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Final Report on the Safety Assessment of Squalane and Squalene

Squalane and Squalene have been identified as natural components of human sebum. Both ingredients are used in a variety of cosmetics at concentrations ranging from ≤ 0.1 to > 50%.

Animal studies indicate Squalene is slowly absorbed through the skin, while both compounds are poorly absorbed from the gastrointestinal tract. The acute animal toxicity of these ingredients by all routes is low. Both compounds are nonirritants to rabbit skin and eye at 100% concentration. Formulations containing Squalene indicate it is not a significant human skin irritant or sensitizer. Limited contact sensitization tests indicate Squalene is not a significant contact allergen or irritant.

It is concluded that both Squalane and Squalene are safe as cosmetic ingredients in the present practices of use and concentration.

INTRODUCTION

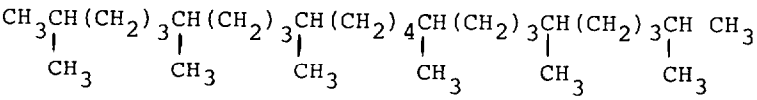
Squalane may be obtained by complete hydrogenation of shark liver oil, Squalene, or other natural oils. This material also exists as a normal constituent of human sebum in amounts up to 2.6%; next to Squalene, it is the most common hydrocarbon in these lipids. Because the human is capable of saturating Squalene, Squalane can be a biogenic product.[1-6]

The triterpene Squalene is a polyunsaturated aliphatic hydrocarbon which is widely distributed in nature. It is found in large quantities in shark liver oil, other fish oils, and in smaller amounts in olive oil, wheat germ oil, rice bran oil, yeast, and in various other foodstuffs. According to a USDA survey taken in 1965, the daily intake of Squalene in the average U.S. diet ranged from 24 to 38 mg per 2,000 calories. The principal hydrocarbon of human surface lipids, it constitutes up to 11% of total surface fat and approximately 5% of adult skin surface sebum. It has also been reported to occur in dermoid cysts, cerumen, and hair fat. In higher vertebrates and humans, it is a precursor of cholesterol. Moderate amounts are found in sites of active cholesterol synthesis, namely the liver (75 μg/g) and the small intestine (42 μg/g).[1,3,5,7-10]

CHEMICAL AND PHYSICAL PROPERTIES

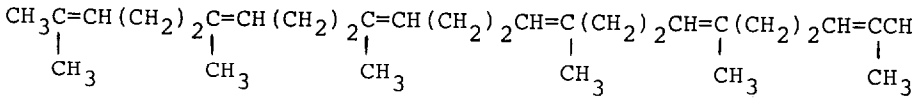
Structure

Squalane: Squalane, (2,6,10,15,19,23-hexamethyltetracosane) is the saturated branched chain hydrocarbon that conforms to the formula:



It has a molecular formula of $C_{30}H_{62}$ and a molecular weight of 422.80. Squalane is also known as dodecahydrosqualene, perhydrosqualene and spinacane.[1-3,6]

Squalene: Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a branched-chain isoprenoid hydrocarbon with six unconjugated double bonds. It conforms to the formula:



Squalene has a molecular formula of $C_{30}H_{50}$ and a molecular weight of 410.70. It is also known as spinacene.[1,3,6]

Properties

Squalane: Squalane is a colorless, odorless, tasteless, transparent oil, stable to air and oxygen. It is readily soluble in ether, gasoline, petroleum ether, benzene, chloroform, and oils. It is slightly soluble in methanol, ethanol, acetone, and glacial acetic acid. It is insoluble in water.[2,3]

Squalene: Squalene is an oil with a faint agreeable odor. It is practically insoluble in water, and freely soluble in ether, petroleum ether, carbon tetrachloride, acetone, and other lipophilic solvents. It is only sparingly soluble in alcohol and glacial acetic acid.[3]

Additional chemical and physical properties for both Squalane and Squalene are presented in Table 1.[2,3,6,11,12]

Reactivity

Squalane: No information was presented to the Panel regarding the reactivity of Squalane. Since Squalane is a saturated compound, it is not as easily oxidized as Squalene.[13]

Squalene: Squalene has the propensity towards instability, ready oxidation, and darkening, and towards becoming viscous and taking on an odor. When Squalene is exposed to air, oxygen is taken up permitting the formation of peroxides.[5,8]

TABLE 1. Chemical and Physical Properties.

	Squalane		*Squalene*	
Properties	*Reported values*	*Ref.*	*Reported values*	*Ref.*
Specific gravity at 20°C	0.805–0.812	11	0.855–0.865	11
Boiling point	approx. 350°C	3,6,11,12	approx. 335°C	11
Melting point	−38°C	3,6,12	−75°C	3
			−60°C	6
			< −20°C	12
Refractive index at 20°C	1.452–1.453	11	1.495–1.500	6,11
Flash point	approx. 230°C	11	approx. 200°C	3,11
Specific heat at 20°C	0.62 cps	3		
Viscosity at 20°C	34 cps			
Viscosity at 25°C			12 cps	3
Acid value	5.0 max.	2,11	5 max.	11
Saponification value	7.5 max.	2,11	0–5.0 max.	6,11
Iodine value	3.0 max.	11	360–380	3,6,11
Iodine no.			360–380	

In a study conducted by Rao and Achaya,[14] Squalene isolated from olive oil initially showed antioxidant properties for methyl oleate and methyl linoleate, but subsequently behaved as an oxidizing agent. Methyl oleate and linoleate solutions with initial peroxide values of 2 and 11, respectively, were incubated at 63 °C for 10 days with and without the incorporation of 0.02% Squalene and tocopherols. During the first four days, Squalene showed "good protective action" with respect to stability. For methyl oleate, the daily peroxide value increases were 7 and 22 units in the presence and absence, respectively, of 0.02% Squalene. For methyl linoleate, the corresponding figures were 11 and 50 units. Within this four-day period, Squalene had a better protective action than the same quantity of mixed tocopherols. In the subsequent six-day storage period the tocopherols continued to exert their protective effect; Squalene did not so continue. The rate of peroxide value increase for Squalene became greater than that for the control. According to the authors, "The oxidation of products of Squalene may perhaps be pro-oxidant, as has also been suggested for other polyene materials, such as carotene. Thus Squalene per se is initially an antioxidant but subsequently behaves as a pro-oxidant."

Squalene reacts exothermically at 185 °C as it begins to polymerize. This exothermic reaction increases suddenly at 300 °C. During thermal cracking, Squalene first undergoes a polymerization and then breaks down into smaller volatile molecules.[15]

Analytical Methods

Squalane: Squalane may be determined by gas chromatographic analysis.[16,17]

Squalene: Squalene may be determined by thin-layer, ion-exchange, gas, and gas–liquid chromatography.[18-22]

An early method involving the formation and isolation of squalene hexahydrochloride proved to be a very "delicate" test for the detection of Squalene.[23]

Tsukida[24] described a procedure that is particularly effective for estimating natural Squalene in the presence of other unsaturated polyenes. This involves

chromatographic separation of the dehydrogenated reaction product of Squalene with N-bromosuccinimide followed by spectrophotometric determination at 395 nm.

A method was reported by Wheatley[25] for estimating Squalene in small amounts of sebum (5 mg) and other lipids; this procedure gives an average recovery of 80% of added Squalene. Following saponification, the hydrocarbon fraction is isolated chromatographically and the unsaturated material in it is determined iodometrically.

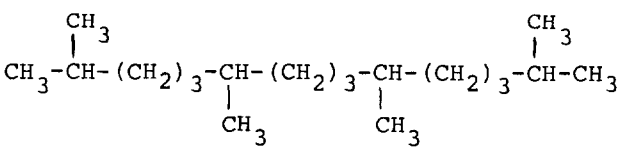
Liu and coworkers[7] described a method for measuring Squalene in human tissues and plasma; this depends on mild saponfication, extraction with petroleum ether, isolation by alumina column chromatography, and measurement by gas-liquid chromatography. Recoveries from all tissues by this technique exceeded 80%, while recoverles from plasma exceeded 96%. Losses were accurately accounted for by appropriate additions of Squalene. The lowest practical detection limit was reported to be approximately 10 ng/mg plasma.

A colorimetric method was reported by Mendelsohn and Mendelsohn[26] for the determination of Squalene in plasma. The plasma is saponified and the Squalene extracted with petroleum ether. The Squalene is then separated and purified by gel chromatography. Color is developed with o-phthalaldehyde in acetic acid + H_2SO_4 heated at 90 °C for three minutes. The color is read at 440 nm. Recovery of added Squalene by this method is 88 ± 5%.

Other reported analytical techniques include: an infrared method for the estimation of Squalene in olive oil; a quantitative colorimetric assay for Squalene eluted from silicic acid columns; a color test for the detection of Squalene on paper chromatograms; a color test for the detection of Squalene using Bezssonoff's Reagent; and a more recent method for measuring Squalene synthesis in man that involves isotope kinetics.[27-30]

Method of Manufacture and Impurities

Squalane: Squalane is obtained by molecular distillation of shark liver oil, hydrogenation of the distillate, and redistillation. This process yields a purity of at least 96% Squalane. Impurities include approximately 1% neutral fat and about 3% pristane. The latter, a stable liquid, is the saturated branched chain hyrocarbon ($C_{19}H_{40}$) that conforms to the formula:[11]



According to older sources, Squalane prepared from direct hydrogenation of shark liver oil may contain some batyl alcohol.[3,31] The latter, another cosmetic ingredient, is the monooctadecyl ether of glycerol.[1]

Squalene: Squalene is obtained by the molecular distillation of shark liver oil, which yields a purity of at least 96% Squalene. Impurities include approximately 1% neutral fat and 3% pristane.[11]

USE

Purpose and Frequency of Use in Cosmetics

Squalane: This ingredient has been in commercial use for over 25 years. Primarily, it functions as an emollient for topical application in creams, lotions, ointments, lipsticks, and other cosmetics. It is also used as a perfume fixative, as a skin lubricant, and as a base or vehicle in the production of creams and other cosmetics.[3,5,6,32,33]

Squalane is reported to be used in 294 cosmetic formulations in concentrations ranging from ≤ 0.1 to $> 50\%$. It is employed in a wide variety of products including bath oils, eye makeups, hair preparations, makeup bases, lipsticks, suntan preparations, body powders, and nail products, and in cleansing, moisturizing and skin-care preparations.[34]

Squalene: Squalene has also been in use for more than 25 years, primarily as a vehicle for topical application.[5,33]

Squalene is reported to be used in 19 cosmetic formulations in concentrations ranging from ≤ 0.1 to > 25 to 50%. It is employed in a wide variety of products including bath preparations, eye makeup removers, blushers, and suntan preparations, and in moisturizing and skin-care products.[34]

Detailed cosmetic product formulation data for both Squalane and Squalene are presented in Table 2. Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21 part 720.4 of the Code of Federal Regulations (1979). Since certain cosmetic ingredients are supplied by the manufacturer at less than 100 percent concentration, the concentration reported by the cosmetic formulator may not necessarily reflect the true, effective concentration found in the finished product; the effective concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for 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 ten-fold error in the assumed ingredient concentration.

The presence of Squalane and Squalene in a wide variety of product types provides the opportunity for contact with most body surfaces including skin, eye, hair, nails, mucous membrane, and respiratory eipthelium; small amounts of Squalene may be ingested from lipstick.[34]

Product formulations containing Squalane or Squalene may be used from once a week to several times per day. Many of the products may be expected to remain in contact with the body for as briefly as a few minutes to as long as a few days. Each product could potentially be applied hundreds of times over the course of several years.

Noncosmetic Use

Squalane: Squalane is used as a high-grade lubricating oil and as an ingredient of watch and chronometer oils. Pharmaceutical applications include use as

TABLE 2. Product Formulation Data.[a]

Ingredient *Cosmetic product type*	*Concentration (%)*	*No. of product formulations*
Squalane		
Lotions, oils, powders, and creams	>0.1–1	2
Bath oils, tablets, and salts	>5–10	1
	>1–5	5
	>0.1–1	3
Other bath preparations	≤0.1	1
Eyeliner	>1–5	1
	>0.1–1	6
Eyeshadow	>10–25	1
	>5–10	4
	>0.1–1	3
Eye makeup remover	>1–5	1
	>0.1–1	1
Mascara	>5–10	2
	>1–5	1
Perfumes	>1–5	2
Powders (dusting and talcum) (excluding aftershave talc)	>0.1–1	1
Other fragrance preparations	>5–10	1
Hair conditioners	>1–5	2
	>0.1–1	2
Hair sprays (aerosol fixatives)	>0.1–1	2
	≤0.1	2
Permanent waves	>0.1–1	15
Rinses (noncoloring)	>0.1–1	1
Shampoos (noncoloring)	≤0.1	2
Other hair preparations	>1–5	1
Blushers (all types)	>10–25	1
	>5–10	2
	>1–5	5
	≤0.1	5
Face powders	>0.1–1	12
Foundations	>5–10	2
	>1–5	4
	≤0.1	1
Lipstick	>25–50	1
	>10–15	1
Makeup bases	>50	2
	>10–25	1
	>1–5	12
	>0.1–1	4
Rouges	>5–10	4
	>1–5	1
Other makeup preparations	>10–25	1
	>5–10	1
	>1–5	9
Cuticle softeners	>5–10	1
Nail creams and lotions	>10–25	1
Nail polish and enamel removers	>1–5	1
Other manufacturing preparations	>0.1–1	1
Other personal cleanliness products	>5–10	3
	>0.1–1	1
Cleansing (cold creams, cleansing lotions, liquids,	>25–50	1
	>10–25	1

TABLE 2. (Continued.)

Ingredient *Cosmetic product type*	*Concentration (%)*	*No. of product formulations*
and pads)	>5–10	2
	>0.1–1	2
Face, body and hand	>25–50	4
(excluding shaving	>5–10	4
preparations)	>1–5	1
	>0.1–1	6
Foot powders and sprays	>0.1–1	1
Moisturizing	>10–25	2
	>5–10	6
	>1–5	46
	>0.1–1	16
	≤0.1	1
Night	>25–50	4
	>10–25	2
	>5–10	7
	>1–5	11
	>0.1–1	5
Paste masks (mud packs)	>5–10	1
	>1–5	1
	≤0.1	1
Skin lighteners	>1–5	2
	>0.1–1	1
Skin fresheners	≤0.1	2
Wrinkle smoothing (removers)	>1–5	1
	≤0.1	1
Other skin care preparations	>10–25	1
	>5–10	2
	>1–5	10
	>0.1–1	3
Suntan gels, creams, and	>10–25	1
liquids	>5–10	2
	>1–5	1
	>0.1–1	1
Indoor tanning preparations	> 1–5	1
Squalene		
Other bath preparations	>0.1–1	1
Eye makeup remover	>1–5	1
Blushers (all types)	>25–50	1
Foundations	>0.1–1	1
Face, body and hand	>1–5	2
(excluding shaving preparations)	≤0.1	1
Moisturizing	>5–10	1
	>0.1–1	2
	≤0.1	3
Night	>1–5	1
Skin fresheners	≤0.1	1
Wrinkle smoothing (removers)	≤0.1	1
Other skin preparations	>1–5	1
	≤0.1	1
Suntan gels, creams, and liquids	>0.1–1	1

[a]Data from Ref. 34.

a skin lubricant, as an ingredient of suppositories, and as a carrier of lipid-soluble drugs.[3,6]

Squalene: Squalene has found use as an intermediate in the manufacture of pharmaceuticals, aromatics, organic coloring agents, rubber chemicals, and surface-active agents. It has been reported that Squalene is also employed as a bactericide. Synthetic squalene derivatives have been reported to be effective as antiulcer agents in clinical practices; however, they failed to show efficacy in large-scale clinical trials.[3,35]

BIOLOGICAL PROPERTIES

General Effects

Squalene: Sobel et al.[36] reported that undiluted Squalene on the surface of Sabourand's agar prevented multiplication of stock cultures of *Microsporum mentagrophytes, M. audouini,* and *M. tonsurans.* Inhibition was sporadic with *M. gypseum* and absent with *Aspergillus terreus.* Squalene that had a high peroxide content as a result of exposure to air was found to be more effective than pure Squalene in inhibiting in-vitro growth.

Mihay and Zackheim[37] reported that both freshly purified or oxidized Squalene exhibited no in-vitro fungistatic properties against cultures of *Trichophyton mentagrophytes, T. sulfureum, Microsporum audouini,* or *M. lanosum. Trichophyton mentagrophytes* was not inhibited in a semi-in-vitro study.

When 21,21-dimethoxyprogesterone (a bacteriostatic and fungistatic compound) was added to growth media containing *Curvularia lunata* at a concentration of 100 μg/ml, a 75–85% inhibition of radial growth was observed. When a mixture of 21,21-dimethoxyprogesterone and Squalene was added to growth media containing the organism at concentrations of 100 μg/ml and 50 μg/ml, respectively, a 65–75% inhibition of growth resulted. According to the authors, addition of Squalene to the mixture did not modify the inhibition of radial growth. In shaken cultures, the inhibition of mycelial synthesis was reversed by Squalene at concentrations between 1 and 20 μg/ml. "No clear explanation can be given for this behavior but experimental evidence obtained so far indicates that under submerged conditions, the presence of Squalene increases the conversion rate of dimethoxyprogesterone to less toxic compounds".[38]

In a study conducted by Kritchevsky et al.,[39] four groups of rabbits were maintained on various diets for seven weeks, after which they were sacrificed and the aortae examined and graded for atherosclerotic plaques. The plaques were graded on a scale from 0–4, and any doubtful plaques were classified as a plus–minus (±). The results are presented in Table 3. The authors stated that unlike cholesterol, dietary Squalene did not induce a significant increase in atheroma, even though it is a precursor in the biosynthesis of the sterol. It was suggested that prolonged feeding of Squalene may produce more atheroma or that ". . . it may develop that under no conditions of Squalene feeding can enough cholesterol be synthesized to effect the appearance of atheroma."

El Ridi et al.[40] described a Squalene-deficient diet which was satisfactory for normal growth and reproduction but inefficient for maintaining successful lactation in the albino rat. The beneficial effect of Squalene on lactation perfor-

TABLE 3. Squalene Feeding in Experimental Atherosclerosis.[a]

Diet	No. of rabbits	Avg. wt. gain (g)	Avg. liver wt. (g)	Plaques 1–4	Plaques ±	Avg. atheroma
Normal	10	328	68	0/10	1/10	0.05
Normal + oil (9%)	9	214	62	0/9	2/9	0.11
Normal + cholesterol (3%) in oil (9%)	8	84	90	8/8	0/8	2.50
Normal + Squalene (3%) in oil (9%)	8	448	91	1/8	2/8	0.25

[a]Data from Ref. 39.

mance in the rat was demonstrated by a comparison of a record of lactation efficiency in animals kept on the purified diets (lactation index 34.9 percent) with a record in similar animals given oral supplementary doses of 0.1 grams of Squalene per day (lactation index 92.1 percent).

Absorption, Metabolism, and Excretion

Squalane: Tritiated Squalane was tested for percutaneous absorption on normal and denuded skin of mice. Over a period of 60 minutes, the average quantity absorbed through the normal skin of 12 mice was 0.12 nmol/cm²/min (3.05 μg/cm²) with a standard error of the mean of 0.94 μg/cm². Over 120 minutes, the average quantity absorbed through the normal skin of five mice was 0.103 nmol/cm²/min (5.25 μg/cm²) with a standard error of the mean of 1.65 μg/cm². On the denuded skin of nine mice, the average rate of absorption was 0.148 nmol/cm²/min (3.75 μg/cm²) over 60 minutes with a standard error of the mean of 1.2 μg/cm². To account for the slight percutaneous absorption, the authors concluded that the main barrier to penetration of Squalane resulted from failure to be removed from the dermis via circulation.[41]

Using an autoradiographic technique, Wepierre[42] showed that tritiated Squalene is able to penetrate mouse skin and migrate via hair follicles into the sebaceous glands. It was found that the compound is not systemically absorbed even when the epidermal barrier is removed.

When it was orally administered in bulk as a solution in corn oil, or fed as a mixture with the standard diet wafer, Squalane was not absorbed from the gastrointestinal tract of rats. Ninety-six to 100% of the Squalane administered to both fed and fasted animals was recovered in the feces collected over a four-day period. Squalane was not detected in the 72-hour urine of 400 g rats given 85 mg doses by stomach tube. Bile collected for eight hours and lymph collected for five hours after administration contained no detectable amounts of Squalane. Seventy-two hours after administration of Squalane, only 120 ± 10 μg (14% of the dose) was recovered from extracts of the gastrointestinal tract.[43,44]

Ingredients commonly used in cosmetic formulations (hydrocarbons of high molecular weight, alcohols, esters, fatty acids and silicones) were studied for possible assimilation by various microorganisms. The microorganisms used in the study were isolated from cosmetic products and included *Penicillum, Candida,* and *Pseudomonas*. These organisms had a strong ability to assimilate some of the ingredients noted; however, Squalane was classified with the ingredients which these organisms do not utilize.[45]

Squalene: Two male subjects were given 1 g Squalene per day for 14 days, while a third male subject received similar doses of cholesterol. Each test substance was mixed with 5 g of butter and eaten with bread. Sebum from the three subjects' backs was then collected and its Squalene content was determined. The Squalene content of sebum did not change significantly upon the ingestion of either Squalene or cholesterol. The mean values (with standard deviations) for the percent of Squalene in sebum were 7.8 ± 0.9 and 8.0 ± 1.1, respectively, for the periods before and during administration of cholesterol. The corresponding percentage for the two subjects who ingested Squalene were 7.4 ± 1.5 before and 8.1 ± 6 during Squalene administration for one subject; and 7.4 ± 1.9 before and 7.2 ± 1.4 during Squalene administration for the second subject. Both subjects receiving Squalene showed a marked fall in the Squalene content of sebum just before the first test dose; however, according to the authors, this may have been fortuitous.[46]

After applying Squalene that contained 1.0% 3-methylcholanthracene or 1.0% menthyl anthranilate to the shaved backs of rats, biopsies of the skin were taken at intervals, fixed, frozen, and sectioned. Subsequent fluorescent microscopy gave no evidence that the materials were absorbed.[47]

Oral ingestion of Squalene by rats failed to potentiate adrenocorticotropin because the material was poorly absorbed from the gut.[48,49]

Four drops of 30% Squalene in acetone were applied daily for five or 14 days, or three times weekly for three weeks to the backs of mice less than 50 days old; this caused an increase in the concentration of both Δ^7-cholestenol and cholesterol in the epidermis. The relative increase of Δ^7-cholestenol in the skin was 18.4% for control of mice and 36.9% for treated mice. Application of Squalene to mice aged 50 days or more caused no consistent change in the concentration of Δ^7-cholestenol, although in some instances the concentration of cholesterol appeared to increase.[50] (The hair cycle of mice was not considered. The latter is known to influence appreciably the absorption of chemicals.)

Mice were fed a purified ration containing 1% Squalene for one or two days. In addition, rats were either fasted for one day and then fed the 1% Squalene ration for two days, or fed 1% Squalene for one day without having been fasted. This dietary Squalene caused no increase in the concentration of Δ^7-cholestenol in the livers of the tested rats and mice.[50]

Rats fed Squalene in amounts equivalent to 1% of the diet for 21 days showed a 50% increase in liver sterols and a 33% increase in fecal sterols, though there was no change in the carcass sterols. The sum of liver and fecal sterol increases equalled approximately one-eighth of the Squalene that had been administered.[51]

Vitamin A deficient rats fed β-carotene with either 10 or 50 mg of Squalene for 12–14 days showed a marked reduction in the vitamin A content of the liver and kidneys. When vitamin A instead of β-carotene was fed with Squalene, the vitamin A content of the organs was unaffected; thus Squalene does not interfere with the utilization of vitamin A, but rather with that of β-carotene, the vitamin A percursor.[52]

Matschner et al.[53] reported dietary Squalene inhibits vitamin K absorption in the rat. A vitamin K-deficient diet of the following composition (given in percentages) was fed for two weeks to individually caged adult male rats: casein, 21; corn starch, 43; glucose monohydrate, 27; corn oil, 5; and a supplement of vitamins and minerals. Other rats were fed a diet that was the same as this one, except that added to it was either (a) 0.5% Squalene; (b) Squalene (0.5%) plus

vitamin K (0.25 μg/g of diet); or (c) vitamin K (0.25 μg/g of diet). Feces were collected daily and assayed for vitamin K. As shown in Table 4, rats fed the basal diet alone for two weeks excreted 1415 μg of vitamin K. Rats fed the diet containing 0.5% Squalene had a fecal vitamin K content of 1095 μg. When both vitamin K and Squalane were added to the diet, the rats excreted 560 μg of the 700 μg ingested. When a similar amount (600 μg) was fed in the diet to which vitamin K alone had been added, only 155 μg of the vitamin was recovered. According to the authors, "These data support a mechanism of interrupted absorption and possible diminished bacterial synthesis of vitamin K for the action of dietary Squalene."

It is known that Squalene, a normal constituent of the liver of most higher animals, is synthesized by animal tissues from acetate, and that it can serve as a direct precursor of cholesterol both in vivo and in vitro. In the biogenesis of cholesterol, acetate is converted to mevalonic acid, mevalonic acid is converted to Squalene, Squalene is cyclized to lanosterol, which, in turn, is converted to cholesterol.[9,10,48,54-57]

Hamsters fed a gallstone-producing diet with 1% added Squalene for 42–44 days showed complete protection against the formation of gallstones. The authors suggested this may have been due to the inhibition of biosynthesis of cholesterol in the liver.[58] This does not appear to be consistent with the work of Bloch,[56] according to which it is "virtually certain that Squalene is an obligatory intermediate in sterol biogenesis." McGuire and Lipsky,[55] who confirmed Bloch's earlier findings that both Squalene and cholesterol inhibited the bioconversion of acetate to cholesterol, postulate the following explanation for this paradox. "Squalene, by causing a 'piling up' of hepatic cholesterol may therefore evoke a homeostatic reduction in cholesterol synthesis from all sources" (feedback inhibition).

Evidence for the in-vivo metabolic conversion of Squalene to glucocorticoids was developed in hypophysectomized male rats given suboptimal injections of ACTH. The resulting increases in adrenal weight and decreases in thymus weight were enhanced when Squalene was injected subcutaneously as little as 24 hours prior to ACTH injection. Squalene alone did not produce statistically significant changes in the organ weights. Orally administered Squalene was not effective. According to the author, these data are consistent with the idea that exogenous Squalene could serve as a ready precursor of glucocorticoids in vivo, and that it may be a potential intermediate in steroid biogenesis.[47,49]

TABLE 4. Fecal Vitamin K in Adult Male Rats.[a]

		Vitamin K				
		Fecal		Eaten	Recovered	
Diet	Feces[b]	μg/g	μg	μg	μg	percent
Basal diet	101	14.0	1415	–[c]	–	–
Basal diet + Squalene	115	9.5	1095	–	–	–
Basal diet + Squalene + vitamin K	114	14.5	1655	700	560	80
Basal diet + vitamin K	100	15.7	1570	600	155	25

[a]Data from Ref. 53.
[b]Total feces (dry weight, grams) collected from 10 rats for 13 days.
[c]– No data.

Rabbits were injected subcutaneously with Squalene twice a day for up to 12 days. In the body of animals, the test material was oxidized to succinic and laevulinic acids. Urine samples showed succinic acid, along with small amounts of benzoic and hippuric acids. In animals sacrificed either four hours or 90 days after the last injection, there were considerable amounts of stored Squalene in liver, muscle, and skin.[59]

In a study with human subjects, a direct relationship was found between plasma levels of Squalene and triglycerides, but not between the levels of Squalene and cholesterol. Levels of Squalene in plasma rose with increased dietary Squalene and varied directly with the cholesterol synthesis rate. That large amounts of Squalene excreted in skin surface lipids was thought to reflect de novo synthesis in the skin rather than transference from the plasma. Small amounts were excreted in the urine and feces.[7]

Animal Toxicology

General Studies

Acute studies: oral toxicity

Squalane: Squalane was given undiluted in single oral doses to a group of 50 mice to determine its LD50. Doses of 5.0, 12.5, 25.0, and 50 ml/kg were given to 10, 10, 10, and 20 mice, respectively. Since no deaths occurred and no toxic effects were noted, the oral LD50 is greater than 50 ml/kg in mice.[60]

Squalene: The single dose oral LD50 was determined to be greater than 50 ml/kg in mice. Groups of 5, 5, 10, and 10 mice received undiluted doses of 5.0, 12.5, 25.0, and 50.0 ml/kg, respectively. In the seven-day observation period, no toxic effects were observed and no deaths occurred.[61]

Squalene/Hydrogenated Shark Liver Oil: In another study in mice, a mixture of 65% Squalene in hydrogenated shark liver oil had a single oral dose of LD50 >100 ml/kg. Doses of 5, 25, 50, and 100 ml were given undiluted to 4, 4, 4, and 20 mice, respectively. Even the highest dose did not produce visible toxic effects.[62]

Subcutaneous administration

Squalane: Subcutaneous injections of 0.5 ml Squalane per 20 g mouse (25 ml/kg) were made in five mice, and 1.0 ml/20 g mouse was given (50 ml/kg) in 10 mice. After a one-week observation period, all animals were sacrificed and the site of injection was examined. Macroscopic examination showed unabsorbed compound present in 3/5 of the low-dose mice, while 10/10 of the high-dose group had identifiable compound present. No toxic response was noted to either dose.[63]

Intramuscular administration

Squalane: Intramuscular injections of 0.5 ml/20 g mouse (25 ml/kg) were made into each of 10 mice. At the end of a one-week observation period, the animals were sacrificed; microscopic examination revealed residual compound present at the injection site in 9/10 animals. No toxic response was noted.[64]

Skin irritation

Squalane: Undiluted Squalane (0.5 ml) did not produce irritation in three

rabbits when applied to intact and abraded skin for 24 hours according to the method of Draize.[4]

An "official" French method was used to determine the skin irritation potential of undiluted Squalane in six albino rabbits.[65,66] The test material was applied to the clipped skin under occlusive patches for 24 hours. The Primary Irritation Index (PII) was 0.29, indicating that the test material was practically nonirritating.

Squalene: According to the procedure of Draize, undiluted Squalene (0.5 ml) was applied to the abraded and intact skin of three rabbits for 24 hours. No irritation resulted.[67]

Eye irritation

Squalane: When the procedure of Draize was employed, undiluted Squalane did not produce irritation or damage in the eyes of rabbits, regardless of whether the eyes had been washed after instillation.[68]

A modified official French method was used to evaluate the eye irritation potential of undiluted Squalane in six albino rabbits.[65,66] Eye readings were taken at one hour and at one, two, three, four, and seven days. The Ocular Irritation Index (OII) was 4.33 at one hour and 0.0 thereafter. The investigators believed that a compound does not provoke any significant injury if the OII is less than 10.

Squalene: The procedure of Draize was used to test undiluted Squalene (0.1 ml) in the eyes of rabbits. The compound did not produce irritation, despite the fact that no attempt was made to wash the eyes after instillation.[68]

Inhalation studies

Squalane: An acute inhalation test was conducted with an antiperspirant spray formulation containing 4% Squalane; investigators used the method of the Federal Hazardous Substances Act (FHSA). Ten (5M, 5F) Wistar-derived albino rats were exposed to a chamber concentration of 181 mg/l for one hour, so that the formulation dose per rat was 45.1 ml/kg. The calculated dose of Squalane was 1.8 mg/kg. Autopsy 14 days after exposure showed "no evidence of compound related tissue abnormality." The test formulation was "not considered toxic" by inhalation to rats under the regulations of the Consumer Product Safety Commission (16 CFR 1500.40).[70]

Ten Sherman-Wistar albino rats were similarly exposed to a deodorant spray containing 4% Squalane. Chamber concentrations were reported to be 345 mg/l, and during the one-hour exposure, the available formulation dose to the rats was 100.9 mg/kg. The calculated dose of Squalane was 9.1 mg/kg. Autopsy 14 days after exposure revealed no abnormalities.[70]

Miscellaneous studies

Squalane: In an anticancer screening program, doses of 350, 400, and 500 mg/kg were administered daily to mice with malignant tumors by intraperitoneal injection for nine to 11 days. The Squalane had no effect on the tumors and no apparent effect on the host animals.[71]

Squalene: As a preliminary investigation to determine whether Squalene had a protective effect against x-irradiation, groups of five mice each were given doses of 500, 1000, 1500, and 2000 mg/kg of the undiluted ingredient by an unspecified route. During the 10-day observation period, no deaths occurred. In the follow-up study, a 10% solution of Squalene at a dose of 2000 mg/kg was given by an unspecified route to 20 mice 15 minutes prior to administration of

575 roentgens of x-radiation. Sixty percent of the animals survived for 30 days. On the other hand, of the mice to which no Squalene was given, only 25% survived the 30-day observation period. When the x-radiation was increased to 800 roentgens, the 10% Squalene solution did not increase survival time. It was concluded that "Squalene is just short of being protective against x-radiation at a mid-lethal dose range . . .".[72]

Subchronic inhalation studies

Squalane: Twice a day, a hair spray containing 2% Squalane was sprayed for 30 seconds into a 200 l chamber containing five immobilized rabbits each weighing 3–4 kg. The rabbits were allowed to remain in the spray atmosphere for 15 minutes after each spraying. Exposure was continued five days a week for 90 days. Throughout the entire experimental period, the test animals ate well and behaved normally. Three of the five rabbits gained weight in the 90 days, while one lost and another remained at its original weight. Hematology findings were within the normal established limits for this strain of rabbit. Organ weights were likewise reported to be "in the range for the size animal employed." (Calculations made of organ weights as a percent of total body weight showed they were essentially normal.) Gross and microscopic examinations of the kidneys, liver, spleen, adrenals, and lungs showed no histopathology. X-rays taken at 30 and 90 days after exposure revealed no pulmonary congestion.[73]

Skin studies

Squalane: To determine the cumulative skin irritation potential of 15% Squalane in aqueous solution and of undiluted Squalane in albino rabbits, investigators employed an official French method.[65,66] Each test material was applied daily for 60 days to the shaved skin of three albino rabbits. Undiluted Squalane gave a mean irritation index of 1.00, indicating that it was "relatively well tolerated." There were vesicles and papules on the skin, but no histological pathology. Squalane in aqueous dispersion was "well tolerated" and produced a mean maximum irritation index of 0.33. There were some vesicles, but no histological pathology.

Squalene: Several studies have shown that Squalene has a reversible depilatory effect on animals.[74-77] In a study by Flesch,[74] a single application of 100% Squalene was made to the skin of rabbits (1 ml), guinea pigs (1 ml), and mice (0.2 ml). The hair in the area of application of all rabbits treated began to fall out in one week; complete baldness in the treated area resulted in 10–12 days. Three of four guinea pigs lost the hair in the area of application after 10 days. The mice showed no depilation. Hair regeneration was visible in all animals at the beginning of the third week, and the fur resumed its normal appearance within a few weeks. No toxic symptoms were observed in any animal. Histological examination of the rabbit skin 12 days after application of Squalene revealed hyperplasia of the cutaneous epithelium. There was no inflammatory reaction of significance. The author's observations apparently did not take into account hair growth cycles at the times he applied Squalene. It is evident that the animals which lost hair had follicles that were in telogen, while those that did not lose hair had anagen follicles. The follicles, apparently in telogen at the time of Squalene application, were stimulated to activity and hair grew back normally.

Flesch[74] found that Squalene inactivated the free sulfhydryl groups of gutathione in human epidermis and mouse liver homogenate. Squalene also inhibited succinic dehydrogenase activity of mouse liver homogenate.

Special Studies

Carcinogenesis

Squalene: Squalene was painted in undiluted form six times weekly for a total of 25 times on the backs of 16 C57B1 mice (the total dose was 1.3 g per mouse). Eight mice survived 100 days, but five of them developed "lymphocytic type of tumors" between days 272 and 849. Tumors were primarily found in the thymus and mesentary of the thymus. Metastases and/or lymphocytic invasions were detected in the peripheral lymph glands, lungs, spleen, liver, and kidneys.[78] This study needs confirmation.

A 20% solution of Squalene in decahydronaphthalene (Decalin) was applied as a tumor promoter twice weekly to the skin of male C3H mice which had been initiated once with 240 μg of 7,12-dimethylbenz(a)anthracene (DMBA). After 30 weeks of application, two out of 12 mice developed malignant skin tumors. In the control group, where 100 percent decahydronaphthalene was used as a promoter, two out of 15 mice developed benign skin tumors.[79]

In a skin painting study, both freshly purified and "aged" Squalene (compound that had been exposed to open air at 37 °C for four weeks) were painted three times weekly for 14 weeks in undiluted form on the backs of C57B1 and C57BR mice. No skin tumors developed. When a similar procedure was used to paint fresh and "aged" Squalene in combination with 0.3% 3-methylcholanthrene (3-MCA), each form of the compound was determined to be inactive as a cocarcinogen. When 0.3% 3-MCA in Squalene was "aged" by being left to stand for four weeks in the open air at 37 °C, 3-MCA lost its carcinogenic effect on mouse skin. According to the authors, these observations suggest that Squalene in human sebum may play a protective role against hydrocarbon carcinogens.[8,80]

Clinical Assessment of Safety

Contact Sensitization

Squalane

Twenty subjects were patch-tested with repeated 48-hour applications made three weeks apart with 8.0% w/w Squalane in a lip emollient. Nineteen responses to both patches were negative; one response was not reported.[81]

Two-hundred forty patients were patch-tested with repeated 48-hour applications made three weeks apart with a formulation (eye pack) containing 16.6% w/w Squalane. All responses were negative.[82]

Ninety-eight patients patch-tested to 15% Squalane in peach kernel oil showed no skin reactions.[83] The procedure used was similar to that described by Fisher.[84]

One-hundred three females patch-tested by a repeated insult procedure to a blushing cream containing 16.8% w/w Squalane demonstrated no contact allergy or irritant reactions.[85]

Twenty females between the ages of 15 and 54 years were patch-tested according to a modification of the Schwartz–Peck 48-hour patch test system to a "night treatment formulation" containing 20.0% w/w Squalane. Two panelists responded with a 2+ reaction on a scale of 0–4 during a second patch-test reading; these scores indicated a "well defined erythema." No other dermal reactions were noted during the 21-day usage of this formulation.[86]

Six-hundred subjects were patch-tested by the modified Draize procedure to

moisture cream containing 7.2% Squalane. None of the 600 subjects demonstrated contact allergy or irritation.[87]

One-hundred female subjects patch-tested to a cream formulation containing 7.0% Squalane did not demonstrate any contact allergy or irritation.[88]

Another 100 female subjects were subjected to prophetic patch-tests with a lipstick formulation containing 20% Squalane. It was the opinion of the investigators that "the product was not a primary irritant, and the sensitizing potential, if existent at all, is exceedingly low".[89]

Ten subjects showed no visible primary skin irritation when tested by the procedure of Kligman and Wooding with 9.0% Squalane in an aerosol antiperspirant spray.[90]

Twenty-five subjects showed no instances of contact sensitization when tested with 9.0% Squalane in an aerosol antiperspirant spray.[91] The test protocols used were those described by Kligman.[92]

Squalene

"Patch-tests" were conducted on an unspecified number of human subjects with both pure Squalene and the nonsaponifiable matter from shark liver oil containing 76% Squalene. The substances were each left in contact with the intact skin for 72 hours. "There was no detectable effect upon the skin or hairs; no change in the degree of pigmentation occurred, nor was any effect on keratinization observed."[46]

Miscellaneous

Squalene

For six weeks, Squalene was tested in a "small group of volunteers" in which the material was applied daily to "various parts of the body" in "free form" and in vehicles. No depilation of other adverse effects were observed. No other data were presented for this study.[5]

Squalene was injected intradermally at a dose of 50 μg into 29 subjects. There was no erythema, induration, or inflammatory skin lesions in any of the subjects. Upon visual inspection at 24 and 48 hours, the sites of injection were normal. Histological examination of biopsies taken at 24 hours demonstrated the presence of a mild, predominantly lymphocytic, perivascular infiltration which was only slightly more intense than the reaction induced by control injections of physiologic saline containing 0.05 percent Polysorbate 80.[93]

In a study conducted by Boughton et al.,[46] undiluted Squalene (0.2 ml) was injected into the skin of an unspecified number of subjects. No effect other than a "short-lived" inflammatory response was observed. In a second study, undiluted Squalene was applied to the unbroken or blistered skin of an unspecified number of subjects, and the treated areas were exposed to ultraviolet irradiation from a mercury vapor lamp. No effect on pigmentation was noted on either unbroken or blistered skin, and all areas of the blistered skin healed normally. According to the authors, "Squalene apparently had no inhibitory or stimulatory effect on either pigmentation or healing." Shark liver oil containing 76% Squalene and pure Squalene were also applied to affected areas of patients with eczema, psoriasis, vitiligo, ichthyosis, and hirsutism; no therapeutic effect was seen.

SUMMARY

Squalane and Squalene have been identified as natural components of human sebum. Both ingredients are used at concentrations ranging from ≤ 0.1 to $> 50\%$ in a variety of cosmetics. Because cosmetics containing Squalane and Squalene are applied to all body surfaces, these compounds may potentially enter the body through the skin, eyes, lungs, mouth, or other routes.

Squalene can form peroxides on exposure to air, while Squalane is stable to air and oxygen. Animal studies indicate Squalane is slowly absorbed through the skin, while both compounds are poorly absorbed from the gastrointestinal tract. Squalene is a metabolic precursor of cholesterol and other steroids.

The acute toxicity of these ingredients by all routes in animals is low. At 100% concentrations, both compounds are nonirritants to rabbit skin and eyes. According to clinical evidence of formulations containing Squalane, the compound is not a significant skin irritant or sensitizer. Limited contact sensitization tests indicate that Squalene is not a significant contact allergen or irritant.

Reversible depilation is reported from topical application of Squalene to animals, but limited human studies did not show any such effect.

No photosensitivity data for the two ingredients were available.

CONCLUSION

On the basis of the available information presented in this report, the Expert Panel concludes that both Squalane and Squalene are safe as cosmetic ingredients in the present practices of use and concentration.

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