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10

Final Report on the Safety Assessment of Cholesterol

Cholesterol is used as an emulsifier in cosmetic skin and hair care products and eye and face makeup formulations at concentrations up to 5%.

The normal metabolism and excretion of Cholesterol is well documented in man and experimental animals. Cholesterol is not a significant dermal or ocular irritant. Cholesterol does not appear to have any genotoxic activity in bacterial or mammalian cell in vitro mutagenic and transformation assays. High doses of Cholesterol were teratogenic in rats. Cholesterol has not been established as a promoter, cocarcinogen, or total carcinogen.

Clinical studies to evaluate the safety of topically applied Cholesterol were restricted to products formulated with the ingredient. Most products were moisturizers containing 1.4% Cholesterol. The highest concentration of Cholesterol tested (6%) was evaluated in a modified prophetic test (110 subjects) and an RIPT (45 subjects); both assays had UVA and UVB exposure incorporated into the protocols. The Cholesterol-containing products were minimal to mild primary and cumulative skin irritants but not sensitizers or photosensitizers.

INTRODUCTION

Cholesterol is one of the most widely studied naturally occurring organic compounds. Cholesterol is found in all tissues of the animal body. It has several important biological functions, such as being a metabolic precursor for steroidal hormones and contributing to the fluidity and/or rigidity of animal cell membranes. Excess Cholesterol, insufficient Cholesterol, and defects in Cholesterol metabolism have all been associated with various pathological conditions that are, and have been, areas of intense research. Cholesterol has also been isolated from plants.[1]

Due to the variety and abundance of literature on Cholesterol, this review was, for the most part, limited to current published literature (~1978–present) listed in the National Library of Medicine's Toxline and Medline computer abstract files, several published reviews and textbooks on Cholesterol including reviews by Kritchevsky,[2,3] an International Agency for Research on Cancer[4] monograph evaluating the carcinogenic risk of Cholesterol, a comprehensive textbook on metabolism and adverse effects of Cholesterol by Sabine,[5] stan-

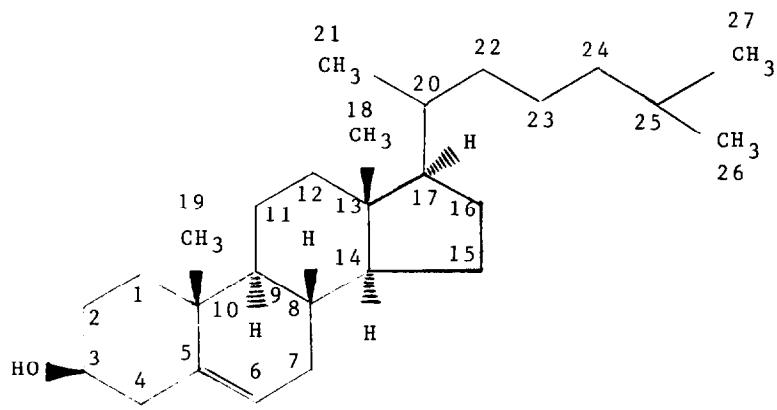
dard textbooks on biochemistry and human physiology, and unpublished data submitted by the Cosmetic, Toiletry and Fragrance Association.[6-41]

Cholesterol is used primarily as an emollient in cosmetic products. It is an ingredient in eye makeup, face makeup, skin lotions and creams, and hair care formulations.

CHEMISTRY

Definition and Structure

Cholesterol is a monounsaturated secondary alcohol of the cyclopentenophenanthrene (four-ring, fused) system. This steroid (CAS No. 57-88-5) has an empirical formula of $C_{27}H_{46}O$, a molecular weight of 386.64, and conforms to the structural formula:



Cholest-5-en-3β-ol and Cholesterin are synonyms for Cholesterol.[41,44,45]

Physical Properties

Cholesterol is a white or faintly yellow, almost odorless solid that occurs in pearly granules or crystals. It is practically insoluble in water, slightly soluble in alcohol, and soluble in organic solvents, oils, fats, and aqueous solutions of bile salts.[46,47] Methods for identification of Cholesterol[47,48] and the UV absorption spectra for Cholesterol and derivatives of Cholesterol[49] have been published. Physical constants for Cholesterol are presented in Table 1.

Reactivity

Sterols are crystalline, neutral, unsaponifiable alcohols of high melting points.[50] The reactive sites of Cholesterol at which most chemical reactions occur are the hydroxyl group, the double bond, and carbon number 7. Cholesterol can be oxidized by exposure to air, heat, x-rays, and UV irradiation. The principal air oxidation products of Cholesterol are 7-ketocholesterol and epimeric

TABLE 1. Physical Properties of Cholesterol[2,44]

Melting point	149°C
Boiling point (bp760)	360°C
Specific gravity (18°C)	1.052
$[\alpha]_D^{29}$ (2.0 g/100 ml ether)	−31.5°
$[\alpha]_D^{29}$ (2.0 g/100 ml chloroform)	−39.5°
ΔH combustion	3978 Kcal/mol
ΔH vaporization	27.4 Kcal/mol
ΔH evaporation	24 Kcal/mol
Electric moment	4.2×10^{-19} esu
Dielectric constant	5.41

Solubility data

t(°C)	*Methanol*	*Ethanol*
0	0.34 g/100g	0.68 g/100g
20	0.65	1.29
40	1.88	3.40
50	2.94	5.25
60	4.42	7.85
Hexane	1.92 g/100g	
Benzene	14.24	
Dioxane	11.26	
i-Butyl alcohol	6.34	
n-Amyl alcohol	10.54	
Furfural	0.33	
Diethylamine	2.21	
Triethylamine	69.16	
Ammonia (liq.-0°)	0.008	

7-hydroxycholesterols.[2] When Cholesterol is heated at 180°C for at least 25 h, 5-cholesten-3β-7β-diol and 5-cholesten-3β-7α-diol are among the oxidation products.[51] The formation of oxidative products produced by irradiation is proportional to exposure time. Cholestan-3β, 5α, 6β-triol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol-α-oxide were identified by chromatographic techniques following irradiation of ^{14}C-Cholesterol by a mercury arc lamp.[52]

Analytical Methods

A large amount of published literature is devoted to the detection and analysis of Cholesterol. These methods are generally variations on a combination of extraction, separation, and quantitation. The analytical scheme used depends on the starting material and the degree of accuracy desired. Extraction with organic solvents isolates the lipid fraction from a sample. The lipid fraction is then separated into its components by any number of chromatographic techniques—column, paper, thin-layer, gas–liquid, or liquid–liquid, and the most common methods for quantitation of the isolated cholesterol are colorimetric.[5]

Method of Manufacture and Impurities

Several methods for the complete synthesis of Cholesterol in the laboratory have been published.[53-55] Cholesterol is commercially prepared from bovine spinal cord and wool grease by extraction with petroleum ether. The extracted material is then purified by repeated bromination. Cholesterol from animal organs always contains cholestanol (dihydrocholesterol) and other saturated sterols.[46,56]

USE

Purpose in Cosmetics

Cholesterol is used primarily as an emulsifier and conditioner in cosmetic products.[57-59]

Scope and Extent of Use in Cosmetics

Cholesterol is used in 145 cosmetic products according to the Food and Drug Administration (FDA) cosmetic product formulation data. Cholesterol is primarily used in skin care products, eye and face makeup, hair care products, and shaving preparations. These products contain $\leq$ 5% Cholesterol, with the majority of products having a Cholesterol content of 0.1–1%.[60]

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 (21 CFR 720.4). Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the concentration reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual 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. See Table 2 for the list of cosmetic products containing Cholesterol.

Surfaces, Frequency, and Duration of Application

Products containing Cholesterol have the potential of being applied to the hair and skin, including the skin of the face and eye area. The major products containing Cholesterol are makeup and skin care lotions; thus, they could be expected to remain in contact with the skin for at least 12 h per day.

Noncosmetic Use

Cholesterol is used as an emulsifying agent in pharmaceutical[48] and veterinary products.[61] A novel experimental pharmaceutical application of Cholesterol is its use in liposomes to encapsulate and deliver chemotherapeutic drugs

TABLE 2. Product Formulation Data[60]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)		
			>1–5	>0.1–1	≤0.1
Cholesterol					
Eyeliner	396	6	–	6	–
Eye shadow	2582	15	–	15	–
Mascara	397	16	5	11	–
Other eye makeup preparations	230	3	2	1	–
Other fragrance preparations	191	1	–	1	–
Hair conditioners	478	7	–	4	3
Hair shampoos (noncoloring)	909	1	–	1	–
Makeup foundations	740	7	–	5	2
Makeup bases	831	12	–	7	5
Other makeup preparations (not eye)	530	14	–	5	9
Aftershave lotions	282	1	–	1	–
Other shaving preparation products	29	1	–	1	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	5	–	3	2
Face, body, and hand skin care preparations (excluding shaving preparations)	832	11	3	6	2
Moisturizing skin care preparations	747	19	7	10	2
Night skin care preparations	219	15	2	10	3
Wrinkle smoothers (removers)	38	2	1	1	–
Other skin care preparations	349	8	2	6	–
Suntan gels, creams, and liquids	164	1	–	1	–
1981 TOTALS		145	22	95	28

to diseased tissue.[62] Cholesterol-^{14}C is used clinically as an organ- or tumor-imaging agent. Organs visualized by this technique include ovaries, adrenals, and spleen.[43,63–66]

BIOLOGY AND METABOLISM

Distribution and Functions of Cholesterol

One hundred forty-five grams, or just over 0.2% of the average adult, 70-kg male is Cholesterol. Most of the Cholesterol is membrane associated, being in and on every plasma membrane of the body. About 8 g, or 5.5% of this Cholesterol is contained in the plasma, and the remainder is distributed in a variety of physiological sites, such as the gut, triglyceride storage droplets, and possibly pathological accumulations.[5]

The overall function of Cholesterol in plasma membranes relates to the fluidity of the membrane. The Cholesterol:phospholipid ratio is inversely propor-

tional to the fluidity of the membrane. Generally, as the Cholesterol content increases, the fluidity decreases and the membrane becomes more rigid.[67]

Cholesterol is also a metabolic precursor for other important steroids, such as adrenal corticosteroids, sex hormones, bile salts, and provitamin D_2.[46]

Biosynthesis

The biosynthesis of Cholesterol has commanded intensive research over the past half-century and is consequently well elucidated and understood. The rudimentary carbon source for Cholesterol is acetyl-CoA. In a complicated, multistep synthesis requiring 26 enzymes, acetyl-CoA is transformed into Cholesterol, with β-hydroxy-β-methylglutaryl-CoA, mevalonic acid, and squalene as some of the important intermediate compounds. As mentioned before, cells can synthesize Cholesterol; however, the vast majority of Cholesterol (~90% in the squirrel monkey) is synthesized in the liver and intestine. In general, the biosynthesis of Cholesterol is regulated by negative feedback where the presence of Cholesterol inhibits its own synthesis.[5,67]

Absorption

Dietary Absorption of Cholesterol

Cholesterol is absorbed from the gut via lymph. The primary site of absorption of dietary Cholesterol is the proximal small intestine. Cholesterol is absorbed from the intestine after incorporation into chylomicrons, which are mixed micelles composed of triglycerides, phospholipids, protein, and free and esterified Cholesterol. Bile salts are necessary for the incorporation of Cholesterol into chylomicrons. The bile salts can increase slightly the solubility of relatively nonpolar molecules, such as Cholesterol and fatty acids. Once the chylomicrons are absorbed into the lymph, the micelles are absorbed into capillaries and degraded, and Cholesterol-containing chylomicron remnants are absorbed into the liver. The liver incorporates Cholesterol (absorbed Cholesterol as well as the Cholesterol synthesized by the liver) into very low-density lipids (VLDL), intermediate-density lipids (IDL), and low-density lipids (LDL). Cholesterol in the form of LDL then enters the circulatory system, where a variety of metabolic pathways are available, as well as excretion in bile, feces, urine, milk, or skin.[5,67,68] A schematic diagram of the fate of ingested Cholesterol is given in Figure 1.

Skin Absorption of Cholesterol

Jones and Murray[69] investigated the influence of various lipids and sterols on water loss from human skin. Cholesterol in alcohol, petrolatum, or wool fat did decrease water loss from the skin, and 2% Cholesterol in petrolatum penetrated the skin 33% more than did Cholesterol-free petrolatum.

Cholesterol is a component of skin surface lipids and sebum. Skin surface lipids contain 2–20% sterols, 90–95% being Cholesterol.[5] Sebum from the forearm generally contains about 5% Cholesterol, half in the free form and half esterified.[70]

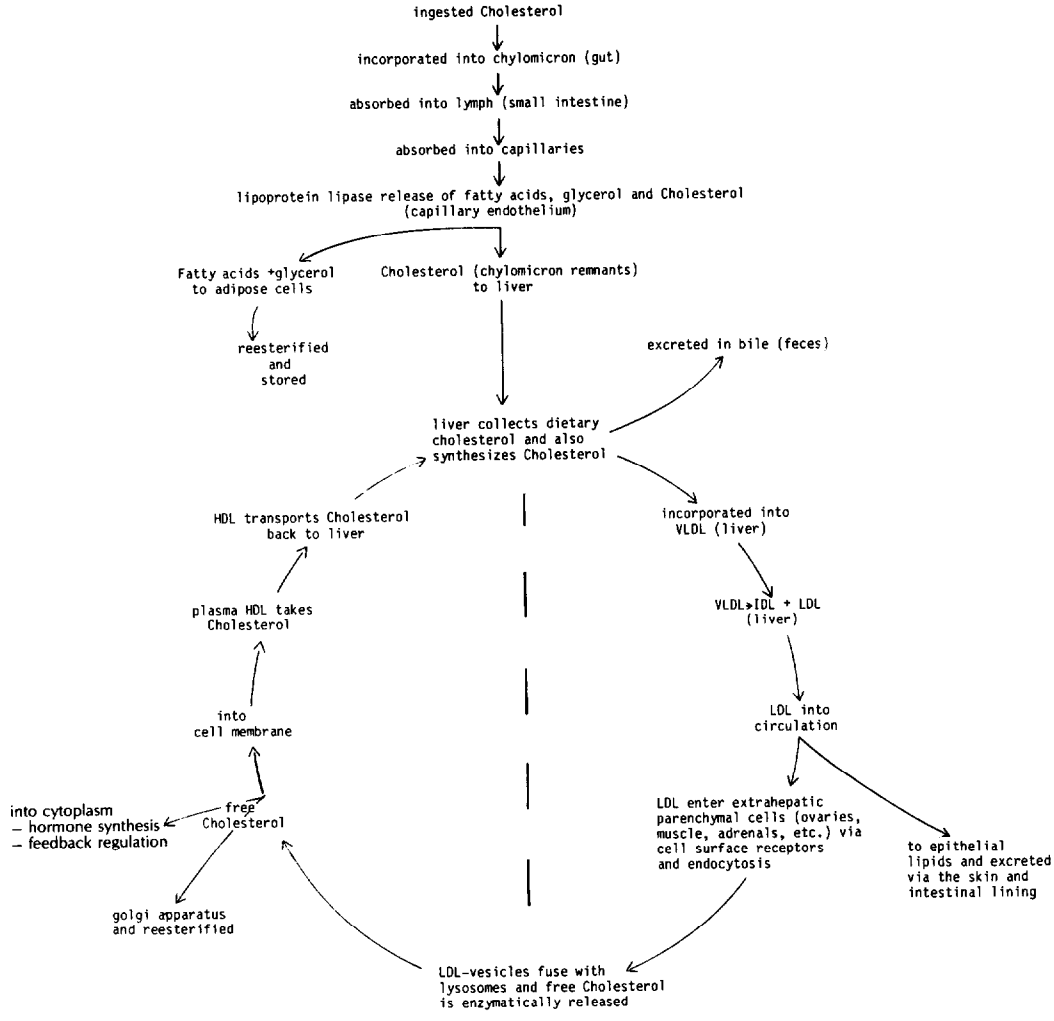


FIG. 1. Fate of Dietary Cholesterol.

Anabolism

There are two major metabolic pathways of Cholesterol in mammals: metabolism to bile acids in the liver, and metabolism to steroid hormones in the adrenals, testes, ovaries, and placenta. The largest amount of Cholesterol lost to anabolism (approximately 600 mg/day) is converted to bile acids. Bile acids are produced more to remove excess Cholesterol than from a need for large quantities of bile salts. The synthesis of steroid hormones requires much less Cholesterol, about 40 mg/day in humans.[5]

Cholesterol is also metabolized into Cholesterol esters, Cholesterol sulfate, cholestanol, and vitamin D_3, a compound essential to calcium and phosphorus

homeostasis. Vitamin D_3 is interesting in that it is produced in the skin by the reaction of Cholesterol-derived 7-dehydrocholesterol and UV radiation.[5]

Cholesterol in the gut is also metabolized by intestinal flora to several compounds, predominantly coprostanol and coprostanone.[5] This metabolism of Cholesterol by gut bacteria has been demonstrated with in vitro and in vivo animal studies and is markedly suppressed by administration of antibiotics in the rat and guinea pig.[71]

Excretion

Cholesterol is eliminated from the body via the feces, urine, and skin surface. The Cholesterol and Cholesterol derivatives excreted in the feces are in the form of bile salts, cellular and membrane Cholesterol from cells sloughed from the intestine, unabsorbed dietary Cholesterol, and the metabolic products of gut bacteria. Cholesterol and its derivatives excreted in the feces accounts for about 50% of the total Cholesterol turnover in man. Free and conjugated Cholesterol is a normal constituent in urine. Cholesterol is also eliminated in the milk of lactating females, primarily in the membranes of milk fat globules.[5]

ANIMAL TOXICOLOGY

Subchronic Oral Toxicity

Albino SPF mice were used in a subchronic feeding study. Thirty mice were fed diets supplemented with 1% (w/w) Cholesterol and 0.45% (w/w) choline, and 30 mice were fed a control diet with choline but no Cholesterol. After 4 weeks, 10 mice from each group were killed, and the livers were evaluated microscopically. Hydroxyproline and glycosaminoglycan concentrations were determined. Twenty mice (10 test and 10 control) were fed their respective diets for another 20 weeks, and the remaining 20 mice were fed the control diet for another 4 weeks. The mice fed Cholesterol for either 4 or 24 weeks had significantly heavier livers, 20- to 25-fold increases in hepatic Cholesterol accumulation, significantly higher concentrations of hepatic hydroxyproline and glycosaminoglycans (as reflected by total hexosamines), and diffuse fatty change without hepatocellular necrosis or fibrosis. Mice fed the Cholesterol diet for 4 weeks, then switched to the control diet for another 4 weeks, differed from controls only by a slightly raised hepatic Cholesterol concentration, indicating that the hepatic changes were reversible.[72]

Skin Irritation

The skin irritation of undiluted Cholesterol was evaluated in nine female albino rabbits. A single, occlusive patch was applied to the clipped skin of each rabbit, and test sites were scored for irritation 2 and 24 h after removal of the 24-h patch. All irritation scores were 0, and the group primary irritation index (PII) was 0 (max 8). Undiluted Cholesterol was not a skin irritant in rabbits.[6]

A moisturizer containing 1.7% Cholesterol was evaluated for skin irritation using nine female albino rabbits. Test sites were scored for irritation 2 and 24 h

after removal of a 24-h occlusive patch containing the full-strength product. At the 2-h reading, one animal was found dead, five animals had scores of 0.5 (max 4), and one animal had a score of 1. At 24 h, two and one animals had irritation scores of 0.5 and 1, respectively. The moisturizer was a slight skin irritant with a group primary skin irritation (PSI) value of 0.56 (max 4).[20]

Cholesterol crystals are skin irritants when injected subcutaneously into rat footpads. The irritation and swelling caused by a 0.20 ml subcutaneous injection of Cholesterol was not reduced by injections of prostaglandin E_1, prostacyclin, or thromboxane (B_1). Injection of Cholesterol crystals produced less severe irritation and swelling in rats with essential fatty acid deficiencies.[73]

Ocular Irritation

The ocular irritation of a 5% solution of Cholesterol in corn oil was evaluated in two groups of six albino rabbits according to the method of Draize.[74] One-tenth milliliter of the test material was instilled into one eye of each rabbit, and the other eye served as the control. The eyes were not rinsed. All ocular irritation scores were 0 one day after instillation in one group, and two animals in the second group had mild conjunctival irritation. All irritation scores were 0 on Day 2. The group ocular irritation score was 0 (max 110) in the first group and 1 in the second group of rabbits. Five percent Cholesterol in corn oil was a minimal eye irritant.[7,8]

A face cream containing 6% Cholesterol was evaluated for ocular irritancy in nine rabbits. One-tenth milliliter of the product was instilled in one eye of each rabbit, and the contralateral eye served as the control. The treated eyes were rinsed in three rabbits 30 seconds after instillation of the test material. Eyes were scored for irritation 1, 2, 3, 4, and 7 days after treatment. In nonrinsed eyes, slight corneal stippling and conjunctival redness were observed in 2/6 and 5/6 rabbits, respectively. No irritation was observed on Days 3–7. Two of the three animals whose eyes had been rinsed had minimal conjunctival redness on Day 1. The face cream was a slight eye irritant.[43]

A moisturizing formulation containing 1.7% Cholesterol was tested full strength for eye irritancy in six albino rabbits. Test eyes were not rinsed following instillation, and the contralateral eye served as the control. Four of five remaining rabbits had mild conjunctival irritation when scored at 24 h, but no irritation was observed 48 h after instillation. The moisturizer was a minimal eye irritant, with a group irritation score of 2 (max 110).[21]

See Table 3 for animal oral, dermal, and ocular toxicity studies.

Mutagenicity

Bacterial Assays

Cholesterol was among a large group of chemicals tested in an Ames *Salmonella*/microsome mutagenicity assay. All chemicals were tested using four strains of *Salmonella typhimurium*, TA1535, TA1538, TA98, and TA100, with metabolic activation (S9 fraction from liver homogenates from rats induced with Aroclor 1254). Cholesterol, up to 2500 μg/plate, was not mutagenic in any bacterial strain tested.[75]

TABLE 3. Oral, Skin, and Ocular Toxicology

Test type[a]	*No. of animals and species*	*Concentration of cholesterol in vehicle/product*	*Dose*	*Comments*	*Reference*
Subchronic oral	30 test and 30 control SPF mice	1% Cholesterol + 0.45% choline in diet or 0.45% choline in diet (control group)	N/A[b]	10 mice per group killed after 4 weeks; 10 mice per group remained on respective diet for 20 weeks; 10 mice per group fed control diet for 4 weeks; Cholesterol-fed mice had significantly heavier livers and 20- to 25-fold increases in hepatic cholesterol; mice fed cholesterol diet for 4 weeks then switched to control diet had only slightly elevated hepatic Cholesterol concentrations	72
Primary skin irritation	9 female, albino rabbits	100%	---	Group PII = 0 (max 8); not a primary irritant	6
Ocular irritation[c]	6 albino rabbits	5% in corn oil	0.1 ml	No irritation, not an eye irritant	7
Ocular irritation[c]	6 albino rabbits	5% in corn oil	0.1 ml	Group irritation index = 1 (max 110); minimal eye irritant	8
Subcutaneous injection in footpad	Rats	Cholesterol crystals	0.20 ml	Injections caused irritation and swelling; irritation not affected by injections of PGE_1, prostacyclin, or thromboxane; Cholesterol produced less irritation in animals with essential fatty acid deficiencies	73
Primary skin irritation	9 female, albino rabbits	1.7% in a moisturizer	---	Group PSI = 0.56 (max 4); Product was slight skin irritant	20
Ocular irritation[c]	9 rabbits	6% in face cream	0.1 ml	Eyes washed in 3/9 rabbits; slight corneal stippling and conjunctival redness in unwashed eyes; slight conjunctival redness in washed eyes; all eyes normal by Day 3; product was a slight ocular irritant	43
Ocular irritation	6 albino rabbits	1.7% in a moisturizer	0.1 ml	Group irritation index = 2 (max 110); minimal eye irritant	21

[a]See text for more details of experimental procedures.
[b]Not applicable.
[c]Eyes were unwashed unless otherwise specified.

The mutagenicity of ozonation products of several compounds was tested in Ames assays using six strains of *S. typhimurium*, TA98, TA100, TA1535, TA1536, TA1537, and TA1538, with and without metabolic activation (rat liver homogenate from rats induced with Aroclor), and in a mitotic recombination assay using the yeast *Saccharomyces cerevisiae* D3. Cholesterol was tested at a very low concentration. Cholesterol did not have any mutagenic activity pre- or postozonation.[76]

A bacterial mutagenicity/genotoxicity assay involving isolated DNA from *S. typhimurium* (strains TA1538, TA1537, TA1535) has been developed.[77] Cholesterol, up to 40 μg, did not increase UV absorbance in this assay, indicating a lack of mutagenic/genotoxic activity.

Although Cholesterol was not mutagenic in bacterial assays, some autoxidation products of Cholesterol have mutagenic activity.[78,79]

Mammalian Cell Assays

Cholesterol did not transform Golden Syrian hamster embryo cells in vitro at concentrations up to 10 μg/ml.[80]

In another Syrian hamster embryo cell transformation assay, Cholesterol (10 μg/ml) was inactive in causing transformation, but two metabolites of Cholesterol, cholesterol-α-epoxide (0.31 μg/ml) and cholestan-3β,5α,6β-triol (1.1 μg/ml), were cytotoxic and induced in vitro transformation.[80]

Cholesterol (no concentration given, but "effective" doses of other compounds ranged from 10^{-2} to 10^{-8}M) was not active in a mammalian cell DNA synthesis inhibition test for mutagenic carcinogens.[82,83] However, when Cholesterol plus polysorbate 60 was painted onto mouse skin, mitotic activity in the skin was decreased.[84]

Teratogenicity

Cholesterol is capable of crossing the placental barrier in several mammalian species, including rats, rabbits, baboons, and man. It is synthesized by the placenta as well as by the fetus.[5,85]

There has been much interest among dental scientists in the teratogenic effect of Cholesterol. Steroids, including Cholesterol, are capable of experimentally inducing oral cleft anomalies in the rat. Two groups of 10 female albino rats were used in a study to evaluate the ability of Cholesterol to induce cleft palate. One group received a daily subcutaneous injection of 15 mg Cholesterol in 2 ml vegetable oil, and the other group received a 2 ml injection of vegetable oil only. Both groups received injections from the 8th through the 14th day of pregnancy. Dams were killed on the 18th day of pregnancy, and fetuses were removed and evaluated for gross and histological oral anomalies. Seven of the 10 control animals were pregnant, with an average of 12.5 pups per dam. No malformations were found in control offspring. In the test group, 5 of the 10 rats were pregnant, with an average of 10 pups per litter. Fifty-seven percent of the fetuses from the test group had abnormal palates.[86] Buresh and Urban continued these experiments with lower doses of Cholesterol. Following the procedure outlined above, controls had no cleft malformations, and fetuses from dams receiving daily injections of 5 mg or 10 mg Cholesterol had 27% and 52% incidences of palate

anomalies, respectively.[87] Cleft palate fetal malformations have also been experimentally induced in Sprague-Dawley rats with 15 mg and 20 mg Cholesterol injections to the dams on Days 7 through 14 of gestation.[88]

Two inhibitors of Cholesterol synthesis [1-(p-(2-diethylaminoethoxy)phenyl)-1-(p-totyl)-2-(chlorphenyl) ethanol and trans 1,4-bis(2-dichlorobenzyl-aminoethyl) cyclohexane dihydrochloride] have produced teratogenicity and embryotoxicity in rats. The malformations most commonly observed were of the holoprosencephalic type: cyclocephaly, cyclopia, monorhinia, and hypoplasia or aplasia of the pituitary. There were also some urogenital anomalies. The holoprosencephalic deformities were prevented by a concurrent (maternal) hypercholesterolemia-provoking diet.[89-92]

Carcinogenicity

Cholesterol as a Promoter and Cocarcinogen

The promoting activities for cancer of the colon of sodium lithocholate, Cholesterol, Cholesterol epoxide, and Cholesterol triol were examined using germfree and conventional Fischer 344 rats. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was used as the initiator, and 2.5 mg MNNG in either 0.2 ml of 0.9% saline or corn oil were administered intrarectally (ir) twice a week for 2 weeks. Initiation was followed by ir doses of 20 mg sodium lithocholate, Cholesterol, Cholesterol epoxide, or Cholesterol triol (in 0.2 ml saline or corn oil) three times a week for 46 weeks. Control groups were given MNNG followed by ir doses of the vehicle only, or 48 weeks of the vehicle with no initiation. All animals survived to the end of the experiment, and at 48 weeks, animals were necropsied and examined grossly and microscopically for number and types of tumors. All animals given MNNG plus sodium lithocholate had significantly greater number of colon tumors than had the MNNG controls (78 tumors in 48 rats as compared to 40 tumors in 48 rats), and sodium lithocholate was considered a tumor promoter in this study. Tumor incidences in rats given MNNG plus Cholesterol or then Cholesterol metabolites did not differ significantly from animals given MNNG plus vehicle alone. Cholesterol, Cholesterol epoxide, and Cholesterol triol were not promoters of colonic tumors in this assay.[93]

Cruse et al. concluded that Cholesterol is a potent dietary cocarcinogen to subcutaneously injected 1,2-dimethylhydrazine (DMH), a large bowel carcinogen in rats. The authors compared a Cholesterol-free liquid diet with a liquid diet containing Cholesterol. However, a standard solid diet was as carcinogenic as the liquid diet with Cholesterol in DMH-treated rats.[94]

Human epidemiological data and some animal studies have been combined and used to support the hypothesis that Cholesterol is a cocarcinogen in human colorectal carcinogenesis. Other investigators have indicated that Cholesterol, bile acids, or diets with high fat and/or low fiber content are correlated with higher incidences of colorectal cancer.[95-102]

Cholesterol has also had a protective effect in N-methyl-N-nitrosourea (MNU)-induced colon cancer in rats. Male F344 rats were given one of four diets for 28 weeks: group 1, control chow; group 2, control chow plus 0.2% Cholesterol; group 3, control chow plus 8 mg MNU; group 4, control chow plus 0.2%

Cholesterol plus 8 mg MNU. The groups were made up of 30, 30, 91, and 74 rats, respectively. The carcinogen was administered ir in 0.9% NaCl in 2-mg doses on Days 1, 4, 7, and 10 of the experiment. No deaths occurred during the experiment, and there were no colon tumors observed grossly or microscopically in control groups 1 or 2. Tumor incidence in animals given MNU and no Cholesterol supplement was 43/91 or 47%. Thirty percent (20/74) of the animals given MNU plus dietary Cholesterol had colon tumors. This significant reduction in tumor incidence indicates that dietary Cholesterol may retard colon carcinogenesis.[103]

Cholesterol as a Carcinogen

Hieger[104–109] and Hieger and Orr[110] have evaluated the carcinogenicity of Cholesterol and related sterols administered via subcutaneous injection in mice. Clayson[111] regards the induction of localized sarcomas in mice upon repeated subcutaneous injection of test solutions as an unreliable indicator of carcinogenicity.

Carcinomas and benign tumors of hamster and mouse urinary bladder or gallbladder have also been studied.[112–114] Bischoff reviewed the extensive literature on Cholesterol as a potential carcinogen. He cautioned against overinterpretation of the data without a detailed review of each experimental method and the need to subject the data to a more rigorous statistical analysis.[115]

The International Agency for Research in Cancer (IARC) reviewed the potential role of Cholesterol as a carcinogen. The 1976 monograph stated that "No data are available to assess the carcinogenicity of exogenous Cholesterol in man. Studies of cancer in relation to dietary fat, serum Cholesterol concentrations and the degradation of biliary steroids are not directly relevant to this question."[4] McMichael et al. reviewed the role of dietary and endogenous Cholesterol and human cancer. They concluded that there was a small-to-medium increase in the risk of cancer with increased dietary Cholesterol, but "the close correlation of Cholesterol with other foods and nutrients precludes causal inference."[116]

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation

Twenty-three female subjects completed a study comparing the skin irritation of six cosmetic products, including a moisturizing base containing 1.4% Cholesterol. A petrolatum control was included in the study. Approximately 0.3 g of the test material was applied to the back via an occlusive patch that remained in contact with the skin for 72 h. The test sites were scored for irritation on a 0 (normal) to 4 (ulcerating and blistering) scale at patch removal (72 h) and 24 h (96 h) later. The group mean score was 0.31 at 72 h and 0.22 at 96 h (max 4). The moisturizing base was minimally to mildly irritating.[31]

Three moisturizing bases, each containing 1.4% Cholesterol, were evaluated for skin irritation as described above.[31] The first product was a mild irritant using a panel of 25 subjects with mean irritation scores (max 4) of 0.24 at 72 h and 0.28 at 96 h.[32] The second formulation was tested using 26 subjects and was mildly irritating, with 72-h and 96-h mean scores of 0.17 and 0.23, respec-

tively.[33] The third moisturizer was a weak irritant when tested using a 25-member panel. The group mean irritation scores were 0.42 at 72 h and 0.48 at 96 h.[35]

Cumulative Skin Irritation

A formulation containing 1.4% Cholesterol was evaluated for skin irritation in a 10-day cumulative irritation test according to the procedure of Kligman and Wooding.[117] Ten subjects received occlusive patches on the volar forearm with approximately 0.3 g of the test material. Patches were applied once daily for 10 consecutive days, and test sites were scored for irritation each day on a 0 (normal) to 4 (intense erythema with edema and vesicular erosion) scale. All 10 subjects developed mild erythema (scores of 1) after repeated applications; the earliest irritant reaction was observed after the fourth application and the last in a subject following the ninth application. All panelists had irritant reactions to patches 9 and 10. The group irritation index was 3.5 (max 40). The product was a mild irritant when administered under occlusive patches.[10]

Eight subjects participated in a 21-day cumulative irritation study with eight products and petrolatum. Five products were moisturizing bases containing 2.7% Cholesterol. Patches containing approximately 0.3 g of the test material were applied under occlusive patches daily Monday through Friday for 3 weeks for a total of 15 patches. The patches administered on Fridays remained in place over the weekend. Test sites were scored on a 0 (negative) to 4 (erythema, induration, and bullae) scale each day before application of a fresh patch. All individual positive scores were 1 (erythema) at any one reading. The five Cholesterol-containing products were minimal to mild cumulative irritants, with the number of positive responders (from an eight-member panel) and group cumulative irritation indexes (max 60) of: 8, 10.1; 8, 10.0; 2, 0.8; 7, 3.4; and 8, 9.5 [22]

Cumulative irritation studies as described above[22] were performed on six cosmetic formulations containing 1.4 or 2.7% Cholesterol. The products were minimal to moderate skin irritants with the following test results. A moisturizing base containing 2.7% Cholesterol was a moderate skin irritant using an eight-member panel. All eight panelists had irritant reactions to several patches: 4/8 panelists had erythema and induration (score of 2) and 2/8 panelists had erythema, induration, and vesiculation (score of 3) to one or more patches. The group cumulative irritation index (CII) was 13.5 (max 60).[23] Eight panelists received repeated patches of a moisturizing base containing 1.4% Cholesterol. The product was a minimal skin irritant with a CII of 1.4.[24] A moisturizing base containing 1.4% Cholesterol had a CII of 1.1 using an eight-member panel. The product was a minimal irritant.[25] A moisturizing base containing 1.4% Cholesterol was a minimal irritant using an eight-member panel. The group CII was 1.0, with mild erythema (score of 1) as the strongest reaction.[26] The CII of a moisturizing base containing 1.4% Cholesterol was 2.1 (max 56). Fourteen instead of the routine 15 patches were applied in this study, which accounts for a max CII of 56 rather than 60. The product was a mild irritant with this eight-member panel.[30] A moisturizing base containing 1.4% Cholesterol was a mild cumulative irritant in 25 panelists. The group CII was 9.68.[36]

Sensitization

Repeated Insult Tests

A repeated insult patch test (RIPT) was conducted on a moisturizing cream containing 1.7% Cholesterol using 87 subjects. Twenty-four-hour occlusive patches (~0.1 ml undiluted product) were applied to the same site on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. Test sites were scored (0 to 4 scale) for reactions immediately before application of the next patch. Challenge patches were administered in Week 7 of the study. Challenge occlusive patches were applied to previously unpatched sites for 24 h and scored for reactions 24 and 48 h after patch removal. Three panelists had barely perceptible to mild erythema to one of the nine induction patches. One panelist reacted with barely perceptible to mild erythema to three of the induction patches. There were no reactions to the challenge patch. The moisturizer was a minimal irritant and not a sensitizer under these test conditions.[12]

Fifteen subjects participated in a cumulative irritation test of a moisturizing base containing 1.4% Cholesterol. Occlusive patches containing 0.3 ml undiluted product were applied daily to the same site on the back Monday through Friday for a total of 14 applications. All patches remained in place for 24 h except those applied on Fridays, which remained in place over the weekend. Test sites were scored for irritation immediately upon removal of each patch. The 14-day total score for all 15 subjects was 23, and the group cumulative irritancy index was 1.5 (max 40). The reactions to a challenge patch were negative at the 48-h and 72-h evaluations. The moisturizing base was a minimal cumulative irritant and not a sensitizer.[34]

Seven Cholesterol-containing moisturizing bases were evaluated for cumulative irritation and sensitization using a panel of 10 subjects. All seven products contained 1.366% Cholesterol, and a concurrent product control was included in the test protocol. Occlusive patches containing 0.3 ml or 0.3 g of the test material were applied daily to the same site Monday through Friday for a total of 21 patches. The patches applied on Fridays remained in place over the weekend, and test sites were scored (0–4 scale) daily through the week and on Mondays for the patch applied on Friday. Challenge patches were applied 14 days after removal of the 21st irritancy patch. The challenge patches were applied occlusively to fresh sites, then scored for irritation 1, 24, and 48 h after removal of the 24-h patch. One subject was not available for the challenge patch. All seven products were minimal skin irritants, with group average irritation scores of 4.70, 5.60, 6.00, 3.65, 3.30, 4.30, and 4.70 (max. 60). The group irritation score for the control product was 3.65. There were no reactions to the challenge patches, indicating that none of the products were sensitizers.[11]

Seven formulations containing 5% Cholesterol were minimal skin irritants and nonsensitizers in a 21-day cumulative irritation plus challenge assay. The products were tested as a 5% w/w solution in olive oil (0.25% Cholesterol) according to the above procedure.[11] Eight subjects participated in the study. There were no reactions at challenge, and the group average irritation scores were 0.25, 0.44, 0.31, 0.06, 0.13, 0.56, and 1.31 (max 84). The irritancy score of the olive oil control was 0.13.[9]

Maximization Tests

A moisturizing base containing 1.4% Cholesterol was evaluated for sensitization in a maximization test.[118] Twenty-five panelists were pretreated with 24-h occlusive patches containing 1% sodium lauryl sulfate (SLS), since it had been previously determined that the test lotion was nonirritating. Upon removal of the irritant SLS patch, the first of five induction patches containing 0.3 g product was applied. The induction occlusive patches remained in place for 48 h, with 24 h between each induction patch. Since the lotion was nonirritating, 1% SLS patches were applied to the induction sites during the 24-h interim between induction patches to maintain minimal brisk dermatitis. Ten days after removal of the fifth induction patch, an occlusive patch was applied for 48 h to a fresh site and scored for reaction at patch removal and 24 h later. The challenge site was pretreated with 10% SLS for 1 h. There were no reactions to any induction or challenge patch. The product was not a sensitizer.[27]

Three moisturizing bases, each containing 1.4% Cholesterol, were evaluated for sensitization using 25-member panels in maximization tests as described above[27] except for the concentration of SLS used for the 1-h pretreatment of the challenge site. One study used 2.5%, the second study used 5%, and the third study used 10% SLS to irritate the challenge site. There were no reactions to induction or challenge patches in the three studies.[13,28,37]

Photosensitization

Phototoxicity

The phototoxicity of a moisturizing base containing 1.4% Cholesterol was evaluated in 10 subjects. Five microliters per square centimeter of the test agent were applied to the lower back, allowed to dry, and placed under occlusion for 6 h. Test sites were irradiated at patch removal with a 150 W xenon solar simulator with a Schott WG345 filter (UVA and visible light; total flux 98.2 mW/cm^2; UVA radiance about 25 mW/cm^2), and test sites were scored for phototoxic reactions immediately after irradiation, and 24 and 48 h later. A petrolatum control was run concurrently with the test material. There were no reactions at any test or control site. The moisturizing base had no detectable phototoxicity under these test conditions.[14]

Two moisturizing bases containing 1.4% Cholesterol were assayed for phototoxicity as described above.[14] Each product was tested using a 10-member panel. There were no reactions immediately, 24 h, and 48 h after irradiation. Neither product was phototoxic under the test conditions.[15,38]

Photosensitization

A face cream containing 6% Cholesterol was evaluated for irritation, sensitization, and photosensitization in a Schwartz-Peck prophetic patch procedure and a repeat insult patch test (RIPT). One-hundred ten subjects participated in the prophetic patch test, which involved open and closed patches. A 48-h closed patch was applied to each subject's back along with a simultaneous open patch application behind the right ear. Forty-eight hours later, the test sites were scored for irritation. After a 14-day nontreatment period, second open and

closed patches were applied and scored 48 h later. After the scoring of the second closed application, this test site was irradiated with a Hanovia Tanette Mark I Lamp (300–370 nm continuous emission spectrum) and scored for photosensitization 48 h later. No reactions were observed during any phase of the prophetic patch procedure. Forty-five subjects were used in the RIPT. Ten open and closed patches were applied to each subject as described for the prophetic patch procedure. The patches were applied on Mondays, Wednesdays, and Fridays and scored for irritation 48 h after application. After a 14-day nontreatment period, open and closed challenge patches were administered as in the prophetic patch test after the 1st, 4th, 7th, 10th, and 11th (challenge) insults had been scored. Irradiated sites were scored for UV-induced reactions 48 h after irradiation. There were no reactions at any open, closed, or irradiated test site. The Cholesterol-containing face cream was not an irritant, sensitizer or photosensitizer in these two assays.[42]

Two moisturizing bases containing 1.4% Cholesterol were evaluated for photosensitivity following the same procedure. Ten microliters of the test material were applied under occlusive patches to test sites on the midback of each subject for 24 h. Test sites were irradiated upon patch removal with the equivalent of three MEDs from a xenon solar simulator (UVA radiation). The sequence was repeated at 48-h intervals to the same site for a total of six exposures over 3 weeks. Ten days after the last induction exposure, subjects were challenged with a 24-h occlusive patch containing 5 μl test material followed by exposure to 4 J/cm^2 of UVA radiation from the solar simulator through a Schott WG345 filter. Challenge sites were scored for reactions 48 and 72 h after irradiation. Both products were tested using 25-member panels, and there were no reactions in either study. The two moisturizing bases were not photosensitizers.[16,39]

Product Usage Studies

A moisturizing lotion containing 1.7% Cholesterol was evaluated for efficacy and irritation in an in-use study comparing it to a similar product. Sixty subjects were divided into two groups and instructed in the proper use of the products. One group of 30 subjects used the test product for 3 weeks, then changed to the control product for 3 weeks. The other group used the products in the reverse order for the two 3-week periods. The subjects were clinically evaluated for improvement or worsening of facial condition (mainly dryness), and the panelists subjectively rated the two products. Clinically, two subjects improved and four worsened after using the test material, and six subjects improved and four worsened from the control material. The test lotion was considered comparable to the control product in efficacy and irritancy.[17]

The irritancy and sun sensitivity of a moisturizer containing 1.4% Cholesterol were evaluated in 102 subjects. The test material was used by the subjects for a 4-week period. The subjects were instructed to apply the product to their face and an area of the body not exposed to sunlight twice daily on Days 1–7 and thrice daily through Day 28. One subject quit the study on Day 4 due to test product-related facial burning and erythema. A second subject discontinued use of the test material on Day 12 due to periorbital erythema. The product was a mild skin irritant to approximately 15% of the panelists at some time during the

study but was no more irritating than similar products tested in the same manner. The product was recommended as safe for unsupervised use.[18]

Three moisturizers containing 1.4% Cholesterol were evaluated in 4-week in-use tests. The first product was tested using 50 subjects. Prepatch testing was negative in all subjects, and three subjects had moderate erythema 24 h following postpatch test. Facial erythema was observed in three subjects, and periorbital erythema was observed in one subject on Day 28 of the in-use portion of the study. The product was considered a mild irritant comparable to similar products.[29] The second product was evaluated in approximately 50 subjects, and facial inflammation was observed in 1 subject and conjunctival irritation was observed in 5 subjects. The product had a low potential for causing facial irritation that was comparable to similar products and was considered safe for unsupervised use.[19] Fifty-four subjects were used to evaluate the third product. The product was nonirritating.[40]

One case history of sensitivity to Cholesterol esters has been reported.[119]

See Table 4 for a summary of the clinical studies.

SUMMARY

Cholesterol is a neutral, unsaponifiable, crystalline steroid with a high melting point. It is practically insoluble in water, slightly soluble in alcohol, and readily soluble in organic solvents and aqueous solutions of bile salts. Cholesterol is found in all animals, being a membrane component and an important metabolic precursor for certain hormones, vitamins, and other steroidal compounds.

Cholesterol is used as an emulsifier in cosmetic skin and hair care products and eye and face makeup formulations. It is used in concentrations up to 5%, with the majority of formulations containing 0.1–1% Cholesterol.

The normal metabolism and excretion of Cholesterol is well understood in man and experimental animals. The "average" North American male consumes 0.5 g of Cholesterol daily and synthesizes another 1–1.5 g per day. This Cholesterol is then incorporated into cell membranes, further metabolized into plasma lipoproteins, bile salts, and steroid hormones, metabolized by gut bacteria, or excreted via the skin, urine, and as neutral fecal steroids.

Cholesterol is not a significant dermal (100%) or ocular (5% in corn oil) irritant in rabbits when tested alone or in corn oil. Moisturizing products containing up to 6% Cholesterol were slight dermal and ocular irritants in rabbits. In a subchronic oral study, excess dietary (4–24 weeks) Cholesterol produced reversible fatty changes in the murine liver along with higher concentrations of hepatic hydroxyproline and glycosaminoglycans.

Cholesterol does not appear to have any genotoxic activity in bacterial or mammalian cell in vitro mutagenic and transformation assays. However, Cholesterol plus polysorbate 60 does decrease mitotic activity when painted onto the skin of mice.

High doses of Cholesterol were teratogenic in rats. Subcutaneous administration of Cholesterol to pregnant dams will consistently result in palate anomalies in the pups, and it has been used as an experimental model to study cleft

TABLE 4. Clinical Assessment of Safety

Test type[a]	*No. of subjects*	*Concentration of Cholesterol/Product type*	*Results*	*Reference*
Primary irritation, single occlusive patch	23	1.4% in moisturizing base	Group mean irritation scores were 0.31 (max 4) and 0.22 at 0 and 24 h following patch removal; a mild skin irritant	31
Primary irritation, single occlusive patch	25	1.4% in moisturizing base	Group mean irritation scores were 0.24 (max 4) and 0.28 at 0 and 24 h following patch removal; a mild skin irritant	32
Primary irritation, single occlusive patch	26	1.4% in moisturizing base	Group mean irritation scores were 0.17 (max 4) and 0.23 at 0 and 24 h following patch removal; a mild skin irritant	33
Primary irritation, single occlusive patch	25	1.4% in moisturizing base	Group mean irritation scores were 0.42 (max 4) and 0.48 at 0 and 24 h following patch removal; a mild skin irritant	35
Cumulative irritation, 10 consecutive 24-h patches	10	1.4% in formulation	Group irritation index was 3.5 (max 40); a mild skin irritant	10
Cumulative irritation, 15 24-h patches over 3 weeks	8	2.7% in 5 moisturizing bases tested simultaneously	Cumulative irritation indexes (CII) for the 5 products ranged from 0.8 to 10.1 (max 60); the 5 products were mild cumulative skin irritants	22
Cumulative irritation, 15 24-h patches over 3 weeks	8	2.7% in moisturizing base	CII = 13.5 (max 60); moderate skin irritant	23
Cumulative irritation, 15 24-h patches over 3 weeks	8	1.4% in moisturizing base	CII = 1.4 (max 60); minimal skin irritant	24
Cumulative irritation, 15 24-h patches over 3 weeks	8	1.4% in moisturizing base	CII = 1.1 (max 60); minimal skin irritant	25
Cumulative irritation, 15 24-h patches over 3 weeks	8	1.4% in moisturizing base	CII = 1.0 (max 60); minimal skin irritant	26
Cumulative irritation, 14 24-h patches over 3 weeks	8	1.4% in moisturizing base	CII = 2.1 (max 56); mild skin irritant	30
Cumulative irritation, 15 24-h patches over 3 weeks	25	1.4% in moisturizing base	CII = 9.68 (max 60); mild skin irritant	36
RIPT	87	1.7% in moisturizing cream	3 panelists had barely perceptible erythema at induction; No reactions at challenge; minimal irritation, not a sensitizer	12

TABLE 4. (Continued)

Test type[a]	No. of subjects	Concentration of Cholesterol/Product type	Results	Reference
Cumulative irritation plus challenge	15	1.4% in moisturizing base	CII = 1.5 (max 40); no reactions at challenge; minimal irritant, not a sensitizer	34
Cumulative irritation plus challenge	10	1.366% in 7 products tested simultaneously	CII ranged from 3.30 to 6.00 (max 60); no reactions at challenge; the 7 products were minimal irritants and not sensitizers	11
Cumulative irritation plus challenge	8	5% in 7 products tested simultaneously; products were tested at 5% in olive oil (0.25% Cholesterol)	CII ranged from 0.06 to 1.31 (max 84); no reactions at challenge; the products were minimal irritants and not sensitizers	9
Maximization (with SLS)	25	1.4% in moisturizing base	No reactions to any induction or challenge patch; not a sensitizer	27
Maximization (with SLS)	25	1.4% in moisturizing base	No reactions to any induction or challenge patch; not a sensitizer	37
Maximization (with SLS)	25	1.4% in moisturizing base	No reactions to any induction or challenge patch; not a sensitizer	28
Maximization (with SLS)	25	1.4% in moisturizing base	No reactions to any induction or challenge patch; not a sensitizer	13
Phototoxicity (xenon solar simulator–UVA and visible)	10	1.4% in moisturizing base	No reactions to 6-h occlusive patch followed by irradiation; not a phototoxin	14
Phototoxicity (xenon solar simulator–UVA and visible)	10	1.4% in moisturizing base	No reactions to 6-h occlusive patch followed by irradiation; not a phototoxin	15
Phototoxicity (xenon solar simulator–UVA and visible)	10	1.4% in moisturizing base	No reactions to 6-h occlusive patch followed by irradiation; not a phototoxin	38
Photosensitization, Schwartz-Peck prophetic patch (300–370 nm)	110	6% in face cream	Occlusive challenge patch site, only, was irradiated; not a sensitizer or photosensitizer	42
Photosensitization, RIPT (300–370 nm)	45	6% in face cream	Occlusive patch sites were irradiated after the 1st, 4th, 7th, 10th, and challenge patches; no reactions to any open, closed, or irradiated test site; not a photosensitizer	42

Photosensitization, RIPT (xenon solar simulator–UVA)	25	1.4% in moisturizing base	6 induction patches and 1 challenge patch; all insults followed by irradiation; no reactions; not a photosensitizer	16
Photosensitization, RIPT (xenon solar simulator–UVA)	25	1.4% in moisturizing base	6 induction patches and 1 challenge patch; all insults followed by irradiation, no reactions; not a photosensitizer	39
Product usage test, comparison to competitive product	60	1.4% in moisturizing lotion	Test material comparable to competitive product in efficacy and irritancy; test material was a mild irritant	17
Product usage test, comparison to competitive product	102	1.4% in a moisturizer	2 subjects discontinued use due to facial burning and erythema; test material was a mild skin irritant to ~15% of the panelists at some time during the 4-week study, but comparable to competitive products in irritancy; a mild irritant	18
Product usage test, comparison to competitive product	50	1.4% in a moisturizer	Facial erythema (3 subjects) and periorbital erythema (1 subject) were observed during the 4-week study; test material was comparable to competitive product in irritancy; a mild irritant	29
Product usage test, comparison to competitive product	~50	1.4% in a moisturizer	Facial inflammation (1 subject) and conjunctival irritation (5 subjects) were observed during the 4-week study; test material was comparable to competitive product in irritancy; a mild irritant	19
Product usage test, comparison to competitive product	54	1.4% in a moisturizer	Test material was nonirritating in the 4-week study	40
Sensitivity, case history	1	"Cholesterol Esters"	Case history of sensitization to a cosmetic traced to "Cholesterol Esters"	119

[a]See text for more details on testing procedures and results.

malformations. The holoprosencephalic teratogenic effects of inhibition of Cholesterol synthesis can be prevented by a concurrent maternal hypercholesterolemia-provoking diet.

Cholesterol has not been established as a promoter, cocarcinogen, or total carcinogen. In several rat studies, the results have varied: it was not a colon cancer promoter in one study when administered ir after initiation with MNNG, it was a dietary cocarcinogen with DMH, and dietary Cholesterol had a protective effect in MNU-induced colon cancer. Increased dietary Cholesterol increased tumor incidences over that found in control mice. Subcutaneous injections of a supersaturated solution of Cholesterol into mice produced sarcomas at injection sites. Undersaturated solutions of Cholesterol in an oily vehicle or as an aqueous suspension were not carcinogenic in mice or rats.

Clinical studies to evaluate the safety of topically applied Cholesterol were restricted to products formulated with the ingredient. Most products were moisturizers containing 1.4% Cholesterol. The highest concentration of Cholesterol tested (6%) was evaluated in a modified prophetic patch test (110 subjects) and an RIPT (45 subjects); both assays had UVA and UVB exposure incorporated into the protocols. The Cholesterol-containing products were minimal to mild primary and cumulative skin irritants but not sensitizers or photosensitizers.

CONCLUSION

On the basis of the available information, the CIR Expert Panel concludes that Cholesterol is safe as presently used in cosmetic products.

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