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Final Report on the Safety Assessment of Choleth-24

Choleth-24 is the polyoxyethylene ether of the cholesterol fraction of lanolins, and is used in cosmetics as a surfactant, dispersant, stabilizer, and emulsifier at concentrations up to 5%. Acute animal studies have shown Choleth-24 to be slightly toxic when ingested, nonirritating to mildly irritating when applied by the Draize method to skin at concentrations of 0.5–100%, and practically nonirritating when instilled in the eyes of rabbits at concentrations up to 100%. Clinical studies have determined Choleth-24 to be only slightly irritating and nonsensitizing when applied to human skin in concentrations up to 50%. Choleth-24 at 0.5% concentration was neither phototoxic nor photoallergenic when tested in 201 subjects. It is concluded that Choleth-24 is safe for topical application to humans in the present practices of use and concentration.

INTRODUCTION

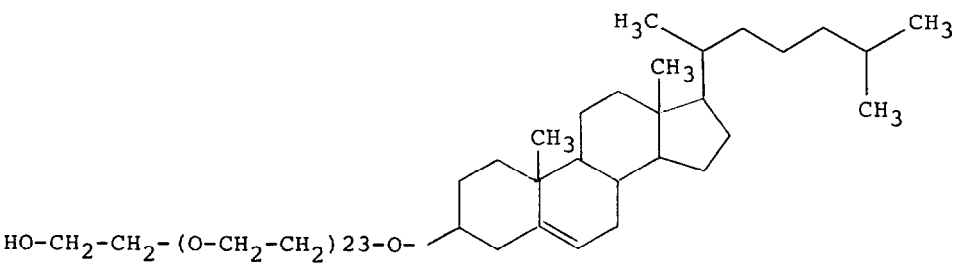
CHOLETH is the ethoxylated cholesterol fraction of lanolin alcohol. Since lanolin alcohol may contain as much as 38% cholesterol, ethoxylating these alcohols to produce Laneths also produces Choleths.[1] For completeness, the safety data on Laneths-5, -16, and -25 have been included in this report.[2]

CHEMISTRY

Structure

Choleth-24 is the polyoxyethylene ether of cholesterol with an average ethoxy chain length of 24. Other names for this ingredient include: PEG-24 Cholesteryl Ether, Polyethylene Glycol (24) Cholesteryl Ether, and Polyoxyethylene (24) Cholesteryl Ether.

Choleth-24 conforms to the following structure:[3,4]



Choleth-24 is prepared by reacting the cholesterol fraction of lanolin alcohol with ethylene oxide in the presence of an alkaline catalyst, until an average of 24 moles of ethylene oxide has been added to each mole of cholesterol. The catalyst is then acid-neutralized and the product vacuum stripped to remove any unreacted ethylene oxide. Choleth-24 is then filtered to remove the insoluble salts that form as a result of catalyst neutralization.[4]

Properties

Choleth-24 is an off-white or pale yellow waxy solid with a faint odor. It is soluble in both water and alcohol and has the properties of a nonionic surfactant (Table 1).[4-7]

Analytical Methods

Spilker and Richey[8] have described a number of analytic techniques useful for the determination of lanolin and lanolin derivatives. These generally involve hydrolysis, fractionation, separation by chromatography, and identification. Infrared spectroscopy (IR) is also used to identify Choleth-24.[9] Scotney and Truter[10] used paper chromatography to determine the components of Choleth-24.

Impurities

Choleth-24 may contain related lanolin alcohols, inorganic salts, and related lanolin sterols such as dihydrocholesterol, 7-keto cholesterol, lanosterol, dihydrolanosterol, and 7-keto lanosterol and possibly ethoxylated forms of these minor lanolin sterols.[1,4] Trace amounts of 1,4-dioxane, a reaction product of the ethoxylation process, may be present, as well as pesticides and trace metals found in crude lanolin.[2]

Additives

The antioxidant/preservative butylated hydroxytoluene (BHT) may be added by the manufacturer to Choleth-24 in concentrations of 0.05%.[4] BHT is a Generally Recognized as Safe (GRAS) ingredient for which regulations have been issued under the Food, Drug and Cosmetic Act (21 CFR 121.101). Its use in food products is limited to 0.02%.

USE IN COSMETICS

Choleth-24 is used in cosmetic products as an emulsifier, stabilizer, dispersant, and nonionic surfactant. The solubility characteristics of cholesterol are changed by ethoxylation. Choleth-24 is used in some oil-in-water lotions to retard the viscosity build-up commonly associated with cholesterol-containing products. The viscosity of anionic lotions can be reduced effectively by the addition of less than 1% Choleth-24.[6,11,12]

TABLE 1. CHEMICAL AND PHYSICAL PROPERTIES OF CHOLETH-24.[a]

Property	*Value*
Hydroxyl value	35–45
Acid value	1.5 (maximum)
Saponification value	3.0 (maximum)
Iodine value	12–19
Moisture	0.5% (maximum)
pH (10% aqueous)	4.5–7.5
Cloud point	88°–95°C
Heavy metals	20 ppm (maximum)
Arsenic	2 ppm (maximum)
Ash	0.25% (maximum)
Hydrophile/Lipophile balance	14

[a]From Refs. 4–7.

Choleth-24 is used in over 130 cosmetic formulations in concentrations up to 5% (Table 2). Products containing Choleth-24 may come in contact with the hair and skin, particularly of the face. These products may be used daily or occasionally and their use may extend over a period of years. Daily contact with Choleth-containing products may last from seconds to all day.[13]

Laneths-5, -16, and -25, which contain Choleths, are used in cosmetics at maximum concentrations of 10%.[2]

BIOLOGICAL PROPERTIES

Animal Toxicology

Acute Oral Toxicity

Five groups of one male and one female albino rat each were administered by gavage a 50% Choleth-24 solution in vegetable oil, in doses of 0.625–10.00 g/kg and observed daily for two weeks. Rats receiving 1.25–10.00 g/kg died within 24 h, whereas all those receiving 0.625 g/kg survived. The approximate acute oral LD50 was determined to be 0.9 g/kg, which corresponds to that of materials classified as slightly toxic.[14,15]

Laneths-5, -16, and -25, which contain Choleths, were administered by gavage to groups of five rats each. Acute oral LD50s for these three ingredients were 25.9–45.0 ml/kg, 9.33–12.2 ml/kg, and >3.0 g/kg, respectively. These resulting LD50s correspond to those of materials classified as practically nontoxic.[2,15]

TABLE 2. FDA PRODUCT FORMULATION DATA FOR CHOLETH-24.[a]

Product type	*Conc. Range (Percent)*	*Number of formulations*
Bubble baths	>1–5	1
Eyeliners	>1–5	14
Eye shadows	>0.1–1	2
Mascaras	>1–5	4
	>0.1–1	2
Other eye makeup preparations	>0.1–1	1
Hair conditioners	>0.1–5	3
	>1–5	3
	>0.1–1	4
Shampoos	>1–5	1
	>0.1–1	2
Tonics and Dressings	>1–5	1
Moisturizers	>0.1–1	2
Night skin preparations	>0.1–1	2
Hair dyes	>0.1–1	71
Blushers	>0.1–1	1
Leg and body paints	>1–5	1
Makeup bases	>0.1–1	1
Other makeup preparations	>1–5	1
Aftershaves	>1–5	1
Skin cleansing preparations	>0.1–1	4
Face, body, and hand preparations	>0.1–1	4
	≤0.1	1
Skin fresheners	>1–5	1
	>0.1–1	1
Other skin care preparations	>0.1–1	8

[a]From Ref. 13.

Primary Skin Irritation

The Draize method was used to test the skin irritancy of undiluted Choleth-24 when applied to the clipped intact and abraded skin of six albino rabbits; irritation was scored at 24 and 72 h. Choleth-24 produced very slight to well-defined erythema in all intact and abraded sites; irritation persisted throughout the 72-hour test period. There was very slight to well-defined edema on all six abraded sites and on four of six intact sites; edema also persisted throughout the test period. The primary irritation index (PII) was 1.29 (maximum score = 8) which indicated mild skin irritation.[16,17]

Motoyoshi et al.[18] compared the skin irritancy of undiluted Choleth-24 in rabbits, guinea pigs, rats, and miniature swine. Choleth-24 was applied to the clipped intact skin of each of six rabbits, rats, and guinea pigs. The test sites were rinsed after 24 h, scored, and the compound reapplied. This procedure was repeated daily for three days. After the final reading, each animal was injected intravenously with 40 mg/kg Evans Blue dye. The animals were sacrificed one hour later and the skin sites excised and scored for erythema, edema, and capillary permeability. In miniature swine, 0.05 g Choleth-24 was applied under an occlusive patch to the shaved intact skin of each of six animals. Patches were removed at 48 h and the skin was evaluated as above. The sum of the averages of the erythema, edema, and capillary permeability scores was referred to as the PII. Choleth-24 was reported to be severely irritating to rabbit skin, moderately irritating to guinea pig and rat skins, and nonirritating to the skin of miniature swine. When these results and those of 19 other cosmetic ingredients were compared to results of human skin patch tests, the authors concluded that in this assay the skin of miniature swine was more reliable than rabbit, rat, or guinea pig skin in screening human skin irritants under their test procedures. The skin irritation test reported by Motoyoshi et al.[18] is not comparable to the Draize test. The former involves a three-day application of the test substance and a scoring system which includes capillary permeability grading; the latter assay contains neither of these procedures. Irritation scores on compounds other than Choleth-24 indicate that values obtained by the methods of Motoyoshi et al. are greater than those obtained under the Draize test.

An eyeliner containing 5% Choleth-24 was tested for skin irritation in three rabbits. Test material was applied daily for four days to the clipped intact and abraded skin of animals. Sites were scored daily for seven days. No skin irritation was observed.[19]

A modified Draize method was used to study the skin irritancy of a nail conditioner containing 0.50% Choleth-24. The product was applied to the clipped intact and abraded skin of six albino rabbits. Skin sites were examined and scored after 24 h and the solution reapplied. This procedure was repeated for three days. There was no reaction in any of the animals.[20]

Undiluted and ten percent Laneths-5, -16, and -25, were tested for primary skin irritation in rabbits. PIIs resulting from application of undiluted materials were: Laneth-5, 0.8 to 1.3; Laneth-16, 1.0 to 2.43; and Laneth-25, 3.83. These values correspond to those of materials classified as slightly to mildly irritating, mildly to moderately irritating, and severely irritating, respectively. The PIIs of 10 percent solutions of these Laneths were 0.04–1.0, or practically nonirritating to mildly irritating to rabbits' skin. Undiluted Laneths-5 and -16 were reported to be nonirritating to rabbits' skin in Department of Transportation tests.[2]

Primary Eye Irritation

The Draize method was used to evaluate the eye irritation of undiluted Choleth-24 on albino rabbits. The test material (0.1 ml) was instilled into the right eye of each of nine animals; the left eye remained untreated. The eyes of three animals were washed with water four seconds after instillation. In the unwashed group, one of six rabbits developed slight conjunctivitis which disappeared by the second day. None of the animals in the washed group developed eye irritation. The average ocular irritation indices (AOIIs) for the washed and unwashed groups were 0.7 and 0, respectively (maximum score = 110).[21]

An eyeliner containing 5 percent Choleth-24 was tested for eye irritation in six rabbits. The test material (0.1 ml) was instilled into each animal's left eye; the right eye served as an untreated control. Eyes were observed daily for one week. Slight conjunctivitis after one hour was observed in some animals but disappeared within 24 to 72 h.[19]

The Federal Hazardous Substance Act (FHSA) method was used to test the eye irritancy of a nail conditioner containing 0.50% Choleth-24. The test material (0.1 ml) was placed in one eye of each of six rabbits; the other eye served as an untreated control. Eyes were examined after 24, 48, and 72 hours; there was no irritation (AOII = 0) and the product was determined to be nonirritating.[20]

Two undiluted and one 10 percent Laneth-5 solutions were tested for primary eye irritation on rabbits. One undiluted and the 10% solutions were nonirritating; in another test, however, undiluted Laneth-5 was minimally irritating.[2]

Undiluted Laneth-16 was instilled into rabbits eyes; some eyes were subsequently washed after instillation. In the washed group, Laneth-16 was nonirritating to minimally irritating. In the unwashed group, Laneth-16 caused minimal irritation; in one study, irritation persisted throughout the seven-day observation period.[2]

Undiluted Laneth-25, instilled into six rabbits' eyes, caused minimal irritation which persisted throughout the seven-day observation period.[2]

Clinical Assessment of Safety

Irritation and Sensitization

A repeated insult patch test was used to study the skin irritation and sensitization of Choleth-24 in 51 human subjects. The subjects were divided into five groups; each group was initially patch-tested with 10%, 20%, 30%, 40%, or 50% Choleth-24 in water. After the fourth application, the concentration was raised to 50% for all subjects. The test material was applied under occlusion for 24 h; the patch was then removed, the site scored, and a fresh patch reapplied. This procedure was repeated four days per week for three weeks for a total of 12 patches. After a rest of 15 days, a 24-hour challenge patch was applied to an untreated site. Sites were scored at 24, 48, and 72 h. There were five reactants (1+ to 2+) to the first four induction patches (10–50% Choleth-24), three of ten subjects at 10%, and two of eleven subjects at 50% Choleth-24. During the next two weeks of induction (all at 50% Choleth-24), eight subjects reacted (1+ to 2+) to one or more patches. No reactions were elicited by challenge patches. It was concluded that 10–50% Choleth-24 is nonirritating to minimally irritating but 50% is capable of being a "fatiguing agent" (a material which elicits a skin response resulting from its cumulative effect on the skin). A concentration of 50% Choleth-24 was nonsensitizing.[22]

Motoyoshi et al.[18] did a comparative study on the irritancy of cosmetic ingredients to the skin of various animals and man. In the human single insult patch tests, undiluted Choleth-24 was applied under occlusion to 50 men for 48 h. Sites were scored 24, 48, and 72 h later. Under an unusual classification scheme, undiluted Choleth-24 was determined to be mildly irritating since it elicited 5 to 20 reactions (including "questionable" responses). The authors concluded that, of the species tested, the irritancy and penetrability of human skin most closely resembles that of miniature swine skin.

A modified Draize-Shelanski-Jordan patch test was used to determine the irritancy and sensitizing potential of a nail conditioner containing 0.50 percent Choleth-24. The test material was applied under occlusion to the upper back of each of 201 subjects. Sites were scored at 48 h and a fresh patch reapplied. This procedure was repeated three days per week for three weeks. Two weeks after the removal of the last induction patch, two consecutive 48-hour challenge patches were applied to a single untested site. Sites were scored at 48 and 96 h. None of the patches, including challenge patches, elicited a reaction. It was concluded that the product was nonirritating and nonsensitizing.[20]

A repeated insult patch test was used to study the irritancy and sensitizing potential of 50 percent aqueous Laneth-5, -16, and -25 solutions. In each study, 50 subjects were divided into five groups of 10 to 12 each. In preliminary studies, each ingredient was tested in concentrations of 10%, 20%, 30%, 40%, and 50%. The test sample was applied four times and the results scored. From these initial studies, one concentration (50%) was selected for the actual test. Test sites were exposed to

sample for four consecutive days, read on the fifth day, and rested the next two days. This protocol was repeated three times and followed by a 15-day rest period. At this time, 24-hour occluded challenge exposures were made at new sites and evaluated for hypersensitivity reactions. Twenty-four, 48, and 72 h later evaluations for delayed hypersensitivity reactions were made at the new sites. It was concluded that 50% Laneth-5, Laneth-16, and Laneth-25 were nonirritating and nonsensitizing, but that Laneth-5 and Laneth-16 were mild "fatiguing" agents.[2]

Photosensitization

A nail conditioner containing 0.5% Choleth-24 was tested for photosensitization in 27 men and women. The test material was applied under an occlusive patch for 24 h; patches were then removed and sites were scored. Skin sites were then irradiated with three minimal erythemal doses of ultraviolet light (200–400 nm) with a xenon arc solar simulator (150 W). Sites were scored 48 h later. The entire procedure was repeated twice weekly for three weeks. Following a 10-day rest period, a challenge patch was applied to a previously untreated site. Sites were scored and irradiated at 24 hours and scored again 15 min and 24, 48, and 72 h post-irradiation. No reactions to any of the patches were observed either before or after irradiation. The investigators concluded that the product containing 0.50% Choleth-24 is nonphototoxic and nonphotoallergenic.[20]

SUMMARY

Choleth-24 is the polyoxyethylene ether of the cholesterol fraction of lanolins with an average of 24 moles ethylene oxide added to each mole of cholesterol. Another related group of cosmetic ingredients, the Laneths, may contain as much as 38 percent Choleth. Choleth-24 is an off-white waxy solid which is soluble in water and alcohol, and acts as nonionic surfactant. Impurities which may be found in Choleth-24 include related lanolin alcohols and sterols, inorganic salts, and 1,4-dioxane. BHT, a GRAS ingredient, may be added by the manufacturer to Choleth-24 at a concentration of 0.05%.

Choleth-24 is used in cosmetics as a surfactant, dispersant, stabilizer, and emulsifier. It is used in concentrations up to 5%. Choleth-containing products may come into contact with most external surfaces of the body and may be applied for a period of years.

Acute animal studies have shown Choleth-24 to be slightly toxic when ingested, nonirritating to mildly irritating when applied by the Draize method to skin at concentrations of 0.5–100%, and practically nonirritating when instilled in the eyes of rabbits at concentrations up to 100%.

Clinical studies have determined Choleth-24 to be nonirritating to mildly irritating and nonsensitizing when applied to human skin in concentrations up to 50%. Choleth-24 (as 0.5% in a nail conditioner) was neither phototoxic nor photoallergenic when tested in 201 subjects.

DISCUSSION

Lanolin alcohol, a saponification product of whole lanolin may contain as much as 38% cholesterol.[1] This cholesterol fraction is ethoxylated to Choleth. When ethoxylated alone, lanolin alcohol produces Laneth, a substance which can contain up to 38% Choleth. This means that data applicable to the safety assessment of the Laneths are also applicable to that of the Choleths. The Expert Panel has already determined the Laneths to be safe at concentrations presently used in cosmetic formulations.[2]

Traces of 1,4-dioxane, a product of the ethoxylation process, may be present as an impurity in Choleth-24. Administration of 1% 1,4-dioxane in drinking water of rats for 13 months induced liver lesions and hepatomas.[23] The cosmetic industry is aware of the problem and is making an effort to lower or remove 1,4-dioxane in cosmetics.[24]

Laneths are used in a variety of cosmetic products at concentrations up to 10%. Thus, a product containing 10% Laneth may contain as much as 3.8% Choleth.[2] The maximum product use concentration of Choleth-24 is 5%.

Acute animal toxicology data available indicate that Laneths are practically nontoxic when in-

gested; undiluted Laneths, equivalent to 38% Choleths, are slightly to severely irritating to rabbits' skin, whereas 10% Laneth solutions, equivalent to 3.8% Choleths, are practically nonirritating to mildly irritating. Undiluted Laneths (38% Choleths) are nonirritating to minimally irritating when instilled into rabbits' eyes (irritancy is reduced if eyes are rinsed). The available animal toxicity data for Choleth correspond well with those for the Laneths. Undiluted Choleth-24 was determined to be slightly toxic when ingested, nonirritating to mildly irritating to rabbits' skin, and practically nonirritating to rabbits' eyes.

In clinical irritation and sensitization tests Laneths-5, -16, and -25 proved to be nonirritating and nonsensitizing at 50% (19% Choleths). Similarly, Choleth-24 (undiluted and in formulation) was found to be nonirritating to mildly irritating and nonsensitizing.

Available test data on phototoxicity and photoallergenicity of Choleth-24 are not in themselves adequate, but clinical experience with it and the Laneths reveals no evidence suggesting that this ingredient is phototoxic or photoallergenic.

CONCLUSION

On the basis of available data, the Panel concludes that Choleth-24 is safe for topical application to humans in the present practices of use and concentration.

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