] In vivo tests produced different results for different Polysorbates. Intraperitoneal injection of Polysorbate 80 into mice inoculated with Ehrlich ascites carcinoma cells or of Polysorbate 60 into rats inoculated with Morris hepatoma cells significantly reduced the formation and size of tumors and increased survival time of the animals.[216-219] One author concluded that the cytotoxicity of Polysorbate 80 for the tumor cells was related to the oleic acid component, since substitution of the polyoxyethylene sorbitan residue by diethanolamine did not eliminate the cytotoxic action.[219] On the other hand, Polysorbates 20 and 40 did not exhibit in vivo tumor growth inhibition activity when assayed in mice with Ehrlich ascites carcinomas.[215,220]

Other Physiologic Effects

The Polysorbates have been shown to produce other physiologic effects in biological assay systems. Polysorbate 80 reduced the size of litters when administered orally to rats at doses of approximately 0.8 to 3.0 g/kg.[221] At 0.01 percent in human serum, it decreased the binding of atropine sulfate to serum albumin.[222] Polysorbates 60 and 80 increased the incorporation of inorganic phosphate into rat pituitary glands and several other tissues by inducing the release of pituitary glands and several other tissues by inducing the release of pituitary corticotropin.[223] Polysorbate 20 decreased the plateletlike thromboplastic activity of bovine brain cephalin due to a change in the physical properties of the dispersion.[224] It also induced reticuloendothelial system (RES) effects in a pattern typical of many RES-activating materials; small doses stimulated and high doses depressed RES function. The low doses were thought to stimulate the RES by way of a surface active effect on RES cell membranes.[225]

Animal Toxicology

Oral Toxicity

Acute Studies

The acute oral toxicity of the Polysorbates has been reported in primary studies[134,226-232] and in reviews of published and unpublished literature.[233-236] The acute oral LD_{50} values for the Polysorbates are listed in Table 12. The doses tolerated by rodents in these studies show each of the Polysorbates to be relatively harmless by acute oral administration. Product formulations, each containing one of the Polysorbates at concentrations of 1.0 to 8.4 percent, have also been tested with similarly high LD_{50} values.[237-243]

Long-term Studies

Numerous long-term feeding studies have been carried out using a variety of animal species. Animals were fed Polysorbates at dietary levels of up to 25 percent, for periods of up to 2 years, and, in some cases, over multiple generations. Most of these studies included detailed clinical, gross pathologic, and histopathologic observations. One such study is reported below, and others are summarized in Table 13. After reviewing many of these studies, the FAO/WHO Committee on Food Additives[244] concluded that the Polysorbates cause no toxicological ef-

TABLE 12. Acute Oral Toxicity.

Ingredient	*Species*	*LD_{50}*	*Reference*
Polysorbate 20	Rat	>38.9 g/kg	233,234
	Rat	36.7 ml/kg	231
	Rat	>34.7 g/kg	233
	Rat	>30 ml/kg	226
	Rat	>5 g/kg	229
	Rat	>4.6 g/kg	228
	Mouse	>30 ml/kg	226
	Mouse	>25 g/kg	232
	Hamster	18.0 ml/kg	231
Polysorbate 21	Rat	>38.0 g/kg	233
	Rat	>33.8 g/kg	233,236
	Rat	>10 ml/kg	235
Polysorbate 40	Rat	>38.4 g/kg	233,234
	Rat	>34.2 g/kg	233,236
	Rat	>20 ml/kg	235
	Rat	>5 g/kg	230
Polysorbate 60	Rat	>38.0 g/kg	233,234
	Rat	>33.8 g/kg	233,236
	Rat	>20 g/kg	235
	Rat	>5 g/kg	227
Polysorbate 61	Rat	>39.8 g/kg	233,236
	Rat	>35.5 g/kg	233
	Rat	>8 g/kg	235
Polysorbate 65	Rat	>39.8 g/kg	233,234
	Rat	>35.5 g/kg	233
	Rat	>10 g/kg	235
Polysorbate 80	Rat	54.5 ml/kg	231
	Rat	>38.0 g/kg	233,234
	Rat	>33.8 g/kg	233
	Rat	>20 ml/kg	235
	Mouse	>25 g/kg	232
	Mouse	>20 ml/kg	134
Polysorbate 81	Rat	>36.6 g/kg	233,236
	Rat	>32.6 g/kg	233
	Rat	>20 ml/kg	235
Polysorbate 85	Rat	>36.4 g/kg	233,236
	Rat	>32.4 g/kg	233
	Rat	>20 ml/kg	235

fects at a level of 5 percent in the daily diet of test animals. Indeed, many species tolerated much greater quantities for extended periods of time.

A definitive long-term study was conducted by Oser and Oser[71,245-247] in which the effects of Polysorbates 60, 65, and 80 at dosage levels of 5, 10, and 20 percent in the diet of rats were observed for 2 years and over four successive generations. The rats were evaluated by various criteria, which can be summarized under the headings of growth, feeding efficiency, clinical observations, reproductive efficiency, hematology, urology, and histopathology. The 20 percent dosage level was chosen as one that "was expected to induce an adverse response." The most notable effect at this level was diarrhea; there were also some effects on postnatal survival, lactation efficiency, breeding activity, growth rate, and longevity. The 10 percent dosage level produced only diarrhea. The problems with diar-

TABLE 13. Subchronic and Chronic Oral Toxicity.

Ingredient	*Length of Study*	*Species*	*Number of Animals*	*Level of Polysorbate in Diet*	*Results*	*Reference*
Polysorbate 20	7 weeks	Chick	12	0.1%	No adverse effects	256
	7 weeks	Chick	12	1.0%	No adverse effects	
	7 weeks	Chick	12	2.0%	No adverse effects	
	8 weeks	Rat		3%	Slow weight gain attributed	257
	8 weeks	Rat		5%	to mild diarrhea; no gross abnormalities nor significant histopathological findings	
	10 weeks	Hamster	36	5%	High mortality, perhaps due	150,151
	10 weeks	Hamster	36	15%	to diarrhea	
	21 weeks	Rat	10	25%	1 fatality; significant gross and histopathological findings in bladder, kidney, spleen, and GI tract	231
	28–39 weeks	Hamster	10	5%	18/30 fatalities, 14 before	231
	28–39 weeks	Hamster	10	10%	week 12; significant gross	
	28–39 weeks	Hamster	10	15%	and histopathologic findings in bladder, kidney, spleen, and GI tract	
	17 months	Monkey	4	1 g/day	No significant gross or histopathologic changes	257
	Lifespan	Rat		0.5–2.0%	No significant gross, hematologic, or histopathologic changes	235,258
		Hamster	10	5% 10% 15%	Diarrhea and retarded growth, high mortality, perhaps due to diarrhea	152
Polysorbate 21	2 years	Rat		2%	No significant gross or histopathologic changes	235
Polysorbate 40	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235
		Rat		2%	No gross or histologic abnormalities	259

TABLE 13. (Continued.)

Ingredient	*Length of Study*	*Species*	*Number of Animals*	*Level of Polysorbate in Diet*	*Results*	*Reference*
Polysorbate 60	7 weeks	Chick	12	0.1%	No adverse effects	256
	7 weeks	Chick	12	1.0%	No adverse effects	
	7 weeks	Chick	12	2.0%	No adverse effects	
	8 weeks	Rat		2%	No adverse effects	235
	8 weeks	Rat		5%	No adverse effects	
	8 weeks	Rat		10%	Diarrhea after first few days with recovery after continued feeding	
	12–16 weeks	Mouse	10–12	2.5%	No adverse effects	156
	12–16 weeks	Mouse	10–12	5%	No adverse effects	
	12–16 weeks	Mouse	10–12	10%	No adverse effects	
	12–16 weeks	Mouse	10–12	15%	Some GI disturbance with reduced food intake and growth retardation	
	14 weeks	Weanling rat	12	5% in purified casein	Diarrhea and growth retardation	249
	14 weeks	Weanling rat	12	5% in soybean meal	No adverse effects	249
	14 weeks	Weanling rat	12	10% in soybean meal	No adverse effects	
	14 weeks	Adult rat	12	5% in soybean meal	No adverse effects	
	14 weeks	Monkey		2 g/day	No adverse effects	235
	15 weeks	Rat		25%	Growth retardation and transient diarrhea; normal hematology and no other gross or histopathologic findings	257
	12 months	Mouse		2.5%	No adverse effects	156
	12 months	Mouse		5%	No adverse effects	
	12 months	Mouse		10%	No adverse effects	

	12 months	Mouse		15%	Reduced food intake and growth retardation; no other adverse effects	
	12 months	Hamster	12	1%	Normal levels of mortality;	156
	12 months	Hamster	12	5%	diarrhea at 5%; kidney changes at autopsy due to water imbalance from chronic diarrhea	
	14 months + 3 generations	Rat		0.25%	No differences from controls	261
	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235
	2 years	Rat	24	2%	All groups showed normal	260
	2 years	Rat	24	5%	patterns of mortality; no	
	2 years	Rat	24	10%	adverse effects at 2 and 5%;	
	2 years	Rat	24	25%	marked diarrhea and enlargement of cecum at 10 and 25%; questionable fatty change in liver at 25%; no other microscopic changes at any feeding level	
	2 years	Beagle puppies		5%	No adverse effects	156
				10%	No adverse effects	
	2 years	Basenji puppies		5%	No adverse effects	156
	2 years + 4 generations	Rat	22	5%	No adverse effects	71,245–247
	2 years + 4 generations	Rat	22	10%	Diarrhea	
	2 years + 4 generations	Rat	22	20%	Diarrhea and some minor effects on growth, longevity, and reproduction	
		Rat		2%	No gross or histologic abnormalities	262
Polysorbate 61	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235

TABLE 13. (Continued.)

Ingredient	*Length of Study*	*Species*	*Number of Animals*	*Level of Polysorbate in Diet*	*Results*	*Reference*
Polysorbate 65	12 months	Dog	2	13.5%	Periods of diarrhea and dehydration; dog fed 34% showed phosphate kidney stones on autopsy as result of dehydration; no other gross, hematologic, urologic, or histopathologic findings	156
	12 months	Dog	1	34%		
	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235
	2 years + 4 generations	Rat	22	5%	No adverse effects	71,245–247
	2 years + 4 generations	Rat	22	10%	Diarrhea	
	2 years + 4 generations	Rat	22	20%	Diarrhea and some minor effects on growth, longevity, and reproduction	

Polysorbate 80	3 months	Rat	12	1.5 ml at 1%	1.5 ml of solution given daily; congestion and degenerative changes in heart, liver, and kidney, thought to result from capillary wall damage	263
	3 months	Rat	12	1.5 ml at 2%		
	3 months	Rat	12	1.5 ml at 4%		
	17 months	Monkey	2	1 g/day	No significant gross or histopathologic findings	257
	2 years	Rat	30	2%	No adverse effects	134
	2 years	Rat	22	5%	No adverse effects	71,245–247
	2 years + 4 generations	Rat	22	10%	Diarrhea	
	2 years + 4 generations	Rat	22	20%	Diarrhea and some minor effects on growth, longevity, and reproduction	
	3 generations	Rat	30	2%	No alteration in fecundity or growth pattern; no histopathologic findings in liver and kidney	264
	3 generations	Rat		2%	No adverse effects	265
Polysorbate 81	6 weeks	Rat		1%	No adverse effects	235
	6 weeks	Rat		4%	No adverse effects	
	6 weeks	Monkey	2	2 ml/day	No adverse effects	235
	2 years	Rat		2%	No adverse effects	235
Polysorbate 85	6 weeks	Rat	12	1%	No adverse effects	235
	6 weeks	Rat	12	4%	No adverse effects	
	2 years	Rat		2%	No adverse effects	235

rhea and reproduction at high dosage levels were alleviated by the addition of fat to the diet. The 5 percent level was chosen as a "substantial multiple of the maximum conceivable human level"; there were no adverse effects noted at this level. Even the highest levels of the Polysorbates gave no evidence of cumulative toxicity or of progressively changing physiologic response through the four consecutive generations.

It is likely that the diarrhea noted in many feeding studies with the Polysorbates resulted from having high concentrations of the unabsorbed polyoxyethylene sorbitan moiety within the intestinal lumen.[248] This diarrhea may either directly or indirectly cause many of the other observed adverse effects. The symptom of diarrhea by itself is of questionable significance, for it was found by Chow et al.[249] to depend directly upon the type of basal diet fed to the test animals. A purified casein diet that contained 5 percent Polysorbate 60 caused diarrhea and growth retardation in rats, whereas a soybean meal diet with up to 15 percent Polysorbate 60 caused neither diarrhea nor any other adverse reactions. More recent studies have confirmed this protective effect against toxicity by certain diets and have attributed it to dietary fiber.[250-254]

Dermal Toxicity

Acute Studies

Undiluted Polysorbate 20 was tested for acute dermal toxicity following a single percutaneous exposure.[255] Each of six albino guinea pigs received 3 g/kg on the clipped intact or abraded skin of the back and flank, and the material was allowed to remain in contact with the skin for 24 hours under occlusion. No deaths resulted from the exposure, all animals appeared normal throughout the study, and there was no observable gross pathology at necropsy on the seventh day.

Six albino guinea pigs were clipped free of abdominal hair and immersed up to their axillae in a 0.5 percent aqueous solution of a product formulation containing 8.4 percent Polysorbate 20; the effective ingredient concentration was 0.042 percent.[266] The animals were immersed 4 hours per day for 3 consecutive days. There were no signs of systemic toxicity, and all skin appeared normal 48 hours after the last exposure.

Subchronic Studies

A body lotion containing 4 percent Polysorbate 40 was tested for percutaneous toxicity in a 28-day study.[267] Doses of 0.3 or 0.9 ml/kg/cm^2 body surface area were applied to the backs of albino rabbits 5 days a week for a total of 20 applications. The two test groups and one control group each contained eight animals, four of which received epidermal abrasions twice a week. No deaths occurred in test or control animals during the study. Slight peripheral leukocytosis was observed in several male and female rabbits after 2 weeks of treatment but was not observed after the last application; the cause of the leukocytosis is unknown. All other hematologic, urologic, and histopathologic findings were within normal ranges. A dose-related dermatitis was seen in treated rabbits as evidenced by mild to moderate erythema and edema and scaly desquamation.

A cream product formulation containing 2.5 percent Polysorbate 80 was tested for percutaneous toxicity in a 90-day study.[268] Doses of 6 mg/cm^2 of the product were applied to the backs of 10 rabbits for 90 consecutive days; 10 untreated ani-

mals served as a control. Three of the 20 animals (2 control and 1 treated) died during the study from intestinal and respiratory problems not considered to be treatment-related. Hematology, clinical chemistry, urinalyses, and histopathology revealed no unusual findings, and no signs of systemic toxicity were observed. Dermal lesions were characterized clinically by moderate edema and erythema, with slight to moderate desquamation, and histologically by mild dermatitis.

A cream product formulation containing 1 percent Polysorbate 85 was tested

Single 1 ml injections of 5 percent Polysorbate 80 in water were made into the central artery of the rabbit ear, intravenously into the margin ear vein of the rabbit, and intradermally at two different sites on the abdomen of the rabbit. The resultant mild intra-arterial irritation was entirely clear within 24 hours, there was no abnormal reaction intravenously, and the intradermal wheals were completely absorbed within 1 hour.[270]

The minimum lethal dose with intravenous administration of Polysorbate 80 was 1.0 g/kg for rats and 0.5 g/kg for cats and dogs. After reviewing this and other parenteral toxicity data, Badden et al.[271] recommended that the maximum concentration of Polysorbate 80 for use in pharmacologic testing without any solvent toxicity should be 6.8 percent.

Subchronic Studies

Grossman et al.[272] investigated the acute and subchronic intravenous toxicity in rabbits of Polysorbate 60 alone and of fat emulsions made with Polysorbate 60. Daily injection of 10 ml/kg of a fat emulsion containing 0.5 percent Polysorbate 60 for 3 weeks produced no biochemical or histologic abnormalities. Daily intravenous Polysorbate 60 at unspecified doses for 9 weeks led to a marked elevation of serum cholesterol and phospholipid, a slight enlargement of the adrenals, and a marked enlargement of the spleen.

Payne and Duff[273] injected intravenously a 20 percent solution of Polysorbate 80 in saline into each of 10 rabbits daily for 40 to 65 days; the daily dose of 25 ml was split into two separate injections of 10 and 15 ml. Six of the animals died between 40 and 61 days of treatment. Histologic examination showed greatly enlarged spleens with tremendous foam cell accumulation in the reticuloendothelial system and marked lipid infiltration of the renal tubular epithelium.

Chronic Studies

In a study by Huy et al.[274] on tetrahydrocannabinol toxicity, a 4 percent solution of an unidentified Polysorbate in saline was administered daily by intraperitoneal injection (0.3 ml) for 6 months to a group of 10 guinea pigs as a solvent control. Physical and biochemical parameters did not differ significantly from absolute controls receiving saline solution alone.

A reported lethal subcutaneous dose of Polysorbate 80 administered to rats over 40 weeks was a cumulative dose of 49 g/kg.[275]

Skin Irritation

Acute Studies

The potential for primary skin irritation caused by Polysorbates 20, 40, 60, and 80 was evaluated using the Draize rabbit skin patch test technique in several studies.[233,279-285] In each study, 0.5 ml samples were applied and occluded for 24 hours, after which the patch sites were graded for erythema and edema on the Draize scale or another, similar scale. The results and other details of these studies are summarized in Table 15. The undiluted Polysorbates produced no or only mild skin irritation. When Polysorbate 60 was diluted to 15 percent in water, the test showed no signs of irritation.

In another test system used by Lansdown and Grasso,[286] a single 0.5 ml application of 10 percent Polysorbate 80 in distilled water to the backs of 15 mice produced no gross or histologic changes in the skin.

TABLE 15. Primary Rabbit Skin Irritation.

Ingredient	*Conc. (%)*	*No. of Rabbits*	*Primary Irritation Index**	*Comments*	*Reference*
Polysorbate 20	100	6	0.0	No signs of irritation	233
	100	9	0.17 (max = 4.0)	Minimal irritation; modified Draize scoring	281
	100	1	0.0	No signs of irritation; three intact and three abraded patch sites	283
Polysorbate 40	100	1	0.0	No signs of irritation; three intact and three abraded patch sites	284
Polysorbate 60	100	6	0.0	No signs of irritation	233
	100	6	0.29	Minimal irritation	285
	100	9	0.50 (max = 4.0)	Mild irritation; modified Draize scoring	280
	100	1	0.0	No signs of irritation	282
	15 in water	6	0.0	No signs of irritation	285
Polysorbate 80	100	6	0.0	No signs of irritation	233
	100	9	0.17 (max = 4.0)	Minimal irritation; modified Draize scoring	279

*Maximum = 80 unless otherwise noted; those results reported on a scale with maximum = 4.0 were calculated as the mean of individual Primary Skin Irritation (PSI) scores.

Cosmetic formulations containing the Polysorbates have also been tested for rabbit primary skin irritation. Formulations containing Polysorbates 20 and 60 at concentrations of 2.0 and 2.5 percent, respectively, produced minimal to mild irritation in a Draize 24-hour test.[287,288] Formulations containing Polysorbates 40, 60, 80, and 85 at concentrations of 1.0 to 4.0 percent produced mild to moderate irritation when applied for 4 successive days.[237,240-242]

Subchronic Studies

Repeated application of 1 percent or 2.5 percent Polysorbate 80 in distilled water to the backs of 15 mice for up to 9 days produced no macroscopic or histologic changes.[286]

When 100 percent Polysorbate 60 was applied daily to the backs of three rabbits for 60 days, it was "relatively well tolerated" with only slight dermal congestion. A 15 percent solution of Polysorbate 60 in water was "very well tolerated" under the same test conditions, resulting in a normal histologic picture.[285]

Polysorbates 20, 60, 80 and 85 were applied daily to the backs of rabbits for 30 days to determine their irritative potentials and physiologic properties. They were applied either undiluted or diluted to 10, 5, or 1 percent; the solvents used were water, petrolatum, and a hydrophilic ointment. Each of the Polysorbates at 100 percent produced erythema by the third day and thickening of the skin by Day 10. Microscopic examination after 10 days revealed minimal to mild inflammation but neither acanthosis nor necrosis. After 30 days application, microscopic examination showed mild to moderate inflammation with acanthosis and, for Polysorbate 80, necrosis. At dilutions of 10, 5, and 1 percent, the Polysorbates produced only slight erythema after 10 days of application. Microscopic evaluation at 10 days showed minimal inflammation. After 30 days of application, the degree of inflammation ranged from minimal to marked for the various Polysorbates, with Polysorbate 60 producing some necrosis at 10 percent in water.[289]

A series of further studies was conducted by the same investigators in an attempt to determine the mechanism by which 10 percent Polysorbate 85 in petrolatum damages the skin of rabbits upon daily application.[290-293] Polysorbate 85 increased the phospholipid content of the rabbit epidermis[290] and increased the in vitro incorporation of labeled inorganic phosphorus into phospholipids.[291] The composition of epidermal phospholipids remained unchanged, but the biosynthetic and turnover rates of all identified phospholipids were greatly increased.[292] There were also increases in the biosynthetic rates of nucleic acids and acid-soluble materials.[293] It was concluded that the damage to rabbit skin caused by the Polysorbates reflected a direct effect on the cell membranes of the epidermis.

Eye Irritation

The Draize rabbit eye irritation procedure or a modification of the test was used to evaluate the Polysorbates in a number of studies.[233,282-284,294-296] In each study, a 0.1 ml sample was instilled into one eye of each rabbit with no subsequent washing; some rabbits received a water wash, as noted in Table 16. Treated eyes were examined and graded on the Draize eye irritation scale at 1, 2, 3, 4, and 7 days. The results and other details of these studies are summarized in Table 16. The undiluted Polysorbates produced, at most, only minimal eye irritation that cleared by Day 3. Some of the Polysorbates diluted to 75 or 30 percent in water

TABLE 16. Draize Rabbit Eye Irritation.

Ingredient	Conc. (%)	No. of Rabbits	Ocular Irritation Index (max = 110)					Comments	Reference
			Day 1	Day 2	Day 3	Day 4	Day 7		
Polysorbate 20	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	100	6	1.0	0	0	0	0	Minimal irritation	296
	100	1	0	0	0	0	0	No irritation	283
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 21	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 40	100	1	0	0	0	0	0	No irritation	284
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 60	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	100	6	2.0	1.0	0	0	0	Minimal irritation	295
	100	1	0	0	0	0	0	No irritation	282
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 61	60 w/v in distilled water	6 unwashed	0.33	0	0	0	0	Minimal irritation	233
		3 washed	0.67	0	0	0	0	Minimal irritation; eyes washed with water after 2 seconds	

TABLE 16. (Continued.)

Ingredient	Conc. (%)	No. of Rabbits	Ocular Irritation Index (max = 110) Day 1	Day 2	Day 3	Day 4	Day 7	Comments	Reference
Polysorbate 65	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	10 w/v in light mineral oil	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 80	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	100	6	2.0	1.0	0	0	0	Minimal irritation	294
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 81	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	10 w/v in light mineral oil	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 85	100	6 unwashed	0.33	0	0	0	0	Minimal irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	75 in water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	30 in water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	10 in light mineral oil	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	

or 10 percent in mineral oil produced no signs of irritation; Polysorbate 61 at 60 percent in water produced a minimal transient irritation at 1 hour after instillation, which cleared before subsequent readings.

Hazleton[297] found undiluted Polysorbates 20, 40, 60, 65, and 80 to be "tolerated" by the rabbit eye. Guillot et al.[285] tested undiluted Polysorbate 60 in a Draize eye irritation test on six rabbits with scores indicative of minimal transient irritation. Draize and Kelley[298] reported the "maximum tolerated concentration" of Polysorbate 80 to be 100 percent.

Polysorbate 60 was also tested for rabbit eye irritation by a method that involved the determination of dry tissue weight as a measure of corneal and conjunctival edema and the estimation of dye diffusion as a measure of vascular leakage in the conjunctiva and aqueous humor. The results indicate minimal to mild irritation with instillation of 100 percent Polysorbate 60 for up to 3 days and "only very minor" effects on the eye with instillation of 25 percent Polysorbate 60 in water for up to 13 days.[299]

Several product formulations containing Polysorbate 20, 40, 60, 80, or 85 at concentrations of 1.0 to 8.5 percent have also been tested in the Draize rabbit eye irritation test with scores indicative of minimal to mild irritation.[237,240-242,300-302] A bubble bath formulation produced severe irritation when instilled into rabbit eyes as evidenced by damage to the conjuctiva, iris, and cornea.[303] A subsequent test with the same formulation diluted to 0.5 percent in water showed minimal transient eye irritation.[304]

Mucosal Irritation

In an attempt to produce a pathophysiologic model of an intestinal malabsorption syndrome, a series of studies was conducted in which detergents were used to damage the intestinal mucosa. Yonezawa[305,306] applied aqueous solutions of Polysorbate 80 to the intestinal mucosa of adult male albino rabbits. Exposure to 10 percent Polysorbate 80 for up to 4 hours produced no morphologic changes in the intestinal villi. There was a hypersecretion of mucus from goblet cells, but there was no desquamation or necrosis. Exposure to 20 percent Polysorbate 80 facilitated glucose absorption and stimulated goblet cell mucus secretion, but this concentration too caused no histopathologic changes. Oshumi[307] used transmission electron microscopy and other methods to determine the histologic and biochemical effects of a 30-minute treatment with 10 percent aqueous Polysorbate 80 on the rat intestinal mucosa. Marked deformation and desquamation of the microvilli occurred after treatment, without removal of the enteric surface coat. Histologically, the destruction was strongly localized within the level of the brush border, while the terminal web and level beneath remained intact. Glucose absorption was accelerated as a result of this mucosal damage. Further tests were conducted by Oshumi et al.,[308] with the duration of treatment lasting 3 months. Polysorbate 80 was given at 10 percent in the drinking water of rats for the duration of the test. As compared to controls, Polysorbate 80 produced body weight gain and increased glucose absorption, which declined after long-term treatment, hypersecretion of goblet cells, deformation of villi, destruction of microvilli, and changes in mitochondria. Nadai et al.[309] studied the effect of Polysorbate 80 on the intestinal mucosa of the rat by measuring the exsorption rate of intravenously administered sulfaguanidine. Polysorbate 80 increased the transfer of sulfaguanidine from the blood vessel to the intestinal lumen, indicating damage to the mucosa.

A study on the effects of Gastrografin, a water-soluble radiographic contrast material, on the colonic mucosa of rats suggested that Polysorbate 80 played a major role in the production of inflammation.[310] A later study in which rats were given enemas of 10 percent aqueous Polysorbate 80 showed no deleterious effects with volumes sufficient to fill the colon. Severe changes did result, however, from volumes that produced overdistention.[311]

Studies have been carried out to determine the effect of Polysorbates on mucosa other than that of the intestine. Polysorbate 20 produced no inflammation when applied to the cheek pouch mucosa of hamsters at an unspecified volume and concentration,[312] and 10 percent aqueous Polysorbate 40 produced neither inflammation nor toxic effects when infused into the urinary bladder of guinea pigs.[313]

Mucosal irritation tests have been conducted on product formulations containing the Polysorbates. A lotion containing 4 percent Polysorbate 40 and a cream formulation containing 1 percent Polysorbate 85 produced no signs of irritation after 0.1 ml doses were applied to the penile and vaginal mucosae of rabbits.[241,314] A bubble bath containing 6 percent Polysorbate 20 produced severe irritation when instilled into the vaginal vaults of three beagle dogs once daily, 5 days a week, for 3 weeks. Mucosal lesions were characterized by redness, blistering, mucosal sloughing, and, in one case, fibrous adhesions. Histologic examination revealed marked diffuse erosion of the vaginal mucosa with necrotic and fibrinous exudate on the mucosal surface and a marked infiltration of polymorphonuclear leukocytes in the submucosal tissue.[315] A subsequent, similar test with the same bubble bath diluted to 0.5 percent in water produced no visible findings that were attributable to treatment. Gross pathologic and histopathologic observations revealed no differences from saline-treated controls.[316]

Skin Sensitization

The Magnusson–Kligman guinea pig maximization test[317] was used to determine the sensitization potential of Polysorbate 20; five assays were completed on three different batches of the material.[318] The procedure consisted of an induction phase of intradermal injection and topical application followed by a series of two or three topical challenges. Animals were injected intradermally with 0.1 ml of 50 percent complete Freund's adjuvant in saline, a 0.1 ml of an irritant concentration of Polysorbate 20 in paraffin oil (5.0 to 7.5 percent), and 0.1 ml of the test material in 50 percent complete Freund's adjuvant in saline. One week following the injections, undiluted Polysorbate 20 was applied topically under occlusion for 24 hours to a site pretreated with 10 percent sodium lauryl sulfate. After a 2-week rest period, undiluted Polysorbate 20 was again applied topically for 24 hours under occlusion. Second and, in one case, third challenge applications were made at 1 week intervals. Four of the five assays evoked responses at challenge indicative of moderate sensitization; one batch of Polysorbate 20 produced strong sensitization under the conditions of the test.

The sensitization potentials of Polysorbates 65 and 80 in guinea pigs were tested by repeated intradermal injections of a 0.1 percent suspension in saline followed by a challenge dose after 2 weeks.[235] There was no indication of sensitization reported.

Clinical Assessment of Safety

Ingestion Studies

Acute Oral Toxicity

Polysorbates 60 and 80 have been given a toxicity rating of practically nontoxic (1/6), with a probable oral lethal dose in humans greater than 15 g/kg.[319]

Chusid and Diamond[320] reported an accidental overdose of Polysorbate 80 fed to a 4 month old male infant weighing less than 8 pounds, wherein 19.2 g of Polysorbate 80 was ingested daily for 2 consecutive days with no other food. The infant passed six loose stools but showed no other evidence of intoxication.

Johnson et al.[321] administered four daily doses of 200 mg Polysorbate 20 to 13 premature and 2 fullterm infants with steatorrhea. No increase in fat absorption was observed, but it was noted that no adverse effects resulted with respect to anorexia, vomiting, defecation, or growth.

Snyderman et al.[322] studied the administration of Polysorbate 80 as a dietary supplement to nine premature infants ranging in body weight from 1.41 to 2.01 kg. Daily doses of 0.179 to 0.335 g/kg for 4 consecutive days were well tolerated with no reported adverse effects.

In an attempt to determine the effect of large doses of Polysorbate 60 on the alimentary tract of man, Steigmann et al.[323] fed a single 20 g-dose to each of 11 subjects of both sexes and various ages. There were no significant changes in gastric motility or gastric acidity and no subjective reports of adverse symptoms.

A single oral dose of 5 ml of Polysorbate 80 was given by Fisherman and Cohen[324] to 86 non-aspirin-sensitive patients with intrinsic chronic rhinitis, nasal polyps, and asthma. A positive reaction was shown by 21 of the patients (only 7 of whom were atopic), as evidenced by exacerbation of their respiratory disease. Comparisons between these patients and those of other groups tested suggest that the incidence of Polysorbate 80 intolerance in patients with intrinsic respiratory disease is about one-half the incidence of aspirin intolerance and twice the incidence of iodide intolerance.[324]

Long-term Feeding

The FAO/WHO Expert Committee on Food Additives[244] has established a maximum acceptable daily intake of Polysorbates of 25 mg/kg/day.

There have been a number of human feeding studies published primarily because of interest in using Polysorbates for therapy in lipid malabsorption syndromes. Krantz and Carr[264] described Polysorbate 80 as useful in promoting fat absorption from the alimentary tract and reported that long-term use for such purposes is "apparently without harmful effects."

Krantz et al.[134] reported that Polysorbate 80 was prescribed to more than 100 patients of both sexes and various ages for its beneficial effect on the intestinal absorption of fats. This group of patients ranged in age from 5 to 72 years and was of approximately equal sex distribution. Doses of 4.5 to 6.0 g were taken daily by 10 patients for 3 to 4 years, 17 for 2 to 3 years, 19 for 1 to 2 years, and more than 54 for less than 1 year. The large body of clinical and laboratory data collected during the course of the study indicated no adverse effects after long-term consumption of Polysorbate 80. It was judged to be harmless for human consumption in amounts of at least 6.0 g per day.

King et al.[325] studied the effects of Polysorbates 20 and 80 in 13 patients with

bile salt-deficient steatorrhea, 8 of whom received Polysorbate 80 and 5 Polysorbate 20. When 2 g were administered three times a day, the Polysorbates increased micellization of ingested fat and improved fat absorption. Therapy reduced steatorrhea in 10 of the 13 patients.

Jones et al.[75] fed as much as 15.0 g of Polysorbate 80 daily for a period of several months to patients with steatorrhea for modification of fat absorption. There were no untoward symptoms and no evidence of toxicity as measured by changes in peripheral blood erythrocytes, leukocytes, liver function, or renal function. The only symptom attributable to the use of Polysorbate 80 has been the rare manifestation of increased bowel activity.

Boyd and Helfrick[326] reported that a 2-year old child with severe celiac disease remained free of any symptoms when treated for over 1 year with 40 mg of Polysorbate 80 for each gram of ingested fat.

Mindrum[327] used Polysorbate 80 at a level of 2 percent in a high-calorie emulsion diet for nine critically ill patients. Ingestion of this emulsion for 30 to 120 days caused no apparent adverse effects.

Waldstein et al.[328] evaluated the pharmacologic effect of Polysorbate 60 per os. A group of 34 patients of an elderly and chronic disease infirmary and a group of 10 normal hospital personnel were fed 6 g of Polysorbate 60 daily for 28 days. Clinical and laboratory tests produced no evidence of adverse effects.

Steigmann et al.[323] fed 6 g of Polysorbate 60 per day for 28 days to each of 10 subjects. No significant effects were found on the physiologic activity of the gastrointestinal tract in any of the subjects.

Preston et al.[329] fed daily 1-g doses of Polysorbate 60 to three normal children; one child received treatment for 13 days, another for 31 days, and the third for 34 days. No harmful effects were observed in any of the patients as reflected by careful clinical examinations, including tests for duodenal enzymes and fecal fat and nitrogen.

Page[330] studied 20 normal adults who were fed 4 g/day for 28 days of an emulsifier mixture containing 20 percent Polysorbate 60 as a supplement to their regular diet. An additional 20 subjects were each fed 8 g/day for 28 days of a mixture consisting of 80 percent Polysorbate 61 and 20 percent Polysorbate 60. A third group of 20 subjects was fed 4 g/day for 28 days of an emulsifier mixture containing 6 percent Polysorbate 60. The test doses were administered in three equal portions daily in conjunction with a chocolate syrup. No significant variations were observed in any of the subjects as evidenced by physical examination, hematology, and urinalysis.

Jeans and Stearns[331] studied the effects of adding emulsifier mixtures containing Polysorbates 60 and 80 to the daily diets of nine infants ranging in age from 1 week to 7 months. Daily administration of approximately 0.2 g Polysorbate 60 with 0.04 g Polysorbate 80 was continued for periods of 1.5 to 5 months, with three of the infants receiving approximately 0.4 g Polysorbate 60 with 0.04 g Polysorbate 80 per day for an additional 1 to 2 months. Careful observation of the patients, including comparative growth curves and nutritional balance studies, indicated no adverse effects as a result of feeding the emulsifiers.

A review of animal studies indicates that the Polysorbates may induce diarrhea when given in the diet at high doses (see Oral Toxicity in Animal Toxicology section). To test the effects of Polysorbates 65 and 80 on gastrointestinal motility in man, Janowitz et al.[332] fed normal individuals of both sexes and various ages 9 g/day for 13 consecutive days; each of the Polysorbates was administered to 12

adult subjects. No subjective reactions or changes in bowel habit, character of stools, or body weight were induced. No evidence was obtained to indicate any effect of the Polysorbates on intestinal transit time or gastrointestinal mucosal patterns.

Polysorbate 80 has been used by McCorriston[333] as an oral therapeutic agent for the treatment of atopic dermatitis, psoriasis, and other dermatoses. Daily doses of 6 g were administered to a total of 85 patients with chronic dermatoses for up to 3 months in an attempt to alter lipid metabolism. There was a significant increase in serum lipid levels but no evidence of toxic effects.

Skin Irritation

Undiluted Polysorbates 20, 21, 40, 60, 80, 81, and 85, and Polysorbates 61 and 65 diluted to 60 percent in water were tested in 50 subject panels by Schwartz.[334] A 72-hour occlusive patch was applied to the skin, followed after 7 days with a second 72-hour patch. No evidence of irritation was observed in any of the patients. Other investigators using a similar technique with 48-hour patches on 10 subject panels found no irritation to undiluted Polysorbates 20, 21, 65, 80, and 81 or to Polysorbates 20, 40, 60, 61, 80, 81, and 85 diluted to 30 percent in water.[335,336] Polysorbates 20 and 80 were also tested in 24-hour single insult patch tests with no resultant irritation in 19 to 20 subject panels.[337,338] These studies are summarized in Table 17; they show no potential for primary skin irritation caused by the Polysorbates.

A number of product formulations containing various Polysorbates at concentrations of 1.0 to 8.4 percent have also been tested for human skin irritation. The results and other details of these studies are summarized in Table 18. Single insult occlusive patch tests on three formulations produced no or only minimal to mild irritation.[339-341] Daily skin patching of eight product formulations for 21 days produced ratings of "essentially nonirritating" to "highly irritating."[342-348] Results indicative of irritation cannot be interpreted without knowledge of the other ingredients in a formulation.

Mezei[349] applied, under occlusion, 10 percent Polysorbate 85 in white petrolatum daily for 4 days to the upper arms of 15 normal individuals (9 male, 6 female; 20 to 32 years old). The other arm was similarly treated with petrolatum to provide the control area. At the end of the treatment, macroscopic observations indicated only minor erythema in 11 cases; no visible changes were noted in the treated areas of 4 persons or in any of the control areas. No definite histologic changes were observed by microscopic evaluation. The results of biochemical assays, however, were more definitive. The content of epidermal phospholipids was elevated 5 to 65 percent as a result of the treatment with the Polysorbate 85 preparation. Radioactive tracer studies indicated higher rates of phosphorus incorporation into epidermal phospholipids, DNA, RNA, and trichloroacetate-soluble fractions of the treated skin. Results resembled those that were documented in earlier studies by the same researcher with rabbit skin (see Skin Irritation in Animal Toxicology section).

Eye Irritation

The effect of Polysorbate 20 on corneal permeability to fluorescein was investigated in human subjects in an effort to find safe and effective agents that increase permeability to drugs. Subjects were normal volunteers of both sexes,

TABLE 17. Clinical Skin Patch Tests with the Polysorbates.

Ingredient	*Test Method*	*No. of Applications*	*Duration of Each Exposure (hours)*	*Conc. (%)*	*No. of Subjects*	*Results*	*Reference*
Polysorbate 20	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	335
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
	Single insult	1	24	40 in water	19	No irritation	338
Polysorbate 21	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	336
Polysorbate 40	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
Polysorbate 60	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
Polysorbate 61	Prophetic patch	2	72	60 in water	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
Polysorbate 65	Prophetic patch	2	48	100	10	One subject had questionable reaction on first application only	336
	Prophetic patch	2	72	60 in water	50	No irritation; no sensitization	334
Polysorbate 80	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	335
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
	Single insult	1	24	100	20	No irritation	337
Polysorbate 81	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	335
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
	Prophetic patch	2	48	12 in water	10	No irritation; no sensitization	335
Polysorbate 85	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335

TABLE 18. Clinical Skin Irritation Tests with Product Formulations Containing a Polysorbate.

Test Method	*Material Tested*	*Conc. of Polysorbate (%)*	*No. of Subjects*	*Results*	*Reference*
24-hour single insult occlusive patch	Unspecified product formulation	8.4—Polysorbate 20	19	Mean PSI = 0.47 (max = 4.0); minimal to mild irritation in 11 subjects	341
	Unspecified product formulation	2.0—Polysorbate 20	18	Mean PSI = 0.08 (max = 4.0); minimal irritation in 2 subjects	339
	Unspecified product	2.5—Polysorbate 60	20	No signs of irritation	340
Cumulative irritancy test (daily 23-hour occlusive patch for 21 days)	Bubble bath	6.0—Polysorbate 20	12	Highly irritating; total composite score was 533/630 max	342
	Bubble bath	0.03—Polysorbate 20 (0.5% aqueous dilution of product containing 6%)	10	Moderately irritating; total composite score was 433/630 max	343
	Lotion	4.0—Polysorbate 40	15	Slightly irritating; total composite score was 71/630 max	344
	Cream	4.0—Polysorbate 40	11	Slightly irritating; total composite score was 80/630 max	345
	Moisturizer cream	4.0—Polysorbate 40	9	Slightly irritating; total composite score was 48/630 max	346
	Moisturizer cream	4.0—Polysorbate 40	9	Slightly irritating; total composite score was 127/630 max	346
	Cream	6.0—Polysorbate 60	11	Essentially nonirritating; total composite score was 16/630 max	347
	Cream	1.0—Polysorbate 85	11	Slightly irritating; total composite score was 72/630 max	348

aged 20 to 46 years. Single drops of Polysorbate 20 at various concentrations in saline were administered to one eye; the other eye served as a control. Polysorbate 20 caused no adverse effects on the eye at concentrations up to 40 percent. During the initial screening for effective agents in this study, Polysorbates 40 and 81 were also administered at 1 percent in saline with no harmful effects.[350]

Skin Sensitization

In 1975 and 1976, a total of 1206 patients with eczema (505 male, 701 female) were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to several common emulsifiers. One of the materials tested consisted of a mixture of 5 percent Polysorbate 60 and 5 percent Polysorbate 80 in petrolatum. Allergic reactions were shown by only 2 of the patients (< 0.2 percent of the test population). Only one of the other emulsifiers tested produced fewer reactions.[351]

Each of the Polysorbates was tested undiluted and/or diluted in water according to the Schwartz prophetic patch test technique.[334-336] The results and other details of these studies are summarized in Table 17. There were no reactions indicative of skin sensitization in a total of 580 subjects.

Several product formulations containing the Polysorbates have been tested for human skin sensitization on a total of 3481 subjects using a variety of testing methods. These studies included: four Schwartz-Peck prophetic patch tests on product formulations containing 0.3 to 2.4 percent Polysorbates 20, 60, or 80; four controlled-use tests on product formulations containing Polysorbate 85 at 1.0 percent or Polysorbates 60 and 80 at 2.5 percent each; 15 Draize-Shelanski repeated insult patch tests on product formulations containing 0.084 to 6.0 percent Polysorbates 20, 40, 60, or 80; and one Kligman maximization test on a product formulation containing 6.0 percent Polysorbate 20. The results and other details of these studies are summarized in Table 19. Of the 3481 subjects reported in Table 19, there were no reactions indicative of sensitization to any of the Polysorbates.

A group of 100 patients who had suffered eczematous eruptions as complications following application of topical preparations for other conditions were tested for allergic contact dermatitis caused by ingredients in the base or vehicle of the topical preparations. The patients were patch tested with 15 such substances, including one of the Polysorbates, and returned to the laboratory 48 hours after the patches were applied. The Polysorbate produced no positive reactions.[352]

Of 130 patients suffering from eczematous complications of lower leg chronic venous insufficiency, 3 were sensitive to topical administration of 2.5 percent Polysorbate 40 in water.[353]

Investigations into an isolated case of contact urticaria caused by a therapeutic cream showed Polysorbate 60 to be the responsible ingredient. The reaction was manifested on the forehead but not on the arm or back.[354]

Intravenous Injection

The intravenous injection of 10 mg/kg of Polysorbate 80 produced hemodynamic changes in five patients. The effects included an increase in cardiac output and a decrease in peripheral resistance.[355]

TABLE 19. Clinical Skin Sensitization Tests with Product Formulations Containing a Polysorbate.

Test Method	*Material Tested*	*Conc. of Polysorbate (%)*	*No. of Subjects*	*Results*	*Reference*
Schwartz-Peck prophetic patch test (open and closed 48-hour patches, repeated after 2 weeks)	Shaving preparation	2.4—Polysorbate 20	197	No irritation; no sensitization	358
	Bubble bath	0.3—Polysorbate 20 (5% aqueous dilution of product containing 6%)	103	Minimal irritation in 3 subjects; no sensitization; supplemental UV exposure after second insult showed no photosensitization	359
	Shaving preparation	0.6—Polysorbate 60	197	No irritation; no sensitization	360
	Makeup	0.6—Polysorbate 80	303	Mild irritation with closed patch in 25 subjects at first exposure and in 14 subjects at second; no evidence of sensitization	361
Prophetic patch and in-use testing (48-hour occlusive patch followed by 4 weeks of daily use and final 48-hour challenge patch)	Shaving foam	2.5—Polysorbate 60 and 2.5—Polysorbate 80	100	No reactions after either patch; 2 subjects discontinued use after 2 weeks because of minor pruritis, but no clinical signs were observed; no evidence of sensitization	362
	Shaving foam	2.5—Polysorbate 60 and 2.5—Polysorbate 80	82	Minor irritation after first patch in 6 subjects; no reactions with use of challenge patch; no evidence of sensitization	363
	Shaving foam	2.5—Polysorbate 60 and 2.5—Polysorbate 80	110	Minimal irritation in 5 subjects and moderate irritation in 1 after first patch; no reactions with use; minimal irritation in 3 subjects after challenge; one subject previously sensitized to fragrance component, no other evidence of sensitization	364
	Moisturizer cream	1.0—Polysorbate 85	204	Minimal to mild irritation in 2 subjects; no other evidence of irritation or sensitization	365

TABLE 19. (Continued.)

Test Method	*Material Tested*	*Conc. of Polysorbate (%)*	*No. of Subjects*	*Results*	*Reference*
Draize-Shelanski repeated insult patch test (24- or 48-hour patches 3 days/week for 10 induction patches; challenge patch after 2 weeks rest)	Shaving preparation	2.4—Polysorbate 20	101	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	358
	Skin care product	2.0—Polysorbate 20	99	Minimal irritation; no sensitization	366
	Skin care product	1.0—Polysorbate 20	98	Minimal irritation; no sensitization	367
	Bubble bath	0.3—Polysorbate 20 (5% aqueous dilution of product containing 6%)	55	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	359
	Shampoo	0.084—Polysorbate 20 (1% aqueous dilution of product containing 84%)	97	Essentially no irritation; no sensitization	368
	Lotion	4.0—Polysorbate 40	206	Isolated transient irritation; no sensitization	369
	Lotion	4.0—Polysorbate 40	206	Isolated transient irritation; no sensitization	370

	Lotion	4.0—Polysorbate 40	205	Isolated transient irritation; equivocal sensitization in 5 subjects	371
	Cream	6.0—Polysorbate 60	107	No irritation; no sensitization	372
	Facial makeup	2.5—Polysorbate 60	116	Minimal irritation; no confirmed sensitization to Polysorbate 60	373
	Baby lotion	1.0—Polysorbate 60	200	Minimal irritation; skin fatigue in 5/200; no sensitization	374
	Shaving preparation	0.6—Polysorbate 60	101	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	360
	Cream	2.5—Polysorbate 80	210	Minimal irritation; no sensitization	375
	Makeup	0.6—Polysorbate 80	149	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	361
	Cream	1.0—Polysorbate 85	210	Essentially no irritation; no sensitization	376
Kligman maximization test (5 successive 48-hour patches with challenge after 10-day rest; sodium lauryl sulfate pretreatment before induction and challenge)	Bubble bath	6.0—Polysorbate 20	25	No sensitization	377

Photocontact Sensitization

Two products containing 2.5 percent Polysorbate 60 were tested by a photomaximization procedure for evidence of photo-induced contact sensitization.[356,357] On Monday of the first week in the test protocol, each patient was patched with a 5 percent aqueous sodium lauryl sulfate solution for 30 minutes. After 6 to 8 hours, a 48-hour open patch and a 48-hour ultraviolet-exposed photopatch of the product were applied at pretreated sites. This was followed on Wednesday by a second 48-hour open patch. This procedure was repeated for a total of 3 weeks, and single 48-hour open and photopatches were applied on Wednesday of the fourth week. In one study,[357] 2 of the 49 subjects showed weak, nonvesicular reactions to the first photopatch. One of these subjects also showed a weak reaction to the challenge photopatch. In the other study,[356] none of the 50 subjects showed positive reactions.

Some of the studies summarized in Table 19 include exposure to ultraviolet (UV) light as a supplement to the Schwartz-Peck prophetic patch test[359] or Draize-Shelanski repeated insult patch test.[358-361] The UV light exposure was to a Hanovia Tanette Mark I quartz lamp at a distance of 12 inches for 1 minute. This lamp has a wavelength coverage of 240 to 370 nM with a peak at 265 nM. One of the 103 subjects in the Schwartz-Peck test on a product containing Polysorbate 20 showed a weak, nonvesicular reaction after a single UV exposure; the significance of this reaction was not interpreted.[359] The Draize-Shelanski tests included UV exposure after induction patches 1, 4, 7, and 10 and after the challenge patch. With products containing Polysorbates 20 and 60, there were few instances of irritation and no reactions indicative of photosensitization.[358-360] The product containing 0.6 percent Polysorbate 80 produced several instances of irritation after UV exposure but no photosensitization.[361] When testing such whole product formulations, positive reactions are of questionable significance with respect to any one ingredient.

Industry Complaint Experience

Complaint experience data are available on three product formulations containing Polysorbate 20. A shampoo containing 8.4 percent Polysorbate 20 had two safety-related complaints in 3 years with an estimated 5.88 million uses.[378] A cuticle softener containing 2.0 percent Polysorbate 20 had 24 complaints in 4 years with 131 million uses.[379] A paste mask containing 2.0 percent Polysorbate 20 had 11 complaints in 4 years with 12.7 million uses.[380] These complaints were listed in the category "allergy/irritation."

Complaint experience data on a moisturizing cream containing 4 percent Polysorbate 40 show five safety-related complaints in 3.5 years with 10.1 million uses; three of these were listed as "rash" and two as "redness or swelling."[381]

There were no safety-related complaints over a 2-year period for a moisturizing product containing 2.5 percent Polysorbate 60 used an estimated 26.7 million times.[382] A shaving preparation containing 2 percent Polysorbate 60 had one complaint over a 2-year period from 3.5 million units sold.[383]

SUMMARY

The Polysorbates are a series of polyoxyethylenated sorbitan esters that differ with respect to the number of polymerized oxyethylene subunits and the num-

ber and type of fatty acid moieties present. They are used as general purpose, hydrophilic, nonionic surfactants in a variety of cosmetic products. Some of the Polysorbates are also approved by the Food and Drug Administration for use in various pharmaceuticals and food products.

Studies employing radioactive tracer techniques show that the Polysorbates are hydrolyzed by pancreatic and blood lipases; the fatty acid moiety is released to be absorbed and metabolized, whereas the polyoxyethylene sorbitan moiety is very poorly absorbed and is excreted unchanged. As expected, the Polysorbates are active at levels of biological structure and function from basic biochemical pathways to the cardiovascular and immune systems. Most or all of these effects can most likely be related to the surface active properties of the intact Polysorbate molecule.

Polysorbate 80 was shown to be nonmutagenic in the Ames and micronucleus tests. The polysorbates have been shown in numerous studies to be noncarcinogenic when administered in a variety of ways to laboratory animals, although Polysorbate 80 produced some neoplastic changes in mixed mouse epidermal and dermal in vitro tissue culture. Multiple studies have shown that the Polysorbates enhance the activity of known chemical carcinogens while not actually being carcinogenic themselves. Proposed mechanisms of this tumor enhancement* effect include induction of cellular hyperproliferation, inhibition of DNA repair, and others. The Polysorbates also exhibit tumor growth inhibition activity under certain conditions.

Extensive testing for acute and long-term oral toxicity in animals has resulted in evidence indicating the low order of toxicity with oral ingestion of the Polysorbates. Most of the reported toxicity can be attributed either directly or indirectly to the osmotic diarrhea caused by the polyoxyethylene sorbitan moiety retained within the intestinal lumen. Polysorbate 20 and product formulations containing 1.0 to 8.4 percent of Polysorbate 20, 40, 80, or 85 produced no evidence of acute or subchronic percutaneous toxicity, the only effects being erythema, edema, and desquamation at the site of application. Acute intravenous and intraperitoneal injection of the Polysorbates into rats or mice resulted in LD_{50} values indicative of a low order of parenteral toxicity. Daily intravenous injections of Polysorbates 60 and 80 into rabbits for up to 65 days produced pathology limited mainly to the renal and reticuloendothelial systems.

The Polysorbates showed little potential for rabbit and mouse skin irritation in acute studies. Those of the Polysorbates that were tested in subchronic skin irritation tests for up to 60 days produced local skin reactions ranging from minimal inflammation to necrosis. These changes were attributable to damage of epidermal cell membranes by the emulsifying action of the Polysorbates. The Polysorbates produced no more than minimal, transient eye irritation in Draize rabbit eye irritation tests. Polysorbate 80 produced superficial, mild damage to the intestinal mucosae of rabbits and rats. Polysorbate 20 produced no inflammation when applied to the hamster cheek pouch, and Polysorbate 40 caused no inflammation when infused into the guinea pig urinary bladder. The Magnusson–Kligman guinea pig maximization test showed moderate to strong skin sensitization to Polysorbate 20 in one study. Another guinea pig skin sensitization assay reported no evidence of skin sensitization to Polysorbates 65 and 80.

* The term "tumor enhancement" encompasses both tumor promotion and cocarcinogenesis.[146]

The Polysorbates have been ingested by human beings in situations ranging from an accidental administration of 19.2 g of Polysorbate 80 to an infant on 2 consecutive days to daily therapeutic administration of up to 6.0 g of Polysorbate 80 to adults for up to 4 years. These studies consistently showed little or no adverse effects from oral ingestion of the Polysorbates. Extensive clinical skin testing in the Schwartz prophetic patch test showed little potential for human skin irritation and no evidence of skin sensitization in a total of 580 subjects. A total of 1206 patients with eczema were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to a mixture of 5 percent Polysorbate 60 and 5 percent Polysorbate 80 in petrolatum; allergic reactions were shown by only 2 of the patients (< 0.2 percent). Several product formulations containing the Polysorbates have been tested for human skin sensitization on a total of 3481 subjects using a variety of testing methods; there were no reactions indicative of sensitization to any of the Polysorbates in these assays. Investigations with patients known to have skin disease revealed isolated instances of skin sensitization to Polysorbate 40 or 80. Intravenous injection of Polysorbate 80 produced hemodynamic changes in 5 patients. Studies involving exposure to ultraviolet light showed no instance of photocontact sensitization to the Polysorbates, although there were isolated instances of mild irritation following UV exposure when testing product formulations containing the Polysorbates.

DISCUSSION

Polysorbates are not mutagens or complete carcinogens. However, some are known tumor enhancers* in certain laboratory animals. Data are not available on the possible tumor-enhancement activity of Polysorbates in man. The Panel considered the published studies on Polysorbates as a tumor enhancer as well as those comments submitted during the public comment period of the Tentative Report. The FDA has approved Polysorbates 20 and 80 at up to 1.0 percent in ophthalmic preparations and Polysorbate 60 at up to 4.5 percent in foods. It has also approved, without limit to concentration, the use of Polysorbate 80 in vitamin-mineral preparations to a maximum recommended daily consumption of 475 mg/day.

In cosmetic preparations, the preponderance of uses does not exceed the 5 to 10 percent range. Presently available data indicate that these ingredients are used in numerous preparations at these concentrations without clinical reports of significant adverse effects. It is recognized that rinse-off preparations and those that are diluted with use carry a lower potential for adverse effects than might be indicated by the ingredient concentration.

CONCLUSION

On the basis of the available data, the Panel concludes that Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 are safe as cosmetic ingredients in the concentration of present use.

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* The term "tumor enhancement" encompasses both tumor promotion and cocarcinogenesis.[146]

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