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Final Report on the Safety Assessment of Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate

Sodium and Ammonium Lauryl Sulfate are anionic surfactants used in cosmetics as cleansing agents. In absorption, metabolism, and excretion studies, Sodium Lauryl Sulfate had a degenerative effect on the cell membranes because of its protein denaturing properties. Low levels of skin penetration may occur at high use concentration.

Sodium Lauryl Sulfate had an LD50 of 0.8 to 1.10 g/kg in rats. A formulation containing 15% Ammonium Lauryl Sulfate caused depression, labored breathing, diarrhea, and death in four out of 20 animals.

In acute ocular tests, 10% Sodium Lauryl Sulfate caused corneal damage to the rabbits' eyes if not irrigated, or if irrigation was delayed. A Draize test of a product containing 5.1% Sodium Lauryl Sulfate caused mild irritation, and products containing 21% detergent were severely irritating with no rinse, and mildly irritating when rinsed. Ammonium Lauryl Sulfate solutions containing 1.25%–27.4% detergent showed increasing irritation with increasing concentration; rinsing decreased irritation.

Acute animal skin irritation studies of 0.5%–10% Sodium Lauryl Sulfate caused slight to moderate irritation. Applications of 10%–30% detergent caused skin corrosion and severe irritation. Solutions of 2%, 10%, and 20% Ammonium Lauryl Sulfate were highly irritating and dangerous. One percent and 5% Sodium Lauryl Sulfate produced a significant number of comedones when applied to the pinna of albino rabbits.

A chronic oral feeding study in rats of 0.25%, 0.5%, and 1.0% Sodium Lauryl Sulfate in the diet for two years produced no abnormalities. A 91-day percutaneous toxicity study of a shampoo containing 17.5% Ammonium Lauryl Sulfate had no treatment-related abnormalities except for moderate to severe dermal effects.

In mutagenesis studies, rats fed 1.13% and 0.56% Sodium Lauryl Sulfate in the diet for 90 days produced no more chromosomal aberrations or clastogenic effects than did a control diet.

Sodium Lauryl Sulfate was tested for human skin irritation in concentrations ranging from 0.1% to 10%. Open patches were less irritating than closed patches, and irritation increased directly with concentration. Similar results

were obtained when formulations containing Sodium and Ammonium Lauryl Sulfate were tested. No UV light sensitization occurred from any formulation in this latter study.

Both Sodium and Ammonium Lauryl Sulfate appear to be safe in formulations designed for discontinuous, brief use followed by thorough rinsing from the surface of the skin. In products intended for prolonged contact with skin, concentrations should not exceed 1%.

CHEMICAL AND PHYSICAL PROPERTIES

Production

Sodium Lauryl Sulfate, an anionic surfactant, is prepared by the sulfation of commercially available lauryl alcohol with either sulfur trioxide or chlorosulfonic acid:

$$CH_3(CH_2)_{10}CH_2OH \xrightarrow[ClSO_3H]{SO_3 \text{ or}} CH_3(CH_2)_{10}CH_2\text{-}OSO_3H$$

The product of this reaction is then neutralized with aqueous sodium hydroxide:[1-4]

$$CH_3(CH_2)_{10}CH_2\text{-}OSO_3H \xrightarrow{NaOH} CH_3(CH_2)_{10}CH_2OSO_3Na$$

Ammonium Lauryl Sulfate is manufactured by sulfating commercial lauryl alcohol with sulfur trioxide or chlorosulfonic acid, as in step one above. This product is then neutralized with aqueous ammonium hydroxide to produce the ammonium anionic salt:[1,2,5]

$$CH_3(CH_2)_{10}CH_2\text{-}OSO_3H \xrightarrow{NH_4OH} CH_3(CH_2)_{10}CH_2OSO_3NH_4$$

Structure and Synonyms

Sodium Lauryl Sulfate conforms to the formula:

$$CH_3(CH_2)_{10}CH_2OSO_3Na$$

CAS No. 151-21-3
Synonyms include:

Dodecyl Sodium Sulfate
Lauryl Sodium Sulfate
Sodium N-Dodecyl Sulfate
Lauryl Sulfate Sodium Salt.

Ammonium Lauryl Sulfate has the structural formula:

$CH_3(CH_2)_{10}CH_2OSO_3NH_4$

CAS No. 2235-54-3
Synonyms include:

Lauryl Ammonium Sulfate
Dodecyl Ammonium Sulfate
Ammonium Dodecyl Sulfate.[6]

Properties

Sodium Lauryl Sulfate has a molecular weight of 288.4 and a characteristic fatty odor. It is commercially available in a variety of forms including a white to yellow powder, a paste, or a clear, viscous liquid. This compound is very soluble in polar solvents and forms a smooth, opalescent solution with water.[3,4,7-9] For other properties, see Table 1.

Ammonium Lauryl Sulfate is a surfactant with a molecular weight of 283.4.[6] Its commercial forms range from a thin to a viscous liquid with a viscosity of 800 to 8000 cps at 25 °C. It is soluble in water and highly polar solvents such as methanol and ethanol.[5] UV spectra of 28% Ammonium Lauryl Sulfate in concentrations of 5,000 and 50,000 mg/l in distilled water did not show absorption in the UVA or UVB region (approximately 290 to 400 nm).[10]

For other properties, see Table 1.

Reactivity

Sodium Lauryl Sulfate, an anionic detergent, binds to the positively charged side groups of proteins, thereby causing conformational changes (denaturizing) in the protein.[11] It is stable in alkaline conditions, but will hydrolyze at room

TABLE 1. Properties.

Property	*Sodium Lauryl Sulfate*	*Ref.*	*Ammonium Lauryl Sulfate*	*Ref.*
Molecular weight	288.4	3,6	283.4	5,6
Form	Viscous, clear liquid; paste; white to yellow powder	3,7,8	Thin to viscous liquid	5
Viscosity cps at 25 °C	500–8,000 (liquid) 25,000–50,000 (paste)	3	800–8,000	5
Density (g/ml)	0.938 (liquid) 1.01 (paste) 0.396 (powder)	3	0.995	5
pH (% in water)	7.3–8.5 (liquid, paste) 7.5–11 (powder)	3	6.8 (max)	5
Cloud pt (°C)	12.2 (liquid) 25 (paste)	3	18.3	5
Index of refraction	1.461 (alpha) 1.491 (gamma)	9		
Solubility	1 g in 10 ml water	3		
Soluble in:	Water Methanol Ethanol	3	Water Methanol Ethanol	5

temperature below a pH of 5. Hydrolysis also occurs when it is exposed to cold mineral acids in the presence of iron or other heavy metals.[3]

Analytical Methods

Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate, both anionic surfactants, are determined by the Methylene Blue Active Substances Procedure, the azure A colorimetric method, and the two-phase titration method. Thin-layer, paper, and gas chromatography and Infrared and Ultraviolet spectroscopy are useful for the analysis of the lauryl sulfates.[1]

Impurities and Additives

See Table 2 for a compilation of impurities and additives, as well as concentrations of the supplied products.

USE

Cosmetic Uses, Frequency, and Purpose

The cosmetic product formulation computer printout which is made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of Code of Federal Regulations.[12] Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the true concentration found in the finished product; the concentration in such a case would be a fraction of that reported to the FDA. The fact that data are submitted only within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to 10-fold error in the assumed ingredient concentration. Table 3 reports the 1981 FDA

TABLE 2. Ingredients, Impurities, Additives.

Agent	*Sodium Lauryl Sulfate*			*Ref.*	*Ammonium Lauryl Sulfate*	*Ref.*
	Liquid	*Paste*	*Powder*			
Actual ingredient (%)	28–30	30–41	90–98	3	22–54	5
Uncombined ingredients (%):						
(a) Unsulfated alcohol	0.55–2.3	1.8–3.0	0.2–3.5	3	3(max)	5
(b) Sulfate	as sodium sulfate–1.5 max	1.5 max	8 max	3	as ammonium sulfate–3 max	5
(c) Chloride	as sodium chloride–0.4 max	1.8 max	8 max	3	as ammonium chloride–3 max	5
Preservative	formaldehyde–up to 0.1%	–	–	15	–	–

TABLE 3. Product Formulation Data.

Product category	Total no. of formulations in category	Total no. containing ingredient	No. product formulations within each concentration range (%)							
			Unreported concentration	>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Sodium Lauryl Sulfate										
Baby shampoos	35	2	–	–	–	1	–	1	–	–
Bath oils, tablets, and salts	237	7	–	1	4	–	1	1	–	–
Bubble baths	475	73	–	–	4	9	55	2	3	–
Bath capsules	3	2	–	–	–	–	2	–	–	–
Other bath preparations	132	24	–	–	5	7	7	2	3	–
Eye makeup remover	81	2	–	–	–	–	–	2	–	–
Mascara	397	9	–	–	–	–	–	–	3	6
Sachets	119	20	–	–	–	–	–	–	20	–
Other fragrance preparations	191	3	–	–	–	–	1	–	–	2
Hair conditioners	478	6	–	–	–	1	1	3	1	–
Hair straighteners	64	9	–	–	–	–	–	8	1	–
Permanent waves	474	9	–	–	–	–	1	2	5	1
Hair rinses (noncoloring)	158	1	–	–	–	–	–	–	1	–
Hair shampoos (noncoloring)	909	226	–	10	33	64	´88	25	5	1
Tonics, dressings, and other hair grooming aids	290	2	–	–	1	–	–	–	1	–
Other hair preparations (noncoloring)	177	6	–	–	–	–	–	2	1	3
Hair dyes and colors (all types requiring caution statement and patch test)	811	61	–	–	–	–	–	5	12	44
Hair tints	15	1	–	–	–	–	–	1	–	–
Hair shampoos (coloring)	16	9	–	–	1	–	1	7	–	–
Hair bleaches	111	21	–	–	–	1	2	11	6	1
Other hair coloring preparations	49	5	–	–	–	–	2	–	2	1
Blushers (all types)	819	1	–	–	–	–	–	–	1	–
Makeup foundations	740	14	–	–	–	–	–	–	12	2
Makeup bases	831	8	–	–	–	–	–	–	7	1
Rouges	211	1	–	–	–	–	–	–	1	–
Cuticle softeners	32	1	–	–	–	–	–	–	1	–

TABLE 3. (Continued.)

Product category	Total no. of formulations in category	Total no. containing ingredient	No. product formulations within each concentration range (%)							
			Unreported concentration	>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Sodium Lauryl Sulfate (cont'd.)										
Other manicuring preparations	50	1	–	–	–	–	–	–	1	–
Dentifrices (aerosol, liquid, pastes, and powders)	42	28	–	–	–	–	2	18	8	–
Mouthwashes and breath fresheners (liquids and sprays)	53	4	–	–	–	–	–	–	4	–
Other oral hygiene products	3	2	–	–	–	–	–	1	1	–
Bath soaps and detergents	148	11	–	–	1	5	–	–	3	2
Deodorants (underarm)	239	6	–	–	–	–	–	–	6	–
Douches	26	1	–	–	–	–	–	1	–	–
Other personal cleanliness products	227	7	–	1	1	–	–	2	3	–
Aftershave lotions	282	2	–	–	–	–	–	–	2	–
Shaving cream (aerosol, brushless, and lather)	114	5	–	–	–	–	–	4	1	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	28	–	–	4	1	2	4	11	6
Depilatories	32	8	–	–	–	–	2	2	1	3
Face, body, and hand skin care preparations (excluding shaving preparations)	832	27	–	–	–	–	–	7	16	4
Moisturizing skin care preparations	747	23	–	–	–	–	–	–	16	7
Night skin care preparations	219	4	–	–	–	–	–	1	2	1
Paste masks (mud packs)	171	9	–	–	–	–	–	5	–	4
Skin lighteners	44	1	–	–	–	–	–	–	–	1
Skin fresheners	260	1	–	–	–	–	–	–	1	–
Other skin care preparations	349	6	–	–	–	–	–	1	3	2
Suntan gels, creams, and liquids	164	4	–	–	–	–	–	–	3	1
Other suntan preparations	28	2	–	–	–	–	–	–	1	1
1981 TOTALS		703	–	12	54	89	167	118	169	94

Ammonium Lauryl Sulfate										
Baby shampoos	35	2	–	–	–	2	–	–	–	–
Bath oils, tablets, and salts	237	1	–	–	1	–	–	–	–	–
Bubble baths	475	19	–	–	5	13	1	–	–	–
Hair shampoos (noncoloring)	909	131	–	–	18	48	57	7	1	–
Hair shampoos (coloring)	16	2	–	–	–	2	–	–	–	–
Bath soaps and detergents	148	4	–	–	2	1	–	1	–	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	4	–	–	1	1	–	2	–	–
Other skin care preparations	349	1	–	–	–	–	–	–	1	–
1981 TOTALS		164	–	–	27	67	58	10	2	–

Data from Ref. 13.

voluntary submission of data from cosmetic manufacturers on formulations containing Sodium and Ammonium Lauryl Sulfate. There were 703 different products using Sodium Lauryl Sulfate in concentrations of less than 0.1% to greater than 50% and 164 with Ammonium Lauryl Sulfate as an ingredient in concentrations of 0.1%–50%.[13]

The types of cosmetics in which the Lauryl Sulfates are used include antiperspirants, bubble baths, foundations, hair colorings, hand creams, and shampoos.[14] In these products, they are used as detergents, wetting, foaming, and/or emulsifying agents.[3,5]

Surfaces to which Commonly Applied

Since the surfactants Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate are used primarily in shampoos, bath products, facial makeup, deodorants, perfumes, and shaving preparations, these ingredients may contact the general body surface, hair, nails, skin of the hand and face, and mucosal surface.[13]

BIOLOGICAL PROPERTIES

Microbiological Effects

Sodium Lauryl Sulfate can have varying effects among different types of microorganisms. Foot and mouth disease virus is highly resistant to Sodium Lauryl Sulfate, but TGE virus is sensitive to it. It is fungistatic to *Candida* and *Tricophyton spp.*, and concentrations of 2% and over eliminate drug resistance and sex transfer factors in *E. coli*. It also inhibits the growth of many Gram positive bacteria, but is ineffective against Gram negative strains.[16]

Absorption and Excretion

The absorption by and subsequent damage to the intestine caused by Sodium Lauryl Sulfate was studied using male white rabbits. A 3 cm length of jejunum was removed from the animals and served as the control. A 15 cm portion of intestine, tied at both ends by ligatures, received a 10 ml injection of 5% Sodium Lauryl Sulfate. After 5, 10, 15, 30, and 60 min, 3 cm segments of the intestine were excised and examined. It was concluded that Sodium Lauryl Sulfate had caused generation changes of the cell membranes of the intestinal epithelium, of intracellular organelles, and of microvilli. The tissue-damaging effect of Sodium Lauryl Sulfate was a result of its protein-denaturing properties. It is suggested that Sodium Lauryl Sulfate can cause an abnormality in intestinal absorption becuse it causes a disturbance in enzyme activation within lysosomes.[17]

The effect of Sodium Lauryl Sulfate on intestinal absorption was studied in the rat. It was found that 17 m*M* of Sodium Lauryl Sulfate increases the absorption of ouabain, phenolsulphonphthalein and pralidoxime in jejunal loops of anesthetized rats. The absorption increases were potentiated by theophylline, increased by dibutyryl cyclic AMP, and reduced by imidazole. Sodium Lauryl

Sulfate caused an increase in cyclic AMP content in the rat intestinal mucosa; the authors believe that this increased cyclic AMP level is the basis of the ingredient's effect on absorption.[18]

The effect of Sodium Lauryl Sulfate on blood sugar was determined using 26 rabbits. The animals received via gavage 0.41 g/kg (body weight) in a 2.5% solution 24 h after fasting. A control group received only water. The Sodium Lauryl Sulfate solution produced a significant rise in blood glucose.[19]

The percutaneous absorption of Sodium Lauryl Sulfate through guinea pig skins was studied in vivo. The detergent was radiolabelled with [^{14}C] at the α(1)-Carbon of the alkyl chain. The surfactant, in a concentration of 16.3 μCi, was applied in 0.6 ml of water to a 22.5 cm^2 area of the flanks of the animals. The sample was rubbed into the skin for 10 min, washed with water, and then covered with nonocclusive patches for 24 h. No radioactivity (0.0%) was found in feces, liver, kidney, or carcass; 0.1% was found in both exhaled CO_2 and urine; 50.2% was found on the skin at the site; the patch contained 23% and the rinsings held 53.4% of the radioactivity. The investigators concluded that the presence of a strongly anionic terminal group in the surfactant impairs its ability to penetrate through the skin.[20]

The in vitro penetration of [1-^{14}C] labeled Sodium Lauryl Sulfate was studied using rat and human epidermis. Clipped dorsal skin was excised from rats and immersed in a compartment containing 0.25 ml of a 25 m*M* [1-^{14}C] solution of Sodium Lauryl Sulfate. Ten ml of saline added to the sampling compartment against the rat dermis were monitored hourly for [1-^{14}C] by removing 1.0 ml and replacing with fresh saline, thereby maintaining the 10 ml volume in the sampling compartment. After 24 h, the epidermis was washed and measured for the radiolabel. From the 1.0 ml samples taken hourly, it was found that up to 24 h after contact, no measurable penetration of Sodium Lauryl Sulfate occurred. After 24 h, 30% of the labeled Sodium Lauryl Sulfate was recovered in the rinsing and 70% remained associated with the skin. No controls were reported. Human female abdominal skin was exposed to 0.1 ml of 25 m*M* [1-^{14}C] Sodium Lauryl Sulfate. Eight ml of saline added to the sampling compartment were monitored for [1-^{14}C] at 0.5, 1, 2, 3, 4, 6, 7, 8, 24, and 48 h. The epidermal sample was washed after 48 h. No measurable penetration of Sodium Lauryl Sulfate occurred until 24 h after application, at which time 3.9 $\pm$ 3.6 μg/cm^2 penetrated. At 48 h, 87.2 $\pm$ 24.1 μg/cm^2 was absorbed. Swelling of the sample occurred and 75% of the [1-^{14}C] sample was absorbed by the epidermis after the 48 h rinsing. No controls were reported.[21] Similar low penetration results were obtained with rats.[22]

Labeled [1-^{14}C] Sodium Lauryl Sulfate was applied in 0.5 ml aliquots as a 25 m*M* solution to 10 cm^2 of rat skin for 15 min. Expired CO_2, urine, feces, and excised skin was monitored for ^{14}C at 24 h after treatment. Autoradiography of the skin showed heavy deposition of Sodium Lauryl Sulfate on the skin surface and in the upper and lower regions of the hair follicles; the urine also contained quantifiable amounts.[21]

Sodium Lauryl Sulfate sorption by neonatal stratum corneum was studied using skin from newly sacrificed young rats. It was found that this surfactant's diffusion through rat stratum corneum was rapid and increased with concentration,

and the authors postulate that high absorption and diffusion of Sodium Lauryl Sulfate are caused, in part, to structural changes in the membranes produced by this surfactant.[23]

Bleached and unbleached human hair was exposed to a 10% solution of radiotagged Sodium Lauryl Sulfate. Bleached hair absorbed 8% of the compound in 9 h, and virgin brown hair took up 1.2% during the same time. Uptake increased with increasing concentration. One percent of a 0.1 percent solution of Sodium Lauryl Sulfate was taken up by bleached hair in 8 h; during the same time, 4.5% of a 1.0% solution was taken up, and 6.6% of a 10% solution was absorbed.[24]

The effect of Sodium Lauryl Sulfate on the permeability of epidermis was studied using human abdominal epidermis and varying concentrations of the surfactant. One percent Sodium Lauryl Sulfate increased water permeability and caused some damage after skin was soaked in the solution for 22 h. Increasing the concentration of surfactant to 5% increased both the rapidity and severity of the damage; effects were noted after 2–6 h. The mechanism of action is believed to be protein denaturation, membrane expansion, hole formation in the epidermis, and loss of waterbinding capacity.[25]

Three female Colworth Wistar rats were injected intraperitoneally and another three were injected subcutaneously with 3.64 mg [1-^{14}C] Sodium Lauryl Sulfate. Animals were sacrificed 24 h after injection. The rate and route of excretion of intraperitoneally administered surfactant were identical to the subcutaneously injected solution. Most of the injected ^{14}C was recovered in the urine at 24 h after dosing (77 ± 4%); the exhaled CO_2 contained 1.5 ± 0.4%, the feces 2.6 ± 0.7%, and the carcass 15 ± 3%.[21]

Swelling of Stratum Corneum

The effect of Sodium and Ammonium Lauryl Sulfate on swelling of the stratum corneum was studied using Hartley guinea pig epidermis. The concentration of surfactant was 0.05 *M* and squares of excised skin were soaked in the solution for 16 hours. When compared with water-treated controls, Sodium Lauryl Sulfate produced a 13.1% increase in surface area (swelling) and Ammonium Lauryl Sulfate, a 9.1% increase. The authors concluded that the mechanism of swelling was not denaturation caused by the detergent, but that swelling is caused by a reversible conformation change resulting from cooperative binding of the detergent.[26]

Effect of Biological Membranes

The effect of Sodium Lauryl Sulfate on biological membranes was studied using yeast cells[27] and human and dog erythrocytes.[28] The first study investigated the interaction of Sodium Lauryl Sulfate with yeast cells and its effect on pH, ionic strength, and lipid content in the cells. It was found that the micelles of surfactant may penetrate into the membrane through pores in the cell wall, and that micelle-like complexes of surfactant and proteins can destroy the membrane.[27] The second study investigated the lytic action of surfactants, including Sodium Lauryl Sulfate, on erythrocyte membranes. It was concluded that the ef-

fect of the surfactant depends upon its structure and charge, as well as on the components of the target cellular membrane.[28]

Miscellaneous Skin Effects

Powdered human callus was used to study Sodium Lauryl Sulfate-induced liberation of sulphydryl groups from keratin. Powdered callus was exposed to water (control) and 1.0% and 10.0% Sodium Lauryl Sulfate. When compared with the control, the 1% concentration produced no increase in the percent of sulphydryl groups liberated from the callus; however, the 10% concentration yielded a 78.9% increase in liberated sulphydryl groups. It was concluded that the anionic terminal portion of the surfactant is the principal cause of the release of sulphydryl groups. This release is a measure of protein unfolding during keratin denaturation.[20]

In a similar study, isolated human heel keratin was incubated in 0%, 0.05%, 0.5%, and 5.0% Sodium Lauryl Sulfate solution at 37 °C for 20 and 120 min. Large amounts of solubilized protein and sulphydryl groups were liberated from the keratin, and similar amounts of detergent were absorbed onto the keratin. Treatment of keratin with 5% detergent for 20 min decreased its hygroscopicity.[29]

The extraction of soluble proteins and amino acids by Sodium Lauryl Sulfate was studied in vivo using guinea pig stratum corneum. The left dorsal flank of the animal was washed with 20 ml water (control), and the right flank was washed with 20 ml of 25 m*M* Sodium Lauryl Sulfate. Both wash liquors were assayed for soluble proteins and amino acids. The detergent solution eluted 75% more soluble proteins and amino acids than did water.[20]

Sodium Lauryl Sulfate-mediated histamine release from mast cells was studied in vitro using rat peritoneum. When the peritoneal mast cells came in contact with Sodium Lauryl Sulfate at concentrations starting at 0.03 m*M*, up to 85% of stored histamine was released.[20,30]

Animal Toxicology

Acute

Oral

The acute oral toxicity of Sodium and Ammonium Lauryl Sulfate was studied using rats and monkeys. The tests are detailed below and summarized in Table 4.

Unfasted female Wistar rats were intubated with doses of 10% Sodium Lauryl Sulfate. Groups of five animals each were fed doses ranging in geometric progression from 0.252 to 7.95 g/kg. Mortality was established over a two-week post-feeding period, and LD50 values were calculated by the Weil modification of the Thompson method.[41] The LD50 was calculated to be 0.8–1.1 g/kg and by the criteria of Hodge and Sterner, it is moderately toxic.[31]

Sodium Lauryl Sulfate, 28.2% concentration, was tested for acute oral toxicity in five groups of Sprague–Dawley rats (5M, 5F). Each group received 4.5, 5.5, 6.0, 6.5, or 7.5 ml/kg (or 4.2, 5.2, 5.6, 6.1, or 7.0 g/kg) of the ingredient in a single ingastric dose, and the animals were observed for 14 days. The LD50 was found to be 6.0 g/kg with pulmonary hemorrhage as the major cause of death found at necropsy.[32]

TABLE 4. Acute Oral Toxicity.

Conc. (%)	Diluted to (%)	Ingredient or formulation	Dose/kg	Species, sex, no. of animals	Observation time (days)	Route	Vehicle	No. dead	LD50/kg[a]	Comment	Ref.
Sodium Lauryl Sulfate											
10	–	ingredient	0.252 g to 7.95 g	Groups of 5F Wistar Rats	14	intubation	–	–	0.8–1.1 g	–	31
28.2	–	ingredient	4.5 to 7.5 ml or 4.2 to 7.0 g/kg	5M, 5F Sprague–Dawley rats per dose	14	intubation	–	–	6.3 ml	95% confidence limits– Upper–7.0 ml/kg Lower–5.6 ml/kg	32
86	25w/v	ingredient	1	5M, 5F Carworth Farm E rats	10	intubation	distilled water	–	1290 mg	No toxic signs; no gross or microscopic lesions.	33
–	–	ingredient	529 mg to 925 mg	Rats	–	–	–	–	–	Diarrhea and death	34
–	–	ingredient	–	Rats	–	–	–	–	1650 mg	Diuresis, diarrhea, lacrimation, salivation, tremors, convulsions, sedation, anesthesia, death. Dead rats showed hyperemia of liver and kidneys.	35
21	–	formulation	2.15 g	5/F Albino rats per group	7	intubation	none	0	3.10 g	No deaths at 2.15 g/kg dosage group. All 5 rats died within 24 hours in 5.0 g/kg group. No gross lesions.	36
			5.0 g	5/F Albino rats per group	7	intubation	none	5			
21	–	formulation	2.15 g	5/F Albino rats per group	7	intubation	none	0	2.71 g	Low-dose group showed depression for 24 hours. All animals died in high-dose group, but no gross lesions were observed.	37
			5.0 g	5/F Albino rats per group	7	intubation	none	5			

Ammonium Lauryl Sulfate											
27.4	–	ingredient	3.0 to 5.5 ml or 3.0 to 5.5 g/kg	5M, 5F Sprague–Dawley rats per dose	14	oral intubation	–	–	4.7 ml	Cause of death:pulmonary hemorrhage	38
15	–	formulation	6.81 ml	5M, 5F Albino rats	14	oral intubation	–	0	8–9 ml	Depression, labored breathing.	39
15	–	formulation	10.0 ml	5M, 5F Albino rats	14	oral intubation	–	4	8–9 ml	Depression, labored respiration, ataxia, ptosis, depressed reflexes, diarrhea. 4 died.	
15	–	formulation	1.0 ml	3F rhesus monkeys	14	oral intubation	–	0	>10 ml	One had emesis	40
15	–	formulation	5.0 ml	3F rhesus monkeys	14	oral intubation	–	0	>10 ml	3 had emesis	
15	–	formulation	10.0 ml	3F rhesus monkeys	14	oral intubation	–	1	>10 ml	One animal died; prior to death it had emesis and diarrhea; One other animal had emesis. No abnormal pathology.	

[a]*Acute Oral Toxicity* *(Prob. lethal dose to humans)*

1. >15 g/kg — Practically nontoxic
2. 5–15 — Slightly toxic
3. 0.5–5 — Moderately toxic
4. 50–500 mg/kg — Very toxic
5. 5–50 — Extremely toxic
6. <5 — Super toxic

Sodium Lauryl Sulfate (86% active material) was administered to groups of five male and five female Carworth Farm E strain rats to test its acute oral toxicity. The compound was diluted with distilled water to 25% w/v and was given in a single dose by ingastric intubation. During the 10-day observation period following dosing, no abnormal symptoms appeared in the rats, nor were there any gross or microscopic changes. The acute oral LD50, with 95% confidence limits, was found to be 1290 mg/kg.[33]

Sodium Lauryl Sulfate administered orally to rats in doses of 529–925 mg/kg produced diarrhea and death.[34]

The acute oral LD50 to rats of Sodium Lauryl Sulfate was found to be 1650 mg/kg. Signs of intoxication included: diuresis, diarrhea, lacrimation, salivation, tremors, convulsions, sedation, anesthesia, and death. Dead rats had hyperemia of the liver and kidneys.[35]

Two formulations, each containing 70% of a 30% solution of Sodium Lauryl Sulfate (actual concentration was 21%) were tested for acute oral toxicity in groups of five female albino rats. Each formulation was administered by intubation in doses of 2.15 and 5.0 g/kg. One formulation produced no deaths or abnormalities at the 2.15 g/kg dose; however, all animals given the higher dose died within 24 h. The acute oral LD50 for the formulation was calculated to be 3.10 g/kg.[36] The second formulation caused depression in the low-dose group, but no deaths occurred. All animals administered the 5.0 g/kg dose died, but there was no observable gross lesions at necropsy. The acute oral LD50 was calculated to be 2.71 g/kg.[37]

A solution of the ingredient containing 27.4% Ammonium Lauryl Sulfate was tested on five groups of 10 male and female Sprague–Dawley rats. Dosage levels were 3.0, 4.0, 4.5, 4.75, and 5.0 ml/kg (or 3.0, 4.0, 4.5, 4.73, and 5.0 g/kg); the number of animals that died in each dosage level were 0, 1, 5, 4, and 10, respectively. The LD50 for the test material was 4.7 ml/kg. Pulmonary hemorrhage was the cause of death.[38]

A shampoo formulation containing 15% Ammonium Lauryl Sulfate was administered in 6.81 and 10 ml/kg doses to five albino rats per dosage level. No animals died in the low-dosage group, but four of the five in the high-dose group died within two days. Signs of toxicity occurred within 4 h of intubation. Rats in both groups had signs of depression and ataxia, but those in the high dosage group also suffered ptosis, ataxia, depressed righting and placement reflexes, weight loss, excessive urination and diarrhea. The single survivor at the 10 g/kg level recovered within 8 days. Necropsy findings in animals dying during the study were congested lungs, kidneys, livers, and adrenals; blanching of gastric mucosa and/or inflammation of the tract. At necropsy, sacrificed animals had lesions, hyperplasia of the gastric mucosa, and adhesions between the stomach, spleen, liver, diaphragm and rib cage. The LD50 for the formulation was 8–9 ml/kg.[39]

Three groups of three female rhesus monkeys were used to test the acute oral toxicity of a shampoo containing 15 percent Ammonium Lauryl Sulfate. After an 8 h fast, the animals in each group were given either 1.0, 5.0, or 10.0 ml/kg and were observed for 14 days. One of three monkeys at the 1.0 ml/kg level and all three at the 5.0 ml/kg level showed emesis 2 h after administration; no deaths oc-

curred. The 10 ml/kg dose caused one death 8–16 h after dosing. This animal had emesis and severe diarrhea, and one other animal had emesis. Gross and histopathologic examinations of the GI tract, esophagus, liver, kidney, and spleen were negative. The LD50 of the shampoo was greater than 10 ml/kg.[40]

Ocular

The following studies are outlined in Table 5.

The ocular irritancy potential of Sodium and Ammonium Lauryl Sulfate was tested according to the Draize method. Concentrations of 2%, 10%, and 20% each were instilled in 0.1 ml volumes into the left eye of five rabbits. The right eyes were used as untreated controls. Draize scores were used to assess irritation at 1, 24, 48, and 72 h, and four and seven days. The average eye irritancy scores of Sodium Lauryl Sulfate were as follows: at 2%, the compound was mildly irritating (Draize score = 21) at 1 h and decreased to practically nonirritating during the seven-day observation; at 10%, the compound was moderately irritating at 1–24 h, decreasing to mildly irritating at seven days; at a 20% concentration, the compound was severely irritating at 24 h, decreasing to mildly irritating after seven days. Ammonium Lauryl Sulfate at 2% was mildly irritating, decreasing to practically nonirritating at seven days; at 10% and 20%, the compound was severely irritating at 24 h, and mildly irritating thereafter.[42]

The ocular toxicity of Sodium Lauryl Sulfate was tested on three rabbits per concentration studied (25%, 5%, and 1% aqueous solutions). The eyes were examined for existing corneal damage 24 h prior to treatment with a 5% solution of fluorescein disodium salt. Two drops of test solution were applied to each eye and within 30 sec, one eye was washed with flowing tap water for 2 min; the other eye was left unwashed. Both eyes were observed for immediate effects and then after 1, 24, and 48 h, and one week for conjunctival and corneal injury, iritis, and lenticular damage. Both the irrigated and unwashed eyes showed: at 1%, very slight conjunctivitis; at 5% and 10% there was slight to moderate corneal injury.[31]

The method of Draize and Kelley was used to study the acute ocular toxicity of Ammonium Lauryl Sulfate in concentrations of 1.25%, 2.5%, 5%, 10%, and 20%. Three albino rabbit eyes per concentration were used for the procedure. For each concentration, one eye was unwashed, one eye was irrigated 2 sec after instillation of 20 ml of 37 °C distilled water, and the eye of the third rabbit was rinsed 4 sec after instillation. One untreated eye which served as a control was rinsed with water. Observations were made 1 h and 1, 2, 3, 4, and 7 days after application and standard Draize scores were used for evaluation. The 1.25% and 2.5% concentrations were moderately irritating to minimally irritating when not rinsed; rinsing decreased irritation in all cases. Moderate to severe irritation occurred in the 5%–20% concentrations. Irritation decreased with rinsing and over the seven days of observation.[52]

Sodium Lauryl Sulfate was tested in 10% and 100% concentrations on rabbit eyes. When not rinsed, the 10% concentration caused corneal opacity, stippling, and red and swollen conjunctiva in all three animals at 24 h; one animal had corneal stippling. The eyes of three animals were instilled with 100% Sodium Lauryl Sulfate, followed by a rinse after 4 sec. Corneal opacity and vacularization was

TABLE 5. Ocular Irritation.

Conc. (%)	Dose (ml)	Solution/ Formulation	No. of Albino rabbits	Observation days	Comment[a]	Ref.
Sodium Lauryl Sulfate						
2	0.1	Solution	5	7	Draize criteria; day 1 – mild irritation. At 7 days – practically nonirritating.	42
10	0.1	Solution	5	7	One to 24 h – moderately irritating. At 7 days – mildly irritating.	
20	0.1	Solution	5	7	At 24-h – severely irritating. At 7 days – mildly irritating.	
1	–	Solution	3	7	One eye washed 30 sec after instillation. Both washed and unwashed eyes showed very slight conjunctivitis.	31
5	–	Solution	3	7	One eye washed 30 sec after instillation. Both washed and unwashed eyes showed slight to moderate corneal injury.	
25	–	Solution	3	7	One eye washed 30 sec after instillation. Both washed and unwashed eyes showed slight to moderate corneal injury.	
10	–	Solution	3	35	No rinse. Corneal opacity, iritis, red, swollen, conjunctiva in all three to 24 *hours,* only. Corneal strippling.	43
100	–	–	3	35	Rinse. Corneal opacity, vacuolization, iritis, and red, swollen conjunctiva in all 3 to 24 h.	
100	–	–	3	35	No rinse. Corneal opacity, vacuolization, iritis, red, and swollen conjunctiva in all three.	

28.2	0.1	Solution	9	14	Three animals' eyes were irrigated after 30 sec. Draize criteria. Unwashed eyes scored 35.6; moderate eye irritation. Washed eyes scored 40.6; moderately irritating.	44
30	0.1	Solution	6	7	Draize criteria; No rinse; Day 1 score = 10; Day 2 = 0. Minimal irritation when rinsed. Rinse; Day 1 score = 5; Day 7 = 0.	45
30	0.1	Solution	6	7	Draize criteria; No rinse; Day 1 score = 32; Day 7 score = 5. Rinse; Day 1 score = 30; Day 7 score = 11; Moderate irritant when followed by a rinse.	46
30	0.1	Solution	6	7	Draize criteria. No rinse; Day 1 = 26; Day 7 = 0. Rinse; Day 1 = 18 and 26; Day 7 = 0, and 0. Mild irritant.	47
5.1	0.1	Formulation	6	7	Draize criteria. Day 1 score = 18; Day 4 score = 0. Mild irritation.	48
21	0.1	Formulation	6	7	Draize criteria. No rinse. Day 1 score = 31, Day 7 score = 35. Severe irritant Rinse; Day 1 score = 0; Day 2 score = 5; Day 7 score = 0; Mild irritant.	49
21	0.1	Formulation	6	7	Draize criteria. No rinse; Day 1 score = 33. Day 7 score = 15. Moderately irritating. Rinse: Day 1 score = 7; Day 3 = 0. Minimally irritating.	50
26	0.1	Shampoo formulation	5	7	Draize criteria. Rinsed eyes–Day 1 score = 13; Day 7 score = 1; Mild irritant.	51

TABLE 5. (Continued.)

Conc. (%)	Dose (ml)	Solution/ Formulation	No. of Albino rabbits	Observation days	Comment[a]	Ref.
Ammonium Lauryl Sulfate						
2	0.1	Solution	5	7	Day 1–mildly irritating. At 7 days–practically non-irritating.	42
10	0.1	Solution	5	7	Day 1–severely irritating. At 7 days–mildly irritating.	
20	0.1	Solution	5	7	Day 1–severely irritating. At 7 days–mildly irritating.	
1.25	0.1	Solution	3	7	Method of Draize and Kelley. Standard Draize scores. One eye in each concentration level was unwashed; one eye was washed 2 sec after instillation and the 3rd washed after 4 sec.	52
2.5	0.1	Solution	3	7	1.25 and 2.5% concentrations are minimally to moderately irritating with no rinse. Rinsing decreased irritation in all cases.	
5.0	0.1	Solution	3	7	Moderate to severe irritation in unwashed eyes. Rinsing decreased over the seven days.	
10	0.1	Solution	3	7	Moderate to severe irritation in unwashed eyes. Rinsing decreased over the seven days.	
20	0.1	Solution	3	7	Moderate to severe irritation in unwashed eyes. Rinsing decreased over the seven days.	
27.4	0.1	Solution	9	7	Draize criteria. Rinsed eyes (3 rabbits). Average Day 1 score = 30.7. Moderately irritating. Unwashed eyes–Average Day 1 score = 36.0. Moderately irritating.	53

8.4	0.1	Formulation	9	14	Draize criteria. 6 eyes unwashed (N); 3 eyes washed (W). Both groups had slight conjunctival irritation at 24 h (score = 2.0N, 0.67W) and disappeared thereafter.	54
9.8	0.1	Formulation	9	14	Draize criteria. 6 eyes unwashed (N); 3 eyes washed (W). Slight conjunctival irritation occurred in unwashed eyes at 24 hrs (score = 1.0). No irritation in washed eyes.	55
11.2	0.1	Formulation	9	14	Draize criteria. 6 eyes unwashed (N). 3 eyes washed (W). Slight conjunctival irritation occurred in unwashed eyes for 48 hrs (score = 0.7) and in washed eyes for 24 h (score = 0.7)	56
11.2	0.1	Formulation	9	14	Draize criteria. 6 eyes unwashed (N). 3 eyes washed (W). Slight conjunctival irritation occurred in unwashed eyes for 48 h (score = 0.3). No irritation occurred in washed eyes.	57
15	0.1	Shampoo formulation	10	7	Draize criteria. Rinsed eyes. Day 1 score = 15; Day 7 score = 1; Mild, transient irritation. Unrinsed eyes; Day 1 score = 34, Day 7 score = 6; Moderate, transient irritation.	58
15	0.1	Shampoo formulation	10 Monkeys	7	Rinsed eyes (4 sec). No irritation. One monkey with eyes washed after 15 sec showed moderate irritation. Unrinsed eyes. Moderate irritation to seven days.	59

[a]Draize scoring criteria; scale ranges from 0 (no irritation) to 110 (extreme irritation).

seen in all three animals, as well as iritis and swollen and red conjunctiva at 24 h. The unwashed eyes of three animals instilled with 100% detergent showed corneal opacity (present in one animal to Day 35), corneal vacularization, iritis, and red and swollen conjunctivae in all animals.[43]

Solutions of the ingredient Sodium Lauryl Sulfate in concentrations of 28.2% and 30% were tested with and without rinse, using albino rabbits. Mild to moderate ocular irritation occurred in all eyes. In some cases, rinses mitigated the effects of the detergent. See Table 5 for test results.[44-47]

Sodium Lauryl Sulfate was tested in formulation according to the Draize method. One product contained 68% of the ingredient which was supplied as a 30% solution. When the shampoo was diluted to 25% in water, the actual ingredient concentration was 5.1%. It was a mild irritant to the eyes of the six rabbits tested, and all irritation disappeared by Day 4.[48]

Two products were tested according to the Draize method on albino rabbits. The final test dilution of the Sodium Lauryl Sulfate therein was 21% in both formulations. According to the Draize scores, one formulation was a severe irritant to Day 7 when not rinsed, and a mild irritant following the rinse;[49] the second product was moderately irritating with no rinse and minimally irritating following the rinse.[50]

One product containing 26% Sodium Lauryl Sulfate was tested in five rabbits according to the Draize method, except that all eyes were rinsed after 4 sec. Mild irritation was produced on Day 1; irritation decreased to Day 7. The formulation was a mild irritant.[51]

The ingredient, Ammonium Lauryl Sulfate (active concentration 27.4%), was tested according to the Draize method on nine albino rabbits. The eyes of three rabbits were washed after 4 sec while the remaining six were unwashed. The washed eyes had an average Draize score of 30.7 on Day 1, and the unwashed eyes had an average score of 36.0 in the same time period. Eyes were moderately irritated by both procedures.[53]

Modified Draize eye irritation studies were performed on four products containing 30%, 35%, and 40% (two products) Ammonium Lauryl Sulfate. The detergent was supplied to the manufacturer as a 28% solution so that the actual concentration of Ammonium Lauryl Sulfate in the products was 8.4%, 9.8%, and 11.2%, respectively. In all tests, 0.1 ml of each product was instilled into one eye of each in four groups of nine rabbits. The eye of six rabbits in each group were left unwashed, while washings were performed on the eyes of the remaining groups of three rabbits. Observations were made at 24, 48, and 72 h, and seven and 14 days. The product containing 8.4% detergent produced slight irritation in both unwashed eyes (score = 2.0) and washed eyes (score = 0.67) at 24 h. The irritation disappeared thereafter.[54] The product containing 9.8% Ammonium Lauryl Sulfate caused slight conjunctival irritation in unwashed eyes for 24 h (score = 1.0) but no irritation in washed eyes.[55] Slight conjunctival irritation (score = 0.7) occurred for 48 h in unwashed eyes from one product containing 11.2% Ammonium Lauryl Sulfate. Washed eyes suffered slight conjunctival irritation (score = 0.7) for 24 h.[56] Similar low levels of conjunctival irritation (score 0.3) occurred in the unwashed eyes instilled with the final product containing 11.2% detergent. No irritation occurred in washed eyes.[57]

Two formulations, both containing 15% Ammonium Lauryl Sulfate, were tested for eye irritation. One study used 10 albino rabbits in a Draize test. Unwashed eyes showed mild, transient irritation, while washed eyes showed moderate, transient irritation.[58] The second formulation was tested according to Draize protocol on 10 cynomulgous monkeys. All animals received in one eye 0.1 ml of the shampoo. Four eyes were rinsed after 4 sec, one after 15 sec (inadvertently), and the remaining five were unwashed. Eyes rinsed after 4 sec showed no irritation, while the one rinsed after 15 sec and those eyes left unwashed showed moderate irritation to seven days.[59]

The studies show that irritation increases with increasing ingredient concentration.

The acute ocular irritancy of Sodium and Ammonium Lauryl Sulfate was assessed using the blepharospasm test.[42] The surfactants were instilled in a 0.1 ml volume into the eyes of 10 rabbits. The onset time of each blepharospasm (spasmodic blink) was recorded during the 30 sec after the initial reflex blink. Positive responses were graded according to the number and rate of complete blepharospasms as follows: 100% response = 3 complete blinks; 50% response = 2 complete blinks; 25% response = 1 blink. The response rate for a particular concentration is obtained by multiplying the number of eyes responding by the percent response and dividing by the number of eyes tested. BD50 values, concentration at which 50% of the test animals elicit a 100% response, were also determined. No controls were reported. Results are shown in Table 6.

New Zealand white rabbits were used to study the acute ocular toxicity and maximum delay time for irrigation of Sodium Lauryl Sulfate. A 10% w/v aqueous solution of the compound in a 0.1 ml volume was instilled into one eye of each rabbit. The other eye served as the control. The eyes of 11 rabbits were not irrigated and served as the positive control. In the other rabbits irrigation was performed at 4, 10, 20, 30, 60, or 120 sec after surfactant instillation with either 20 or 100 ml of 37 °C water. The eyes were examined 1 and 4 h after instillation and after 1, 2, 3, 4, 7, 14, 21, 28, and 35 days, or until the reaction disappeared. Scoring was according to 16 CFR 1500.42, and results are tabulated in Table 7.

Where irrigation was delayed for 20 sec or longer, corneal opacity or dulling

TABLE 6. Blepharospasm Test Results.

Surfactant	*Percent conc. ($\times 10^{-6}$)*	*Response Rate*	*BD50 (95% conf. limits)*
Sodium Lauryl Sulfate	1.5	0.4	4.6×10^{-6}(1×10^{-6}– 2.1×10^{-5})
	3.8	0.4	
	7.5	0.625	
	12.8	0.6	
Ammonium Lauryl Sulfate	1.5	0.325	4.1×10^{-6}(2×10^{-6}–8×10^{-6})
	4.5	0.50	
	9.0	0.575	
	16.5	0.825	
	27.0	0.925	

Data from Ref. 42.

TABLE 7. Acute Ocular Toxicity and Effect of Irrigation of 10% Sodium Lauryl Sulfate.

	Number of rabbits showing corneal damage						
	Delay time before irrigation of eye (sec)						
Effects	*4*	*10*	*20*	*30*	*60*	*120*	*No irrig.*
No opacity	7	4	–	–	–	–	–
Lack of luster	4	8	5	7	5	4	2
Opacity grade 1	–	1	3	2	5	4	8
2	–	–	–	1	–	–	1
3	–	–	–	1	–	–	–
Total no. eyes treated	11	13	8	11	10	8	11

Data from Ref. 60.

of the surface occurred in every eye. Reactions of eyes of animals exposed to the surfactant for 20 or 30 sec before wash were similar to those observed in animals subjected to a 120 sec irrigation delay, and only slightly better than eyes receiving no irrigation at all. Eyes irrigated before 20 sec showed fewer instances of damage, and where irritation was evident, it was less severe and less persistent. When irrigation occurred before 10 sec, only one of 24 animals showed corneal opacity. A 4 sec exposure to Sodium Lauryl Sulfate elicited no opacities, but did dull corneal luster in four animals. The critical exposure time before damage is produced in the rabbit eye by this surfactant is between four and 10 sec.[60]

Inhalation

The potential of Sodium and Ammonium Lauryl Sulfate to produce upper respiratory tract irritation was studied using Swiss strain albino mice. Eight to 12 mice were used for each of six surfactant concentrations in air. The surfactants were aerosolized into a 1 L inhalation chamber. Animal responses were determined at various atmospheric concentrations. The mice were exposed to each concentration for two minutes and recovery was monitored for up to 20 min. The average respiratory rate was determined for each animal as a control and the percent change from this control rate was determined for each concentration as peak inhibition. The RD50 value, the chamber concentration at which there was a 50% reduction in respiratory rate, was determined for each compound. The RD50s for Sodium and Ammonium Lauryl Sulfate are 88 μg/l (3.67–259.1 μg/l confidence limit) and 114.5 μg/l (59.3 to 213.9 μg/l confidence limits), respectively.[42]

The irritancy of Sodium and Ammonium Lauryl Sulfate on the upper respiratory tract was studied using mice and rabbits. Irritation of the respiratory tract produces a reflex inhibition of respiration; therefore, the amount of inhibition was taken as a measurement of irritation. The animals were exposed to aerosolized 15% and 25% aqueous solution of the surfactants for 2–5 min by the head exposure method. Four to eight animals were used per exposure level of 130, 175, or 73 μg/l air. The aerosolized particles were less than 12.5 μm in diameter. The rabbits, at all exposure levels, suffered a 50%–60% inhibition of respiration with Sodium and Ammonium Lauryl Sulfates. Mice exposed to both surfactants, at a

concentration of 73 μg/l, showed a 35%–40% inhibition. At 130 μg/l, the inhibition for both surfactants was 2%–35%, and for 170 μg/l, 55%–65%.[61]

Intraperitoneal injection

The mouse writhing test was used to assess the irritancy potential of Sodium and Ammonium Lauryl Sulfate. Both test surfactants were administered IP to 10 animals at each concentration of 0.063%, 0.125%, 0.250%, and 1.0% in a 0.2 ml volume. After injection, the mice were observed for a positive response up to 5 min. Positive responses of classical writhing included: contraction of abdomen with extension of fore and hind limbs, preening at injection site, listlessness, high stepping gait, ataxia, and running with arched back. Untreated animals served as controls. WD50 values (concentrations at which 50% of the animals elicited a response) were calculated. Both Sodium and Ammonium Lauryl Sulfate produced writhing in 30% of the mice at the 0.063% concentration, in 70% at the 0.125% concentration, and in 100% at the 0.250% and 1.0% concentrations. WD50 concentration values for both surfactants are 0.086 percent with 0.06%–0.12% confidence limits.[42]

An intraperitoneal LD50 to rats, reported by Woodard and Calvery,[62] was given as 210 mg/kg.

Skin irritation

The effect of Sodium Lauryl Sulfate on rat skin was studied both grossly and microscopically. Six eight-week old female Colworth/Wistar rats were shaved free of fur on the dorsal region. This shaved area was wetted twice daily for three days with a 1% aqueous Sodium Lauryl Sulfate solution and a solution of 0.5% sodium sulfate was applied to three other rats as a control. A small piece of skin from the treated area was excised for ultrastructural study. After the three days of treatment, the treated areas showed signs of coarsening, reddening, and edema. Electronmicroscopy showed the following epidermal changes: deposition of lipids in mast cells, spongiosis and vesiculation, partial detachment of basal cells from basal lamina, condensation of tonafibrils within cells of the stratum basale and stratum spinosum, protein deposition between the deeper epidermal cells, and thickening of the epidermis and stratum corneum. There was a reduction in the number of keratohyalin granules and some parakeratosis and incomplete keratinization.[63]

The irritancy of Sodium Lauryl Sulfate was tested on rat skin in vivo. The surfactant was applied to the shaved dorsal skin of weanling rats as a 0.25 *M* solution (between 5% and 10% solutions by weight). The solution was applied twice daily for three consecutive days. After one and three days, the irritation was assessed. Inspection of the treated site after Day 1 showed no changes in the skin when compared to the water-treated controls. After three days, however, Sodium Lauryl Sulfate caused thickening of the epidermis with scaling and cracking of the stratum corneum.[20]

Sodium Lauryl Sulfate was applied to two groups of eight-week-old female TFI mice. Their hair was in telogen and was clipped from the back. A 0.5 ml volume of reagent was applied to a 2 cm^2 area of clipped skin. The first group of 15 animals received a single application of surfactant as a 10% w/v concentration in distilled water and controls were treated with distilled water. Tissue samples

were taken from treated animals at 30, 60, and 120 min after treatment. The exposed areas showed slight thickening and erythema 1.5–2 h after treatment. Thirty minutes after treatment, 30%–40% of the squamous cells of the epidermis exhibited an intracellular vacuolation or edema. After 1 h, 80%–90% of the cells of the epidermis displayed this anomaly; however, the stratum corneum was normal despite these epidermal changes. After 2 h, there occurred cellular degeneration and necrosis, edema, leucocyte infiltration and some engorgement of the more superficial blood vessels. The 15 mice in the second group were exposed to repeated applications of the surfactant daily for 2, 5, or 9 days. Concentrations of the surfactant were 1% or 2.5% w/v in distilled water. The mice were sacrificed 24 h after the last treatment and tissue samples were taken at that time. Treatment with 1% percent Sodium Lauryl Sulfate caused erythema after 8–9 applications. With application of 2.5%, minimal epidermal changes were seen after two days. After five days, the epidermal changes were more pronounced. The stratum corneum spinosium were thickened and focal intracellular edema occurred. Widespread edema and capillary engorgement occurred in the dermis. Similar, but less intense reactions occurred at this stage in skin exposed to 1% Sodium Lauryl Sulfate. With both 1% and 2.5% solutions, there occurred further thickening of the stratum corneum, nuclear debris in the keratin layer, and an increase in the number of epidermal cells exhibiting intracellular edema. Approximately 80% of the cells were affected and there appeared numerous foci of cellular degeneration and necrosis.[64]

The acute skin irritation of Sodium Lauryl Sulfate was studied using the shaved, intact and abraded bellies of rabbits. Aqueous dilutions of 1%, 5%, and 25% were applied in 5 ml volumes. Ten such samples were applied to cotton pads and held by bandages over a period of 14 days to the intact skin and three applications were made to the abraded areas. A small amount of material was likewise applied to the ear. Applications were discontinued in the event of substantial burn or eschar formation. Skin reactions were recorded after each application and at intervals up to three weeks from the beginning of the study. The results show that when applied to the belly, a 1% solution of Sodium Lauryl Sulfate caused very slight to slight erythema; a 5% solution caused moderate chemical burns, and a 25% solution caused severe chemical burns. When applied to the ear, the 1% and 5% solution caused very slight erythema; a 25% solution caused severe chemical burns.[31]

Groups of four New Zealand rabbits were used to study the irritancy of Sodium Lauryl Sulfate. The hair on four areas of the dorsal surface of each rabbit was clipped and two of these areas were scarified. The rabbits received Sodium Lauryl Sulfate on one test site, two other test surfactants on two other sites, and water on the last site (control). The areas of application were rotated so each surfactant occupied a different area on each rabbit. A 10% w/v concentration of Sodium Lauryl Sulfate in water, in a 0.5 ml volume, was applied under occlusion for 24 h. The patches were removed and graded according to the Draize method 30 min later, and a second set of readings was made 48 h later. This procedure was repeated three times. The average Draize score (Primary Irritation Index [PII]) for the three repeat applications of the surfactant was 1.48 out of a possible 8. A PII of 2 or less is considered mildly irritating.[65]

The standard Draize skin irritancy test procedure was used to assess the effect of Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate on rabbit skin. The surfactants, tested at 2%, 10%, and 20% concentrations, were applied to the sites and covered for 24 h. Readings were taken at 24 and 72 h, and scored according to the Draize method. The 2% concentrations of Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate gave scores of 5.2 and 5.3, respectively. Scores of this magnitude show that the surfactants are primary irritants and are highly dangerous. The 10% concentrations for Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate scored 6.0 and 5.9, respectively. The 20% concentrations scored 6.0 and 6.0, respectively. Both of these surfactants in the 10% and 20% concentrations, like the 2% concentration, are primary skin irritants.[42]

A 10% solution of Sodium Lauryl Sulfate was tested according to the Modified Draize method on a scoring scale of 0–4. A 0.1 ml volume was applied under occlusion to the intact and abraded skin of nine albino rabbits for 24 h. The ingredient was severely irritating when sites were evaluated 2 and 24 h after application. The Primary Skin Irritation (PSI) score was 3.91 out of 4.0.[66]

Sodium Lauryl Sulfate, in a 28.2% concentration, was tested in a Department of Transportation (D.O.T.) skin corrosion test and in a Draize primary skin irritation test. In the D.O.T. test, 0.1 ml of the substance was applied to intact skin of six New Zealand albino rabbits for 4 h and then washed off. Corrosion readings were made at 4 and 48 h after exposure. The 4 h reading was negative but the 48 h reading showed that the material was a corrosive agent. Corrosion was considered to have occurred if the substance caused destruction or irreversible tissue alteration.[67] This same substance was tested according to the Draize method on the intact and abraded skin of six albino rabbits. A 0.5 ml volume of the material was applied under occlusion for 24 h, and scores were graded on the Draize scale of 0–8. The patches were then removed, sites were washed and then evaluated 24 and 72 h after exposure. In intact and abraded skin at 24 and 72 h, there occurred erythema and eschar. Edema occurred in intact skin at 24 h and in abraded skin at 24 and 72 h. The ingredient was a moderate primary irritant with a score of 4.54 out of 8.0.[68]

A 30% and a 100% concentration of Sodium Lauryl Sulfate were both tested in a Modified Draize test as above, with a scoring scale of 0–4. When applied to the nine albino rabbits, the 30% solution produced severe irritation. The PSI was 4.0.[69] The 100% solution produced a PSI of 2.96; the animals suffered moderate irritation.[70]

The Draize method was used to test the skin irritation of a shampoo containing 26% Sodium Lauryl Sulfate. The formulation was diluted so that the actual concentration of the ingredient was 0.65%. When applied to six albino rabbits, the compound produced mild irritation.[71]

Ammonium Lauryl Sulfate, in a concentration of 27.4%, was tested in both the D.O.T. and Draize Method tests, as above. In the D.O.T. test, Ammonium Lauryl Sulfate produced no corrosion at 4 h, but it was corrosive to the rabbits' skin when observed at 48 h.[72] In the Draize test, the ingredient produced a PII of 4.71; this indicates moderate primary irritation to the abraded and intact skin.[73]

A shampoo containing 15% Ammonium Lauryl Sulfate was tested according

to the Draize method. The material was diluted so that the actual concentration of the ingredient was 1.5%. When tested on four albino rabbits, the compound scored a 6.0, which indicated that the formulation was a severe irritant[74] (see Table 8).

Dermal toxicity

The dermal LD50 to rabbits of Sodium Lauryl Sulfate is greater than 10,000 mg/kg. Signs of intoxication included diarrhea, salivation, incoordination, and death. Dermal irritation included severe erythema and edema with subdermal hemorrhaging.[35]

The acute dermal toxicity of a shampoo containing 26% Sodium Lauryl Sulfate was tested on four New Zealand albino rabbits. One 10 ml/kg dose of the product was applied for 24 h under occlusion to the clipped skin of the back and flanks of each animal. After 24 h, the material was removed and observations of toxicity were made at 4 and 24 h, and daily for a total of 14 days. Necropsies were performed after 14 days. The single 10 ml/kg application caused severe skin irritation, including erythema, edema, desquamation, and blanched, discolored, necrotic and fissured skin. "The damaged skin sloughed during the second week, and the underlying skin was thickened with eschar formations." Signs of systemic toxicity by percutaneous absorption included: depression, labored respiration, abnormal positions of hind legs, and nasal discharge. One animal died on Day 3 of observation. The acute dermal LD50 was greater than 10 ml/kg.[75]

A shampoo containing 15% Ammonium Lauryl Sulfate was tested for acute dermal toxicity on four New Zealand albino rabbits. The material was diluted so that the actual concentration of the ingredient was 0.75%. A dosage level of 10 ml/kg of the diluted material was applied to each animal, and the testing procedure continued as above. After 24 h of contact, gross signs of dermal irritation included: moderate erythema and slight edema, accompanied by small blisters. The edema cleared after 48 h as did the erythema after 11 days. Moderate atonia and desquamation occurred in all animals; these conditions persisted until Day 14 in three animals. There were no gross signs of systemic toxicity; no animals died and the LD50 of the 1:20 dilution was greater than 10 ml/kg.[76]

Sodium Lauryl Sulfate-mediated sensitization

Nilzen and Wikstrom[77] demonstrated that guinea pigs became sensitive to nickel and chrome only when these agents were mixed with Sodium Lauryl Sulfate. In this experiment 25 male and female white guinea pigs were clipped free of fur on one side of the body. Groups of five animals each were painted for eight days with 1% aqueous Sodium Lauryl Sulfate, 0.5% aqueous potassium dichromate, 4% aqueous nickel sulfate, an aqueous mixture of 1% lauryl sulfate and 0.5% potassium dichromate, and an aqueous mixture of 1% lauryl sulfate and 4% nickel sulfate. Paintings with Sodium Lauryl Sulfate, potassium dichromate, and nickel sulfate produced mild to no reactions. Animals painted with the mixtures, however, showed scaling, erythema, and infiltration. Testing 12 days after the start of the experiment with both the metal salts and the mixtures evoked erythema and infiltration. The authors stated that the eczematogenic properties of the metals are "brought about by the permeability of the skin in-

TABLE 8. Skin Irritation: Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate.

Conc. (%)	Dose (ml)	Solution or formulation	Species and number	Protocol	Irritation score / Max. score	Contact time (day)	Observation time (day)	Comment	Ref.
Sodium Lauryl Sulfate									
0.5	–	Solution	6 Colworth-Wistar rats	–	–	3	3	Applications twice daily for 3 days. Skin was coarse, red, edematous. There were microscopic changes.	63
1.0	–	Solution		–	–	3	3		
5–10	–	Solution	Rats	–	–	3	1,3	Applications twice daily for 3 days. After Day 3, thickening of epidermis and scaling and cracking of stratum corneum was observed.	20
10	0.5	Solution	15 Female TFI mice	–	–	30, 60, 120 min	120 min	Slight thickening and erythema 1.5 to 2 h after treatment. Intracellular vacuolization of squamous cells after 1 h.	64
1	0.5	Solution	15 Female TFI mice	–	–	2, 5, 9	10	After 8–9 applications, erythema occurred.	
2.5	0.5	Solution	15 Female TFI mice	–	–	2, 5, 9	10	After 5 days, thickened stratum corneum and intracellular edema.	
1	5.0	Solution	Rabbits	–	–	14	–	Ten applications in 14 days. Slight erythema on abdomen and ear.	31
5	5.0	Solution	Rabbits	–	–	14	–	Ten applications in 14 days. Moderate chemical burns on abdomen; slight erythema on ear.	
25	5.0	Solution	Rabbits	–	–	14	–	Ten applications in 14 days. Severe chemical burns on abdomen and ear.	
10	0.5	Aqueous solution	4 New Zealand rabbits	Draize	1.48/8.0	1	1,2	Three 24 h applications on intact and abraded skin. Skin evaluated after each 24 and 48 h period. Solution was mildly irritating.	65

TABLE 8. (Continued.)

Conc. (%)	Dose (ml)	Solution or formulation	Species and number	Protocol	Irritation score: Max. score	Contact time (day)	Observation time (day)	Comment	Ref.
Sodium Lauryl Sulfate (Cont'd.)									
2	0.5	Solution	Rabbits	Draize	5.2/8.0	1	1,3	Primary skin irritant, highly dangerous.	42
10	0.5	Solution	Rabbits	Draize	6.0/8.0	1	1,3	Primary skin irritant, highly dangerous.	
20	0.5	Solution	Rabbits	Draize	6.0/8.0	1	1,3	Primary skin irritant, highly dangerous.	
10	0.1	Solution	9 Albino rabbits	Modified Draize (PSI)	3.91/4.0	1	2	Applied to shaved skin on filter disc under occlusion. Ingredient was severely irritating.	66
28.2	1.0	Solution	6 New Zealand rabbits	D.O.T.	–	4 hrs	4 h, 2 days	No skin corrosion at 4 h. Positive skin corrosion at 48 h. Material is considered to be corrosive if it causes destruction or irreversible alteration of the tissue.	67
28.2	0.5	Solution	6 New Zealand rabbits	Draize	4.54/8.0	1	1,3	Erythema and eschar in abraded and intact skin at 24 and 72 h. Edema occurred in intact skin at 24 h and in abraded skin at 24 and 72 h. Ingredient was a moderate primary irritant.	68
30	0.1	Solution	9 Albino rabbits	Modified Draize (PSI)	4.0/4.0	1	2	Applied to shaved skin on filter disc under occlusion. Ingredient was severely irritating.	69
100	0.1	–	9 Albino rabbits	Modified Draize (PSI)	2.96/4.0	1	2	Applied to shaved skin on filter disc under 24 h occlusion; 5 min rinse-off. Uniform, red erythema. Ingredient was moderately irritating.	70
0.65	0.5	Shampoo formulation	4 New Zealand albino rabbits	Draize	1.6/8.0	1	3 or 7	Applied under occlusion to intact and abraded skin for 24 h. Compound was mildly irritating to rabbit skin according to Draize scores.	71

Ammonium Lauryl Sulfate									
2	0.5	Solution	Rabbits	Draize	5.3/8.0	1	1,3	Primary skin irritant, highly dangerous according to Draize.	42
10	0.5	Solution	Rabbits	Draize	5.9/8.0	1	1,3	Primary skin irritant, highly dangerous.	
20	0.1	Solution	Rabbits	Draize	6.0/8.0	1	1,3	Primary skin irritant, highly dangerous.	
27.4	0.1	Solution	6 New Zealand rabbits	D.O.T.	–	4 hrs	4 h, 2 days	No skin corrosion at 4 h. Positive skin corrosion at 48 h. Material is considered to be corrosive if causes destruction or irreversible tissue alteration.	72
27.4	0.5	Solution	6 New Zealand rabbits	Draize	4.71/8.0	1	3	Material was applied under occlusion to abraded and intact skin. Material was a moderate primary irritant according to the Draize scores.	73
1.5		Aqueous solution of shampoo formulation	4 New Zealand albino rabbits	Draize	6.0/8.0	1	3	Applied under occlusion to intact and abraded skin for 24 h. According to the Draize scores, the material was a severe irritant.	74

creasing under the influence of lauryl sulphate [sic]." They also conjecture that the power of the allergen is enhanced by the surfactant; it can then combine with protein via the metabolic process to cause the dermatitis.

Subchronic

Oral

A 13-week feeding study in rats was carried out on Sodium Lauryl Sulfate. Twelve male and 12 female individually caged five-week old rats were fed dietary levels of 40, 200, 1000, or 5000 ppm active material. A control group of 18 males and 18 females received the basic diet. Daily observations were made on health, and weekly observations were made on body weight and food intake. Urine samples were obtained from the 5000 ppm group and checked for color, pH, protein, reducing substances, bile salts, and microscopic constituents. Terminal blood samples were made and checked for erythrocyte and leucocyte counts, hematocrit and hemoglobin, total plasma protein, urea concentration, and serum protein fractions. At necropsy, visceral organs were weighed and processed for histopathologic examination. The only significant finding was an increase in absolute organ weights in the rats of the highest dietary level, and increased hepatic weights occurred in females in the 5000 ppm group. No lesions were observed and 1000 ppm is considered the "no effect dietary level."[33]

Weanling male rats were fed for five months drinking water containing 0%, 0.05%, or 0.25% concentrations of Sodium Lauryl Sulfate. At the highest concentration (0.25%), the weights of the lung and kidney were increased. Histopathologic examinations of the major organs gave evidence of bronchopneumonia caused by ingestion of small amounts of Sodium Lauryl Sulfate. Activities of serum enzymes were not affected. At the 0.25% concentration, the hepatic triglyceride concentration increased but the serum triglyceride concentration decreased.[78]

Sodium Lauryl Sulfate was fed to albino rats for four months. At 2% in the diet, this surfactant produced slight but not significant growth retardation; a 4% concentration in the diet produced significant growth retardation, and 8% in the diet was lethal within two weeks, with severe diarrhea and bloating of the intestines. Gross examination showed only gastrointestinal irritation. Tissues were not examined microscopically.[79]

Chronic

Oral

Osborne Mendel strain albino rats were fed 0.25%, 0.5%,or 1.0% Sodium Lauryl Sulfate in the diet for two years. During this time, weight gains were normal. Tissues taken at necropsy were free of gross and microscopic abnormalities. The investigators concluded that Sodium Lauryl Sulfate is not toxic at dosage levels up to 1.0% (10,000 ppm) in the diet.[79]

Another two-year study on rats showed that 2,000 ppm (0.2%) Sodium Lauryl Sulfate in the diet gave "negative results."[80]

The chronic oral toxicity of Sodium Lauryl Sulfate was tested using four groups of two male and two female beagle pups. Each group received for one

year a diet containing 0.0% (control), 0.67%, 1.0%, or 2.0% Sodium Lauryl Sulfate. Blood samples were taken from each dog prior to testing and at 1, 3, 6, and 12 months to determine hemoglobin, hematocrit, total white and red cell counts, differential white counts, red cell fragility and sedimentation rate.

Body weights of dogs receiving 1% Sodium Lauryl Sulfate were comparable to those of control dogs and greater than dogs in the 0.67% group. Dogs in the 2% group had the lowest weight gains. Blood determinations found that, when compared with initial determinations, all dogs, including controls, had increased red cell count, hematocrit and hemoglobin. No abnormalities attributable to Sodium Lauryl Sulfate were found. Gross examinations found no abnormalities that could be associated with ingestion of the compound. One dog in the 2% group died after 24 weeks; it appeared emaciated and there was evidence of pneumonitis. Microscopic examinations of internal organs likewise showed no abnormalities. It was concluded that diets containing 0.67%, 1.0%, and 2.0% Sodium Lauryl Sulfate for one year caused no anatomical injury to dogs.[81,82]

Percutaneous toxicity

A shampoo containing 17.5% Ammonium Lauryl Sulfate was tested in a 91-day percutaneous toxicity study. The shampoo was diluted to 10%, making the ingredient concentration 1.75%. Five male and five female New Zealand albino rabbits received sixty-five 2 ml/kg doses of the dilution to unabraded skin. At the end of the study, the animals were observed for dermal and systemic effects. Skin effects included: erythema (moderate in 2 of 10 sites and severe in 8 of 10), edema in all sites, atonia (slight in 6 of 10 sites), desquamation (slight in 9 of 10 sites to moderate in 1 of 10 sites) and slight fissuring in 3 of 10 sites. Transient body weight loss was noted in three animals, but overall weight gains were recorded for all animals. Hematology showed no abnormalities that could be attributed to treatment and histology showed no treatment-related abnormalities, except those changes found in the skin. There were no deaths due to treatment.[83]

Special Studies

Mutagenesis

Sodium Lauryl Sulfate was fed to Colworth/Wistar rats for 90 days to study the effect of its ingestion on the chromosomes of cells in the bone marrow. Six male and six female rats were used for each dosage level of 1.13% and 0.56% Sodium Lauryl Sulfate in the diet. Control animals were fed the diet without the detergent. At the completion of the feeding phase of this study, the animals were sacrificed and the bone marrow of one femur of each animal was removed for examination. The results showed that feeding rats 1.13% and 0.56% Sodium Lauryl Sulfate in the diet for 90 days produced no more increase in chromosomal aberations nor did the chemical cause a clastogenic effect.[84]

Carcinogenesis

A one-year chronic oral study using beagles showed that Sodium Lauryl Sulfate at concentrations up to 2% in the diet was not tumorigenic or carcinogenic.[81,82]

Teratogenesis

The effect of dermal application of Sodium Lauryl Sulfate on pregnant mice and their fetuses was studied by Takahashi et al.[85] Five groups of 13-week old JCL/ACR type mice were mated; observance of a vaginal plug determined Day "0" of pregnancy. Daily applications of 1.5 ml/kg of 0.4%, 4.0%, and 6.0% aqueous Sodium Lauryl Sulfate were made to a 3 × 3 cm^2 shaved area of the backs of three groups of mice on Days 6–13 of pregnancy. The fourth group served as an untreated control and the fifth as a water-treated control. Mothers were observed from Day 0 of pregnancy to Day 18, at which time they were sacrificed, and the fetuses taken for examination. The following parameters were observed for abnormalities: fetal extremities, oral cavity, body measurement, resorptions, implantations, bones, maternal breasts, intestines, number of eggs, spleen, heart, lung, liver, and body weight. Table 9 lists the findings at Day 18 of pregnancy, Table 10, the effects of Sodium Lauryl Sulfate on the pregnant dams and their offspring, and Table 11, the effect of Sodium Lauryl Sulfate on fetal skeletal development.

Investigators found skin discoloration and a dramatic reduction in maternal body weight increases in the 4.0% and 6.0% detergent-treated group. Determination of the conception rate by detection of a vaginal plug showed that 21 mice in each detergent-treated group and in the water-treated group were pregnant.

Abnormalities found in nontreated mice included open eyelids, polydactylia, bent tail, and clubfoot. Water-treated controls had only abdominal hernia and open eyelids. The 0.4% Sodium Lauryl Sulfate group displayed brain hernia, cleft palate, open eyelids, polydactylia, and clubfoot. The 4.0% group showed cleft palate, open eyelids, and digital anomalies, and the 6.0% group had cleft palate, open eyelids, and bent tail. There was a significant delay in bone ossification with increasing detergent concentration.

In summary, there occurred a reduction in maternal weight and growth rate with application of the detergent, and the rate of pregnancies brought to term was low for mice in the 6% treatment group. Post-implantation examination found that growth retardation occurred in the 4% and 6% detergent application groups. "Open eyes" and "cleft palate" are thought to be growing phenomena in this particular strain of mice; therefore, occurrence of these anomalies in test and control mice may or may not be significant. Delayed ossification and fetal weight and growth were retarded in the 4% and 6% detergent-treated groups.

Comedogenicity

Sodium Lauryl Sulfate, in 1% and 5% solutions, was applied to the internal base of the pinna of the right ear of three albino rabbits. An unspecified number of applications were made on Monday through Friday for two weeks; the left ear served as the control. Clinical severity of lesions was scored for 0 (no increase in visible hyperkeratosis or no comedones) to 5 (severe lesions). One percent Sodium Lauryl Sulfate scored 3 (significant comedones) and 5% scored 4 (comedones of greater severity than score 3).[86]

A 10% aqueous solution of Sodium Lauryl Sulfate was applied in 100 μl amounts twice a day, five times per week, for up to six weeks to the entire dorsal trunk of hairless rhino female mice. The detergent produced a fine scaling of the

TABLE 9. Effect of Sodium Lauryl Sulfate (SLS) on Rates of Pregnancy and Embryonic Development in Mice.

Group	*No. of females with vaginal plug*	*No. of dams (%)*	*Total no. of implantations (mean ± S.D.)*	*Total no. of surviving fetuses (mean ± S.D.)*	*Total no. of dead and resorbed fetuses (%)*	*Mean body weight of surviving fetuses (mean ± S.D.)*
Nontreatment	48	39(81.3)	450 (11.5 ± 2.45)	424 (10.9 ± 2.18)	26(5.8)	1.36 ± 0.11
Distilled water	21	19(90.5)	218 (11.5 ± 2.70)	205 (10.8 ± 2.37)	13(6.0)	1.29 ± 0.12
SLS (0.4%)	21	20(95.2)	244 (12.2 ± 1.79)	228 (11.4 ± 1.85)	16(6.6)	1.28 ± 0.12
SLS (4.0%)	21	17(81.0)	198 (11.6 ± 1.32)	180 (10.6 ± 1.70)	18(9.1)	1.21 ± 0.15[b]
SLS (6.0%)	21	11(52.4)[a]	126 (11.5 ± 1.37)	116 (10.6 ± 1.69)	10(7.9)	1.21 ± 0.15[b]

[a] $p < 0.01$.
[b] $p < 0.001$.
From Ref. 85.

TABLE 10. Effect of Sodium Lauryl Sulfate (SLS) on External Anomalies of Fetuses.

Group	*Nontreatment*	*Distilled water*	*SLS 0.4%*	*SLS 4.0%*	*SLS 6.0%*
No. of fetuses examined	426	205	228	180	116
No. of abnormal fetuses	16(3.8)[a] 13(27.1)[b]	8(3.9) 7(36.8)	9(4.0) 7(35.0)	14(7.8) 9(52.9)	7(6.0) 5(45.6)
Brain hernia	0	0	1(0.4) 1(5.0)	0	0
Abdominal hernia	0	1(0.5) 1(5.3)	0	0	0
Cleft palate	0	0	1(0.4) 1(5.0)	4(2.2) 4(23.5)	5(4.3) 4(36.4)
Open eyelids	10(2.4) 7(14.6)	7(3.4) 6(31.6)	5(2.2) 3(15.0)	9(5.0) 6(35.3)	1(0.9) 1(0.9)
Polydactylia	1(0.2) 1(2.1)	0	1(0.4) 1(5.0)	0	0
Bent tail	2(0.5) 2(4.2)	0	0	0	1(0.9) 1(9.1)
Clubfoot	3(0.7) 3(6.3)	0 0	1(0.4) 1(5.0)	0	0
Subcutaneous bleeding	0	0	0	0	0
Digital anomalies	0	0	0	1(0.6) 1(5.9)	0

[a]No. of fetuses (%).
[b]No. of mother mice (%).
From Ref. 85.

dorsal skin, but no other gross changes. Microscopic changes included: moderately hyperplastic epidermis, hyperkeratotic horny layer, and a slightly more cellular dermis with a mild infiltrate of leukocytes. The horny contents remained unchanged. Untreated controls showed a slight enlargement of pseudocomedones with greater compaction of the horny cells and a marked increase in the number and size of dermal cysts. Gross sagging and wrinkling of the rhino mouse skin increased. This study showed epidermal irritancy, but did not show comedogenicity.[87]

Clinical Assessment of Safety

Ocular

Two shampoo formulations, each containing 11.0% Ammonium Lauryl Sulfate, were tested for ocular irritation on 44 men and women. A group of 21 subjects were used in a pilot study to determine the highest concentration of a product that is minimally to moderately irritating and that can be safely instilled into the eye. A second group of 26 subjects were used in the main study to evaluate the irritation of the highest concentration of the product as determined in the pilot study. A 10% concentration of the shampoo in sterile normal saline

TABLE 11. Effect of Sodium Lauryl Sulfate (SLS) on Skeletal Development of Mouse Fetuses.

Group	*Nontreatment*	*Distilled water*	*SLS 0.4%*	*SLS 4.0%*	*SLS 6.0%*
Degree of Ossification					
No. of caudal vertebrae	8.13 ± 2.465[a]	8.81 ± 1.726	8.70 ± 1.885	8.41 ± 2.617	8.08 ± 2.244
Forepaw					
No. of proximal phalanx (L)	3.90 ± 0.616	3.97 ± 0.192	3.94 ± 0.282	3.84 ± 0.577	3.86 ± 0.456
(R)	3.91 ± 0.601	3.99 ± 0.145	3.95 ± 0.236	3.87 ± 0.458	3.89 ± 0.378
No. of middle phalanx (L)	2.69 ± 1.218	1.97 ± 1.018	1.69 ± 1.188	1.90 ± 1.157	1.55 ± 1.155
(R)	2.81 ± 1.263	2.16 ± 0.932	1.98 ± 1.148	2.07 ± 1.155	1.79 ± 1.198
Hind Paw					
No. of proximal phalanx (L)	4.54 ± 1.383	4.59 ± 0.775	4.53 ± 0.831	3.96 ± 1.486	4.20 ± 1.170
(R)	4.57 ± 1.218	4.64 ± 0.716	4.56 ± 0.819	4.18 ± 1.256	4.18 ± 1.174
No. of middle phalanx (L)	1.02 ± 1.578	0.46 ± 0.927	0.47 ± 1.058	1.02 ± 1.619	0.52 ± 1.026
(R)	1.16 ± 1.609	0.43 ± 0.887	0.51 ± 1.116	1.02 ± 1.598	0.54 ± 1.037
Separation of sternebrae	12(7.4)[b]	3(1.6)	3(1.5)	17(10.8)	12(12.4)
Absence of cervical bodies	21(13.0)	15(7.9)	26(12.6)	32(20.4)	14(14.4)
Defective development of sternebrae	33(20.4)	2(1.1)	6(2.9)	13(8.3)	13(13.4)
Delayed ossification	142 133	177 172	172 171	122 119	84 84
of talus (L) (R)	(87.7)(82.1)	(96.2)(94.0)	(78.2)(87.2)	(89.7)(88.2)	(94.5)(93.3)
of calcaneous (L) (R)	60 55	49 45	53 49	53 48	42 40
	(30.7)(34.0)	(26.6)(24.6)	(24.1)(25.0)	(39.6)(35.8)	(47.7)(44.4)
Variation					
Surplus of sternebrae	15(9.3)	17(9.0)	31(15.1)	17(10.8)	7(7.2)
Asymmetry of sternebrae	56(34.5)	30(15.9)	33(16.0)	23(14.7)	11(11.3)
Cervical rib	54(33.3)	9(4.8)	4(1.9)	16(10.2)	15(15.5)
No. of rib 14	63(38.9)	105(55.7)	132(64.1)	77(49.0)	47(48.5)
No. of lumber vertebrae 7	–	1(0.53)	11(5.34)	1(0.64)	5(5.2)
Split of atlas and axis	–	45(23.8)	62(30.1)	38(24.2)	24(24.7)
Anomalies					
Adhesion of ribs	–	–	2(0.1)	–	–
Absence of cervical vertebrae	–	–	–	1(0.6)	–

[a]Mean ± S.D.
[b]No. of fetuses.
From Ref. 85.

(actual Ammonium Lauryl Sulfate concentration was 1.1%) was instilled into the right or left eye of each subject. Sterile normal saline was instilled into each contralateral eye as a control. Stinging and dryness of the eye was evaluated by each subject and irritation of the conjunctiva, cornea, and iris was evaluated by an ophthamologist. Stinging and irritation was scored on a scale of 0 (none) to 3 (severe). The mean sting score of one product was 2.1 (moderate) and the mean duration of the stinging was 42.9 sec. The mean tearing score was 0.5 (none to mild), while dryness of the eye scored 0.08 immediately after instillation and 0.04 after 1 h. The mean irritation score immediately after instillation was 0.5, after 1 h it was 0.1, and after 2 h, 0.0.[88] When a 10% concentration of the second formulation (1.1% Ammonium Lauryl Sulfate) was instilled into the eyes, the mean sting score was 1.2 (mild) and the mean duration of the effect was 21.4 sec. The mean tearing score was 0.04 (practically none) and the mean eye-dryness scores

immediately after instillation and after 1 h were 0.04 and 0.15, respectively. The mean irritation scores immediately and 1 h after instillation were, respectively, 0.08 and 0.02 (practically none).[89]

Microscopic Effects and Irritation

The effect of Sodium Lauryl Sulfate on human epidermal mitosis was studied using healthy adult male volunteers. Concentrations of 0.5%, 1.0%, and 2.0% Sodium Lauryl Sulfate were applied to several sites on the upper back, and the subjects served as their own controls. The materials were applied every 24 h and biopsies to assess mitotic effect were taken at 24, 48, and 96 h. Six hours prior to the readings, the patch and excess detergent were removed, and 0.5% Calcemid cream was applied under occlusion. At the end of the 6 h, a 3 mm punch biopsy was taken and the mitotic index was ascertained. When compared with controls, the 1% Sodium Lauryl Sulfate solution produced mild erythema and a 30-fold increase in mitotic activity which peaked at 48 h. The 0.5% and 2.0% solutions produced similar changes in mitotic activity but to a smaller degree. It was found that 1% Sodium Lauryl Sulfate is the maximum mitotic response concentration.[90]

The cumulative effect of Sodium Lauryl Sulfate on cutaneous horny layers and on the lysosomal activity of keratin layers was studied in humans in vivo. To study cutaneous roughness and inflammatory reaction, human forearm was treated with Sodium Lauryl Sulfate for four successive days and clinical diagnosis was made every day for five days. The horny layer was then stripped from the arm in order to study the acid phosphatase activity. Homogenized dermis and epidermis from human instep was used to study the lysosome labilizing ability of the surfactant. The detergent produced roughness of the forearm in all subjects by the third day; an erythematous reaction occurred in one person. The acid phosphatase activity of the stripped, detergent-treated horny layer of the forearm decreased before the onset of skin roughness. The epidermal lysosome labilizing was studied by measuring spectrophotometrically the liberation of acid phosphatase and β-glucuronidase from the human lysosome fraction. A concentration of 5×10^{-5} g/kg Sodium Lauryl Sulfate released the greatest amount of these enzymes. The authors suggest that skin roughness could result from the interaction of surfactant molecules with the horny layer to labilize their membranes and cause disruption of water retention and cellular adhesion abilities within the membranes.[91]

Skin Irritation and Sensitization

Irritation and sensitization by Sodium and Ammonium Lauryl Sulfate was studied in humans. These studies are detailed below, and outlined in Table 12.

Sodium Lauryl Sulfate is reported to be "well known as a standard skin irritant."[92] An aqueous concentration of 5% Sodium Lauryl Sulfate was tested in wetting tests[93] on 495 patients with occupational contact dermatitis and cumulative insult dermatitis, and on healthy control persons. Irritant reactions occurred in 52% of the patients with eczema and in 12% of the control subjects. In 12 patients, 11 of whom had contact and cumulative insult dermatitis, "crescendo" reactions occurred. These persons had been exposed previously to

TABLE 12. Human Clinical Studies: Skin Irritation/Sensitization Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate.

Test Conc. (%)	Dose (ml)	Ingredient solution or formulation	No. of subjects	Time on test	Irritation Score Max. score	Comment	Ref.
Sodium Lauryl Sulfate							
Wetting and Patch Test							
5	–	ingredient solution	495	–	–	Subjects had occupational contact dermatitis and cumulative insult dermatitis. A healthy control group was also tested. Irritant reactions occurred in 52% of dermatitis-patients and in 12% of controls	92
0.1	–	ingredient solution	12	–	–	Eczema in 2 of 12 patients.	92
Patch Test							
–	–	ingredient solution	100	48 h	–	No contact dermatitis	35
Closed							
0.25	0.3	ingredient solution	28 women	7 days	0.20/4.0	3–24 h patches in 7 days. Faint, barely perceptible erythema.	95
0.5	0.3	ingredient solution	28 women	7 days	0.36/4.0	Faint, barely perceptible erythema.	
1.0	0.3	ingredient solution	28 women	7 days	0.74/4.0	Faint to definite erythema.	
0.25	50 μl	aq. ingredient solution	20 M,F	24 h patch 72 h obsv.	–	Slight erythema at 48 h, decreased at 72 h.	96
0.5	50 μl	aq. ingredient solution	20 M,F	24 h patch 72 h obsv.	–	Slight erythema at 24 h, increased at 48 h and decreased at 72 h.	96
1.0	50 μl	aq. ingredient solution	20 M,F	24 h patch 72 h obsv.	–	Slight erythema at 24 h, bright erythema at 48 h, slight at 72 h.	96
2.0	50 μl	aq. ingredient solution	20 M,F	24 h patch 72 h obsv.	–	Slight to bright erythema at 24 h. Bright erythema with some infiltration at 48 h, slight to bright erythema at 72 h.	96

TABLE 12. (Continued.)

Test Conc. (%)	Dose (ml)	Ingredient solution or formulation	No. of subjects	Time on test	Irritation Score Max. score	Comment	Ref.
Sodium Lauryl Sulfate (Cont'd.)							
Closed							
0.1	0.2	ingredient solution	2 men	21 days	14.5/84	Continuous 24 h patches.	65
1.0	0.2	ingredient solution	2 men	21 days	71/84	Irritation was almost maximum.	
2.0	0.2	ingredient solution	2 men	21 days	77/84	Irritation was almost maximum.	
4.0	0.2	ingredient solution	2 men	21 days	78.5/84	Irritation was almost maximum.	
6.0	0.2	ingredient solution	2 men	–	–	Concentration was too irritating to continue testing.	
8.0	0.2	ingredient solution	2 men	–	–	Concentration was too irritating to continue testing.	
Open							
1.0	0.2	ingredient solution	2 men	21 days	0/84	No irritation.	65
2.0	0.2	ingredient solution	2 men	21 days	0/84	No irritation.	
4.0	0.2	ingredient solution	1 man	21 days	0/84	No irritation.	
6.0	0.2	ingredient	2 men	21 days	0/84	No irritation.	
Closed							
10.0	25 μl	ingredient solution	5M, 5F	24 h	–	After 24, 48, 72 h – mild inflammatory reaction. After 26, 28 h – significantly greater inflammatory reaction. After 96 h – milder reaction than at 24 h.	97
10.0	0.2	ingredient solution	16 men	24 h	0.12/8.0	PII after 24 and 72 h was indicative of no irritation potential.	65
10.0	0.2	ingredient solution	8 men	21 days	34.1/84.0	Continuous 21-day closed patch on paraspinal skin of back.	65

Formulations							
Patch Tests							
1.02	0.1	formulation	20	48 h	1.3/4.0	24 h patch; 48 h observation. Pink, uniform erythema.	98
21	0.1	formulation	20	48 h	0.53/4.0	24 h patch. 48 h observation. Barely perceptible erythema.	99
21	0.1	formulation	20	48 h	1.03/4.0	24 h patch. 48 h observation. Mild, uniform erythema.	100
Schwartz-Peck							
2.5	–	formulation	599	see comment	–	48 h closed and open patch. 14-day rest. 2nd 48 h patch. UV irradiation. 3rd 48 h patch. First closed 48 h patch produced weak reactions in 22/599. The 2nd closed patch produced weak reactions in 26/599 and strong reactions in 2/599. Six weak reactions occurred after UV radiation. Nonirritating; nonsensitizing; nonphotosensitizing.	101
Repeated Insult Patch Test							
0.21 and 0.105 (see comment)	0.10	formulation aq. solution	115M, F	6 weeks	See comment	0.21 aq. for 1–2 induction patches; 1.05 aq. for 3–9 induction patches and challenge patch. Nine inductions, 2-week rest, 24 h challenge. Irritant reactions in 2/115. No sensitization.	102–104
1.26	–	formulation	57	5 weeks	See comment	Eight 24 h inductions; 24 h challenge. Slight to severe erythema; edema in 1/57. Slight to moderate erythema after challenge.	105
1.26	–	formulation	54	5 weeks	See comment	Eight 24 h inductions; 24 h challenge. Slight to well-defined erythema. No reaction after challenge.	106
1.45	0.4	formulation aq. solution	9F, 3M	21 days	See comment	21–23 h daily occlusive patches to upper back. Highly irritating.	107
1.45	0.4	formulation aq. solution	90F, 62M	6 weeks	–	10 24-h occlusive patches applied to upper arm. Primary irritation, no sensitization.	108

TABLE 12. (Continued.)

Test Conc. (%)	Dose (ml)	Ingredient solution or formulation	No. of subjects	Time on test	Irritation Score: Max. score	Comment	Ref.
Sodium Lauryl Sulfate (Cont'd.)							
2.50	–	formulation	249	–	See comment	10 24-h induction patches. One 48 h challenge. UV-irradiation after the 1st, 4th, 7th, 10th, and 11th patches. Weak reactions occurred in, at most, 12/249; and a strong reaction in one person. Number of weak reactions after the number of the UV patches were: 1 after 1st; 2 after 4th; 3 after 7th; 0 after 10th; and 4 after 11th.	109
Ammonium Lauryl Sulfate							
Patch Test							
0.11	–	shampoo (aq. solution)	53M, F	5 weeks	+1/4	Slight erythema in 1/53 after the 4th and 8th patches, and in 2/53 after the 7th patch. Indicative of fatiguing reactions. No sensitization.	110
0.15	–	shampoo (aq. solution)	52M, F	5 weeks	See comment	Max score = 5 (edema, vesiculation). Slight erythema in 7 people. Moderate erythema in 2 people. Edema in one person. No sensitization, but challenge patch produced slight erythema after 24 h.	111

0.20	–	shampoo (aq. solution)	55M, F	5 weeks	See comment	Max score = 5 (edema, vesiculation). Slight erythema in 15 people; Moderate erythema in 10; Severe erythema in one; Edema in 2.	112
1.68	0.1	shampoo (aq. solution)	162F 47M	5 weeks	+2/4	Very mild to mild primary irritant in 16 individuals. Fatiguing agent in 56 individuals after 2 or more applications. No sensitization.	113
0.84	0.1	shampoo (aq. solution)	162F 47M	5 weeks	–	Not a primary irritant. Fatiguing agent in 2 individuals. No sensitization.	113
0.42	0.1	shampoo (aq. solution)	162F 47M	5 weeks	–	Not a primary irritant. No sensitization.	113
0.84	–	product (aq. solution)	46F 10M	5 weeks	–	No irritation or allergic reactions.	114
0.98	–	product (aq. solution)	41F 11M	5 weeks	–	No irritation or allergic reactions.	115
1.12	–	product (aq. solution)	42F 11M	5 weeks	+1/+4	One person had mild erythema; no allergy.	116
1.12	–	product (aq. solution)	43F 11M	5 weeks	+1/+4	One person had mild erythema; no allergy.	117

Sodium Lauryl Sulfate in household and cosmetic products. These patients were then tested according to the lymphocyte transformation test with 0.005–0.001 mg Sodium Lauryl Sulfate per ml culture.[94] Ten of the 12 patients showed increased incorporation of ^{3}H-thymidine, but there was no increase in DNA synthesis. This, according to Prater, is indicative of certain sensitization in at least 10 of the 12 people. Patch tests with 0.1% aqueous Sodium Lauryl Sulfate caused eczematous reactions in two of 12 patients.[92]

Twenty-eight women tested the irritation potential of Sodium Lauryl Sulfate in concentrations of 0.25%, 0.5%, and 1.0%. Patches containing 0.3 ml of the test substances were fixed to the back of each panelist for three 24 h periods in seven days. Scores were read 72 h after application of the first patch, and 48 h after application of both the second and third. The 0.25% and 5.0% concentrations caused faint, barely perceptible erythema, and scores were, respectively, 0.20 and 0.36 out of 4.0. The 1.0% concentration scored 0.74 out of 4.0, which indicates faint to definite erythema.[95]

Sodium Lauryl Sulfate, in aqueous concentrations of 0.25%, 0.5%, 1.0%, and 2.0%, was patch-tested on the backs of 10 men and 10 women. The occlusive patches were applied for 24 h and observations were made 24, 48, and 72 h after application. Reactions were scored on a scale of 0 (no reaction) to 3 (erythema, infiltration, vesicles, papules). The results showed a dose-response, and a time-response, with stronger responses at 48 h than at 24 or 72 h. The strongest irritant responses included bright erythema and infiltration.[96]

A 21-day continuous closed patch with multiple concentrations was used to test the irritancy potential of Sodium Lauryl Sulfate on twelve men. The men were placed in six groups of two each, and each group was tested with 1%, 10%, 20%, 40%, 60%, or 80% of the original 10% compound. The actual concentrations of the ingredient were, therefore, 0.1%, 1.0%, 2.0%, 4.0%, 6.0%, or 8.0%. The solutions, in 0.2 ml volumes, were placed on patches which were applied to the subjects' backs. Each patch was removed at the end of every 24 h, and sites were read after 30 min. A fresh sample was then applied to the same area. Scoring was from 0 (no reaction) to 4 (bullous reaction). Testing was continued for 21 continuous days or until a grade 4 reaction was observed. Cumulative maximum irritation was 84 (21 days × 4). The results of the test showed that the 0.1% concentration gave a cumulative irritation score of 14.5 out of 84, the 1.0% solution scored a 71, and the 2.0% and 4.0% solutions scored 77 and 78.5, respectively. The higher concentrations were too irritating for continued testing.[65]

In a 21-day open test, Sodium Lauryl Sulfate was tested in concentrations of 1%, 2%, 4%, and 6% on 2, 2, 1, and 2 men, respectively. The compound was applied to the backs of the men and the sites were circumscribed with petrolatum, so as to confine the test liquid while the solvent evaporated. This procedure was repeated daily for 21 days, or until a grade 4 reaction developed; the scoring system used was the same as for the 21-day continuous patch test. None of the concentrations produced any irritation (scores were 0).[65]

The degree of inflammation to human skin caused by Sodium Lauryl Sulfate was assessed by patch testing. A 25 μl volume of 10% aqueous Sodium Lauryl Sulfate was applied under a 24 h patch to the forearms of five men and five women. Upon completion of the 24 h contact period, the patches were removed

and sites were graded immediately (24 h), and at 26, 28, 30, 48, 72, and 96 h. All challenged sites developed inflammatory reactions, with scores at 26 and 28 h significantly higher than at 24 h (immediately upon patch removal). The 48- and 72-hour scores were similar to the 24 h scores, and the 96 h score was significantly lower than the 24.[97]

Sixteen adult white male volunteers tested the effect of Sodium Lauryl Sulfate, among 14 other compounds, in a Draize rabbit type irritation test modified for man. The men were placed into four groups of four each, and the ventral aspects of both forearms were clipped free of hair 24 h prior to testing. A 10% w/v aqueous solution of Sodium Lauryl Sulfate was applied and covered with occlusive patches. Results were read 24 and 72 h later. The irritation index for Sodium Lauryl Sulfate was 0.12 out of a possible 8.0. The compound, as tested, showed no irritation potential.[65]

A 21-day continuous closed patch test of a 10% concentration of Sodium Lauryl Sulfate was conducted using the paraspinal skin of the back of eight white male volunteers. Procedure and scoring were as described for the preceding 21-day continuous closed patch test. The compound scored 34.1 out of a possible 84.[65]

Three shampoo formulations containing Sodium Lauryl Sulfate were tested by 24 h occlusive patch test procedures. The volar surface of the forearm and/or the inner aspect of the upperarm was the site of contact for the 24 h patch. After 24 and 72 h, the sites were graded on a scale of 0 (no effect) to 4 (severe erythema, vesiculation and/or edema). One formulation contained 68% of a 30% concentration of Sodium Lauryl Sulfate. This solution was then diluted to 5% in water (final ingredient concentration 1.02%) and was affixed to the arms of 20 individuals. After 48 h, the formulation scored 1.3 and irritation was manifested by mild to moderate erythema.[98] Two other formulations, each containing 70% of 30% Sodium Lauryl Sulfate (actual concentration 21%) were each tested full strength on 20 individuals. One product scored a 0.53 (barely perceptible erythema) and the other, 1.03 (mild, uniform erythema).[99,100]

The Schwartz-Peck prophetic patch procedure was used to assess the irritation, sensitization, and UV light sensitization potential of a makeup foundation product containing 2.5% Sodium Lauryl Sulfate. The skin of the upper back of 599 individuals was cleansed and a 48 h occlusive patch containing the formulation was affixed to each site. A simultaneous open 48 h patch was applied to the wrist. After a 14-day rest, a challenge 48 h patch was applied to assess sensitization. The sites were then irradiated with a Hanovia Tanette Mark I UV source quartz lamp ($\leq$ 150 W) with a continuous emission of 300–370 nm for 1 min, at a distance of 12 in. These sites were read 48 h later. After the first patch, weak, nonvesicular reactions occurred in 22 panelists. The second patch caused weak reactions in 26 and strong, edematous or vesicular reactions in two people. Weak reactions occurred in six people after UV irradiation. The investigators concluded that the compound was nonirritating, nonsensitizing, and nonphotosensitizing.[101]

A repeated insult patch test was conducted on a shampoo containing 70% of Sodium Lauryl Sulfate, supplied as a 30% solution (actual concentration is 21.0%). A 1.0% aqueous solution of the product (0.21% Sodium Lauryl Sulfate in a 0.1 ml volume) was applied under 24 h occlusion to the backs of the 115

panelists. Since this concentration was too irritating, it was reduced to 0.5% (or 0.105%) Sodium Lauryl Sulfate for induction patches 3–9 and for the 24 h challenge patch applied after a 14-day rest. Mild erythema occurred in two individuals after challenge. The investigators concluded that the reactors were indicative of irritant but not allergic responses.[102-104]

Repeated insult patch tests were performed on two shaving cream formulations each containing 1.26% Sodium Lauryl Sulfate. Eight occlusive 24 h patches were applied to each panelist. A two-week rest ensued, then a challenge 24 h patch was applied. One formulation was applied full-strength to 57 male and female panelists. Sites scored 24 h after each induction showed that both the number of reactions and the severity of the reactions increased with time. At most, 19 people showed slight erythema, 12 showed moderate erythema, three showed severe erythema, and one showed edema. Reactions after the challenge patch showed slight erythema in six at 47 h after application, in four after 50 h, and two after 53 h. One person showed moderate erythema after 50 h. The investigators did not believe these reactions constituted primary irritation.[105] The second formulation was tested on 54 men and women. At most, very slight erythema occurred in one person, slight erythema in three, and well defined erythema in four. Challenge patches produced no irritation.[106]

A hair preparation containing 14.5% Sodium Lauryl Sulfate was tested in a 21-day cumulative irritancy test on nine women and three men. The product was diluted so that the final test concentration of the detergent was 1.45%. Daily occlusive 23 h patches containing 0.4 ml of the solution were applied to the panelists' backs. Patches of baby oil and a deodorant concentration were applied simultaneously to provide low and high-irritation frames of reference. The cumulative irritation scores for each product were: baby oil: 1.67; shampoo: 460; deodorant: 610.1. The investigators concluded that the shampoo was highly irritating.[107] When treated in a Draize cumulative irritancy test, this shampoo (diluted in water to contain 1.45% Sodium Lauryl Sulfate) was applied under occlusion to the arms of 90 women and 12 men. Responses included patterns of primary irritation, but no sensitization was exhibited.[108]

A repeated insult patch test of a foundation formulation containing 2.5% Sodium Lauryl Sulfate was conducted on 249 individuals. Ten 24-hour induction patches, followed by a two- to three-week rest, and one 24 h challenge patch was applied to the back. Open 24 h patches were applied to the forearm. Photosensitivity was tested by irradiating the test sites after the first, fourth, seventh and tenth inductions, and after the challenge patch. Mild, nonvesicular reactions occurred in, at most, 12 individuals, and a stronger reaction occurred in one individual after the induction patches. The challenge patch produced a weak reaction in six people; however, the investigators did not consider the reactions to be indicative of sensitization. After UV irradiation with the aforementioned Hanovia Tanette Mark I quartz lamp, one mild reaction occurred after the first patch, two after the fourth, three after the seventh, none after the tenth, and four after the challenge.[109]

Three shampoo formulations containing Ammonium Lauryl Sulfate were tested for human skin irritation and sensitization using repeated insult patch test procedures. Eight 12 h patches of the products were applied to the arms on each

Monday, Tuesday, Wednesday, and Thursday of two consecutive weeks and the sites were read 24 h after application. After a two-week rest, a ninth 24 h challenge patch was applied to the same site. It was read 24, 48, and 72 h after application. A scoring scale of 0 (no reaction) to 4 or 5 (edema, erythema, vesiculation) was used for evaluation. One shampoo containing 11.0% Ammonium Lauryl Sulfate was tested as above on 53 men and women. The product was diluted to make the test concentration of the ingredient 0.11%. Slight erythema (score = 1) occurred in one individual after the fourth and eighth induction patches and in two people after the seventh; these were considered to be fatiguing reactions. No sensitization occurred.[110]

Another shampoo containing 15% Ammonium Lauryl Sulfate was evaluated as above with 52 men and women. The final test concentration of the ingredient was 0.15%. Visible skin changes characteristic of a fatiguing reaction occurred in seven people (slight erythema), moderate erythema occurred in two people, and edema in one person. No sensitization occurred, but slight erythema was noted in one person after 24 h.[111] The third and final shampoo formulation, tested as above, also contained 15% Ammonium Lauryl Sulfate, but the final test concentration of the ingredient was 0.20%. It produced slight erythema in 15 out of 55 individuals, moderate erythema in 10, severe erythema in one, and edema in two. Challenge patches produced slight erythema in two at 24 h and one at 48 h. These were characterized as fatiguing reactions, but no sensitization occurred.[112]

A shampoo containing 16.8% Ammonium Lauryl Sulfate was evaluated for irritation and sensitization in a repeated insult patch test. Aqueous solutions of the product containing 1.68%, 0.84%, and 0.42% Ammonium Lauryl Sulfate were applied to the cleansed upper backs of 162 women and 47 men in a series of ten 24-hour induction patches. Reactions were read 48 h after contact and scores were based on a scale of 0 (no reaction) to 4+ (erythema, edema, vesiculation, ulceration). Fourteen days after the last induction application, one challenge patch containing 0.84% Ammonium Lauryl Sulfate was fixed to the original test site, and one to a virgin site on the upper arm. These patches were read 24, 48, and 96 h after application. Under the test conditions, a solution of the shampoo containing 1.68% Ammonium Lauryl Sulfate was found to be a very mild to mild primary irritant in 16 subjects, and a substantial fatiguing agent in 56 individuals by the cumulative effect of two or more applications. The 0.84% concentration caused no irritation but it was a fatiguing agent in two subjects. The 0.42% concentration was not irritating. The three test concentrations of the ingredient caused no sensitization.[113]

A modified Draize-Shelanski-Jordan patch test was conducted on two formulations, one containing 30% Ammonium Lauryl Sulfate and the other with 35% detergent. The Ammonium Lauryl Sulfate was supplied as a 28% solution and the actual concentration of detergent in each product was 8.4% and 9.8%, respectively. A 10% dilution of each formulation (0.84% and 0.98% detergent) was applied under open patch conditions for ten alternate 24 h periods to the upper backs of 46 women and 10 men testing one product, and 41 women and 11 men testing the second product. Readings were made after each application. After a 13-day rest, a 48 h challenge patch was applied to the back, and a second 48 h patch was applied seven days later. Challenge sites were read 48 and 72 h

after application. Scoring was based on a scale of +1 (mild erythema) to +4 (intense erythema, edema, vesicles). No irritation or allergic reactions were produced by either product.[114,115]

A similar test was performed on two products each containing 40% of 28% Ammonium Lauryl Sulfate. The actual concentration of detergent was 11.2% in the products and the repeated insult patch tests were performed on 10% dilutions of each formulation. Each product produced a +1 reaction in the 42 women and 11 men testing one product and in the 43 women and 11 men testing the other; however, the investigators stated that the products appear to be safe and are neither irritants nor allergens.[116,117]

Controled Use

A controled use study of a foundation product containing 2.5% Sodium Lauryl Sulfate was conducted using 52 panelists. The product, applied full strength to the face for four weeks, produced no irritation.[118]

The "Sodium Lauryl Sulfate Provocative Test"

Kligman[119] described the "Sodium Lauryl Sulfate provocative patch test . . . a method designed to reveal threshold states of sensitization." This procedure calls for pretreatment of the test site with 10% aqueous Sodium Lauryl Sulfate for 1 h before applying the test-allergen. The use of Sodium Lauryl Sulfate in pretreatment grew from studies that showed it to enhance skin permeability and, therefore, to foster sensitization. Sodium Lauryl Sulfate may also be combined with the test allergen, rather than pretreating the test area, provided no incompatibility exists between the surfactant and the test allergen.

Case Reports

Fisher[15] reported a case in which a 37-year-old woman was allergic to the formaldehyde preservative (0.03%) in Sodium Lauryl Sulfate. The author notes that previous reports of Sodium Lauryl Sulfate sensitivity[92,120,121] may be less valid if the ingredient contained formaldehyde (a known sensitizer).

Cosmetic Experience

The following are cosmetic experience submissions for products containing Sodium Lauryl Sulfate:

a. A hair preparation containing 14.5% Sodium Lauryl Sulfate is reported to have a number of uses totaling 5×10^8, a number of uses per year of 2×10^5, and a number of safety-related complaints totaling 17 over seven years.[122]

b. A shampoo formulation containing 30% Sodium Lauryl Sulfate has per annum sales totalling 398,000 units, and per annum uses of 4,852,620. The number of safety-related complaints was 1 in two years; the type of complaint was an allergic/irritant reaction.[123]

c. A shampoo formulation containing 10% Sodium Lauryl Sulfate is sold at the rate of 390,000 units per annum, and it has 8,580,000 uses per year. No safety-related complaints were received in two years.[124]

d. Shampoo products containing 23%, 25%, 29.2%, and 30.9% Ammonium Lauryl Sulfate have total combined sales of 6,814,127 units per annum. There were six safety-related complaints from these four products combined; they included two complaints each of itchy scalp and allergy, and an instance each of damaged hair and eye irritation.[125,126]

SUMMARY

Sodium and Ammonium Lauryl Sulfate are anionic surfactants. Sodium Lauryl Sulfate is supplied as a powder or a viscous liquid, and Ammonium Lauryl Sulfate appears as a thin to viscous liquid. Both are soluble in polar solvents. The 1981 product data submitted to the FDA show that Sodium Lauryl Sulfate was used in 703 formulations, 158 of which contain the detergent at 0.1%–1% or greater in non-rinse-off products. Ammonium Lauryl Sulfate was used in 164, of which four contain the surfactant at 0.1%–1% or greater and are not rinsed off. Since they are surfactants, Sodium and Ammonium Lauryl Sulfate are primarily used in hair and bath products.

The effects of Sodium and Ammonium Lauryl Sulfate on biological systems have been studied. TGE virus, *E. coli,* and Gram-positive bacteria are sensitive to Sodium Lauryl Sulfate. It is also fungistatic to *Candida* and *Tricophyton spp.* In Absorption, Metabolism, and Excretion studies, 5% Sodium Lauryl Sulfate had a degenerative effect on the cell membranes of rabbit intestinal epithelium, on intracellular organelles, and on microvilli, due to its protein denaturing properties. Sodium Lauryl Sulfate caused an increase in cyclic AMP content in rat intestinal mucosa, and an increase in the blood glucose level in rabbits, when a 2.5% solution was administered after a 24 h fast. In guinea pig skin penetration and absorption studies, investigators found that the strongly anionic head group of Sodium Lauryl Sulfate impairs its ability to penetrate through the skin. Similar low levels of penetration were found using rat and human epidermis. Another report stated that diffusion through rat neonatal stratum corneum was rapid and increased with concentration. The investigator postulated that the surfactant caused structural changes in the membranes, thereby allowing skin penetration.

In a study of detergent uptake by hair, it was found that bleached hair absorbed more Sodium Lauryl Sulfate than untreated hair. Permeability studies using human abdominal epidermis showed that skin permeability to water and skin damage increased in rapidity and severity when the epidermis was soaked in solutions containing increasing amounts of Sodium Lauryl Sulfate. Intraperitoneal and subcutaneous injections of this surfactant into rats showed that the main route of excretion from the body is through the urine. Both Sodium and Ammonium Lauryl Sulfate, in solution containing 0.05 *M* detergents, caused swelling as a result of reversible conformation change in the cell membranes of the stratum corneum. Sodium Lauryl Sulfate penetrates through pores in the cell membranes of yeast, and has a lytic action on dog erythrocyte membranes. This surfactant also liberates solubilized protein and sulphydryl groups from human skin, soluble protein and amino acids from guinea pig stratum corneum, and histamine from rat peritoneum.

In acute oral toxicity studies, a 10% concentration of Sodium Lauryl Sulfate had an LD50 of 0.8–1.10 g/kg in rats, a 28.2% concentration had an LD50 of 6.3 ml/kg in rats, and an 86% concentration produced an LD50 of 1290 mg/kg. Intubation of "purified" 100% Sodium Lauryl Sulfate into rats had an LD50 of 2.7 mg/kg; an "unpurified" sample had an LD50 of 1.0 mg/kg. Two other 100% samples produced diarrhea, diuresis, lacrimation, salivation, convulsions and death in the rats; the LD50 was 1650 mg/kg. Products containing 21% Sodium Lauryl Sulfate caused depression and death of all animals at a dosage level of 5.0 g/kg. Doses of a 27.4% concentration of Ammonium Lauryl Sulfate caused death by pulmonary hemorrhage in 20 out of 50 rats. A formulation containing 15% Ammonium Lauryl Sulfate caused depression, labored breathing, diarrhea, and death in four out of 20 animals. Rhesus monkeys receiving a formulation containing 15% Ammonium Lauryl Sulfate suffered emesis and diarrhea. One died at the 10 ml/kg level.

In acute ocular tests, 10% Sodium Lauryl Sulfate caused corneal damage to the rabbits' eyes if not irrigated, or if irrigation was delayed for longer than 4 sec after instillation. Draize tests of Sodium Lauryl Sulfate in concentrations of 1%–100%, with and without rinse, showed that irritation increased with increasing concentration and decreased when the eyes were rinsed. A Draize test of a product containing 5.1% Sodium Lauryl Sulfate caused mild irritation, and products containing 21% detergent were severely irritating with no rinse, and mildly irritating when rinsed. Twenty-six percent Sodium Lauryl Sulfate in a shampoo was mildly irritating when rinsed. Solutions of the ingredient Ammonium Lauryl Sulfate containing 1.25%–27.4% detergent showed increasing irritation with increasing concentration; rinsing decreased irritation. Two formulations containing 15% Ammonium Lauryl Sulfate was mildly irritating when rinsed from the eyes of monkeys and rabbits, and moderately irritating when not rinsed.

Upper respiratory tract irritation and inhibition of respiration in mice and rabbits was caused by Sodium and Ammonium Lauryl Sulfate aerosols. The concentration of Sodium Lauryl Sulfate which produced irritation in mice was 88 μg/l and in Ammonium Lauryl Sulfate, 114 μg/l. Inhalation irritation occurred in mice exposed to either surfactant at 73 μg/l and in rabbits at 130 and 170 μg/l.

The concentration of both surfactants to cause writhing in mice after intraperitoneal injection is 0.086%.

Acute skin irritation studies of the ingredient, Sodium Lauryl Sulfate, show that application of solutions containing 0.5%–10% detergent cause slight to moderate irritation. Applications of 10%–30% detergent caused skin corrosion and severe irritation; however, one 100% application was only moderately irritating. A shampoo formulation containing the ingredient at 0.65% caused mild irritation. Solutions of 2%, 10%, and 20% Ammonium Lauryl Sulfate were highly irritating and dangerous. A 27.4% solution, when tested according to D.O.T. procedures, caused corrosion and irreversible tissue damage. This same solution, tested according to the Draize Method, produced moderate irritation. A formulation containing 1.5% Ammonium Lauryl Sulfate was severely irritating.

The acute dermal lethal dose of Sodium Lauryl Sulfate to rabbits is greater than 10 g/kg. Signs of systemic intoxication and dermal irritation were noted. A formulation containing 26% Sodium Lauryl Sulfate was tested for acute dermal

toxicity in albino rabbits. A dose of 10 ml/kg caused systemic effects including depression, labored respiration and nasal discharge. Dermal irritation was severe. Fifteen percent Ammonium Lauryl Sulfate in a shampoo caused moderate skin irritation, but no systemic toxicity.

Sodium Lauryl Sulfate facilities skin sensitization to nickel and chrome in guinea pigs.

A thirteen-week subchronic oral toxicity test in rats on dietary levels of 40, 200, 1000, or 5000 ppm Sodium Lauryl Sulfate caused only an increase in absolute organ weight at the highest dose level. No other abnormalities were found. Rats ingesting 0%, 0.05%, or 0.25% Sodium Lauryl Sulfate for five months showed bronchopneumonia and an increase in serum triglyceride levels. Rats fed 2% Sodium Lauryl Sulfate for four months had signs of growth retardation, those fed 4% had greater growth retardation, and those fed 8% died within two weeks. The high-dosed group had signs of diarrhea and gross alterations of intestines.

A chronic oral feeding study in rats of 0.25%, 0.5%, or 1.0% Sodium Lauryl Sulfate in the diet for two years produced no abnormalities, nor did a similar two year study with 0.2% detergent in the diet. A chronic oral one year toxicity study using beagle pups was conducted on 0%, 0.67%, 1.0%, or 2.0% Sodium Lauryl Sulfate. Decreased weight gain occurred in the 2% group, but no other abnormalities were noted.

A 91-day percutaneous toxicity study of a shampoo containing 17.5% Ammonium Lauryl Sulfate had no treatment-related abnormalities except for moderate to severe dermal effects.

In mutagenesis studies, rats fed 1.13% and 0.56% Sodium Lauryl Sulfate in the diet for 90 days produced no more chromosomal abberations or clastogenic effects than did a control diet.

Sodium Lauryl Sulfate (4.0% and 6.0%) applied topically to pregnant mice caused cleft palate and delayed bone ossification in some offspring.

In comedogenicity tests, 1% and 5% Sodium Lauryl Sulfate was applied for two weeks to the skin of rabbits and significant to severe lesions were produced.

When shampoo formulations containing 11.0% Ammonium Lauryl Sulfate were instilled into the eyes of volunteers, mild stinging and eye dryness resulted.

Concentrations of 0.5%, 1.0%, and 2.0% Sodium Lauryl Sulfate applied to the upper backs of male volunteers produced an increase in mitotic activity of the skin at the application sites.

The cumulative effect of Sodium Lauryl Sulfate on cutaneous horny layers and on lysosomal activity was studied in humans in vivo. The detergent produced roughness on the area of the forearm to which it was applied, and a decrease in acid phosphatase activity in the skin of the treated area. It also liberated acid phosphatase and β-glucuronidase from the lysosome fraction.

The ingredient Sodium Lauryl Sulfate was tested for human skin irritation in concentrations ranging from 0.1% to 10%. In all cases, open patches were less irritating than closed patches, and irritation increased directly with concentration. Formulations containing 0.21%–2.5% Sodium Lauryl Sulfate and 0.11%–1.68% Ammonium Lauryl Sulfate were tested under open and closed patch conditions and with UV light. In all cases, the ingredient in open patches and lower concen-

trations caused less irritation than did higher concentrations and in closed patches. No UV light sensitization occurred from any formulation.

Sodium Lauryl Sulfate is used in a provocative skin test which calls for the treatment of the test area with the detergent prior to the application of the test allergen or irritant. Sodium Lauryl Sulfate is used to enhance skin permeability and, therefore, to aid in the assessment of sensitization.

Certain case reports state that sensitivity to products containing Sodium Lauryl Sulfate may be due to other ingredients and not to the detergent.

DISCUSSION

Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate are irritants in patch testing at concentrations of 2% and greater, and that irritation increases with ingredient concentration. In some cosmetic formulations, however, that irritant property is attenuated. The longer these ingredients stay in contact with the skin, the greater the likelihood of irritation, which may or may not be evident to the user.

Although Sodium Lauryl Sulfate is not carcinogenic in experimental animals, it has been shown that it causes severe epidermal changes to the area of skin of mice to which it was applied. This study indicates a need for tumor-enhancing activity assays.

Autoradiographic studies of rat skin treated with radiolabelled Sodium Lauryl Sulfate found heavy deposition of the detergent on the skin surface and in the hair follicles; damage to the hair follicle could result from such deposition. Further, it has been reported that 1% and 5% Sodium Lauryl Sulfate produced significant number of comedones when applied to the pinna of albino rabbits. These two problems—possible hair loss and comedone formation—along with proven irritancy, should be considered in the formulation of cosmetic products.

Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate appear to pose less potential hazard when in products designed for brief, discontinuous use, following which they are thoroughy rinsed from the surface of the skin.

CONCLUSION

Sodium Lauryl Sulfate and Ammonium Lauryl Sulfate appear to be safe in formulations designed for discontinuous, brief use followed by thorough rinsing from the surface of the skin. In products intended for prolonged contact with skin, concentrations should not exceed 1%.

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