Amended Final Report on the Safety Assessment of Hydroxystearic Acid[1]

Hydroxystearic Acid is a fatty acid used as a surfactant–cleansing agent in cosmetic products. Initial review of available safety test data resulted in a finding that there were insufficient data to support the safety of Hydroxystearic Acid for use in cosmetic products. Data needed included concentration of use, chemical characterization, dermal reproductive and developmental toxicity, genotoxicity (and carcinogenicity data if the genotoxicity data were positive), and shin irritation data. Subsequent to that conclusion, new data were received. Use concentrations were reported as high as 10%. Small amounts of other fatty acids are commonly found in preparations of Hydroxystearic Acid. Genotoxicity was not found in bacterial or mammalian systems and only subcutaneous sarcomas at the site of injection were found in carcinogenicity studies. Dermal reproductive and developmental toxicity studies were negative. Skin irritation was produced by antiperspirant prototype formulations containing Hydroxystearic Acid under occluded or semioccluded patch test conditions. It was considered that such formulations under those exaggerated conditions can be irritating, but are generally not irritating in actual use. Because Hydroxystearic Acid and Stearic Acid are structurally similar, data from a previous safety assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid were summarized. On the basis of the animal and clinical data, it was concluded that Hydroxystearic Acid is safe as a cosmetic ingredient in the present practices of use.

INTRODUCTION

Hydroxystearic Acid is a fatty acid used as a surfactant–cleansing agent in cosmetic products. The Cosmetic Ingredient Review (CIR) Expert Panel issued a Final Report on the safety of Hydroxystearic Acid on March 17, 1995, with the following conclusion: The CIR Expert Panel concludes that the available data are insufficient to support the safety of Hydroxystearic Acid for use in cosmetic products. It was determined that the following data were needed in order for the Panel to complete its safety assessment: (1) concentration of use; (2) chemical characterization; (3) dermal teratogenicity study; (4) one genotoxicity test using a mammalian system (if the results of the genotoxicity test are positive, a dermal carcinogenicity test by National Toxicology Program (NTP) standards will be requested); and (5) skin irritation data. Subsequent to the completion of the Final Report, new data inclusive of all of the above were received. According to Section 46 of the CIR Procedures, Amendment of a Final Report, the Expert Panel will reconsider a Final Report that has been issued when new data are available. In order to provide readers with a complete picture of the data available, the data included in the original report are presented in their entirety.

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CHEMISTRY

Chemical and Physical Properties

Hydroxystearic Acid (CAS No. 106-14-9) is the fatty acid that conforms to the formula (Wenninger and McEwen 1995a) shown in Figure 1. Other names for this chemical are as follows: 12-Hydroxyoctadecanoic Acid; 12-Hydroxystearic Acid; and Octadecanoic Acid, 12-Hydroxy- (Wenninger and McEwen 1995b). 12-Hydroxystearic Acid has a molecular weight of 300.48 and crystallizes from alcohol (crystalline form not reported). It also has a melting point of 82°C, and is soluble in ethyl alcohol, ether, and chloroform (Lide and Frederikse 1993). Other values for the melting point of 12-Hydroxystearic Acid, recrystallized from ethanol, that have been reported range from 75 to 77°C (Zevenhuizen 1974). The phase transition of 12-Hydroxystearic Acid (in CCl_4) from gel to sol occured at 36°C, and the transition from sol to gel occured at 18°C (Umezawa, Nobuharu, and Yamabe 1970).

The ultraviolet (UV) absorption maximum for 12-Hydroxystearic Acid in concentrated sulfuric acid occurs at 295 nm (Zevenhuizen 1974). UV irradiation of this compound has resulted in the formation of two main products, an aldehyde, $CH_3(CH_2)_5CHO$, and an acid, $CH_3(CH_2)_9COOH$:12-Hydroxystearic Acid was not oxidized. The UV lamp that was used in this experiment had maxima at 254 and 366 nm (Fedeli and Favini 1980).

Methods of Production

12-Hydroxystearic Acid may be synthesized as follows: 2-hexylcyclododecanone is oxidized with a mixture of peracetic and permaleic acids in methylene chloride to form the lactone of Hydroxystearic Acid. Alkaline hydrolysis of the lactone leads to the production of Hydroxystearic Acid. Acylcyclododecanones, such as 2-hexylcyclododecanone, can be obtained by the addition of cyclododecanone at the double bond of the respective 1-alkene. This reaction occurs through a radical-chain mechanism; organic peroxides are used as initiators (Tanchuk, Kotenko, and Rozhenko 1989).

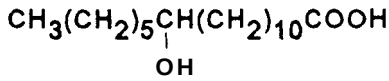

FIGURE 1
Chemical formula for Hydroxystearic Acid (Wenninger and McEwen 1995a).

The catalytic hydrogenation of castor oil to 12-Hydroxystearic Acid has also been reported (Mankovskaya et al. 1970; Maskaev, Simurova, and Mankovskaya 1973). This reaction also yielded small amounts of ricinoleic acid, stearic acid, oleic acid, palmitic acid, arachidic acid, and traces of linolenic acid and linoleic acid (Mankovskaya et al. 1970).

Analytical Methods

12-Hydroxystearic Acid has been identified by mass spectrometry (Murata et al. 1990), two-dimensional thin-layer chromatography (Takatori and Yamaoka 1976), and gas-liquid chromatography (Binder et al. 1970; Zevenhuizen 1974). The structure of 12-Hydroxystearic Acid has also been confirmed by data from infrared (IR) and ^{13}C nuclear magnetic resonance (NMR) spectrometry (Tanchuk, Kotenko, and Rozhenko 1989).

Impurities

Specific information on impurities in 12-Hydroxystearic Acid has not been identified. However, the catalytic hydrogenation of castor oil to 12-Hydroxystearic Acid also yielded small amounts of ricinoleic acid, stearic acid, oleic acid, palmitic acid, arachidic acid, and traces of linolenic acid and linoleic acid (Mankovskaya et al. 1970). Information on the chemical characterization of typical Hydroxystearic Acid samples is included in Table 1. The data, reported as weight percentage, are from two chemical suppliers [Cosmetic, Toiletry, and Fragrance Association (CTFA) 1995a].

TABLE 1
Chemical characterization of typical Hydroxystearic Acid samples (CTFA 1995a)

Chemical detected	Supplier 1	Supplier 2
12-Hydroxystearic Acid (12 HSA)	**84%**	**84.9%**
Stearic Acid	**8.3%**	**9.5%**
Palmitic Acid	**0.9%**	1%
Triglyceride (castor oil)	5%[a]	5%[a]
Polyvinyl Stearate	2%[b]	<1%[b]

[a]Quantitation based on thin layer chromatography (TLC) (±0.5%).
[b]Quantitation based on TLC (±0.5%); material identified by super critical fluid/mass spectrophotometer (SFC/MS) as combination of diester, triester, and tetraester of 12 HSA.

TABLE 2
Product formulation data on Hydroxystearic Acid (FDA 1996)

Product category	Total No. of formulations in category	Total No. containing Hydroxystearic Acid
Body and hand skin care preparations (excluding shaving preparations)	1012	**2**
1996 totals		**2**

USE

Purpose in Cosmetics

Hydroxystearic Acid is used as a surfactant–cleansing agent in cosmetic products (Wenninger and McEwen 1995b).

Scope and Extent of Use in Cosmetics

The product formulation data submitted to the Food and Drug Administration (FDA) in 1996 indicated that Hydroxystearic Acid is used in two cosmetic product formulations (Table 2) (FDA 1996). Concentration of use values are no longer reported to FDA by the cosmetics industry (FDA 1992a). Because concentration of use data are no longer available from FDA, CIR has requested these data be provided directly. Data on Hydroxystearic Acid were submitted indicating that this ingredient is used in lipstick at a concentration of 2.5% (CTFA 1995b) and, in one antiperspirant/deodorant product, in the >5 to 10% concentration range (CTFA 1995c).

Cosmetic products containing Hydroxystearic Acid are intended for application to the hands and torso; however, the potential for application to other parts of the body exists. Product formulations containing Hydroxystearic Acid may be used on a daily basis and are expected to remain in contact with the skin for extended periods of time. Each product has the potential for being applied many times over a period of several years.

international Use

Hydroxystearic Acid is included in the CTFA List of Japanese Cosmetic Ingredients known to be approved for cosmetic use in Japan. The inclusion of any ingredient in the CTFA list does not guarantee either that the ingredient is safe for use as a cosmetic ingredient, or that the use of the substance as a cosmetic ingredient complies with the laws and regulations governing such use in Japan (Rempe and Santucci 1992). Hydroxystearic Acid is not included among the ingredients listed as prohibited from use in cosmetic products marketed in the European Union (EEC 1993).

Noncosmetic Use

12-Hydroxystearic Acid has been used as a textile lubricant (Mankovskaya et al. 1970) and as a saponifiable base for greases (Maskaev, Simurova, and Mankovskaya 1973). 12-Hydroxystearic Acid-polyethylene glycol block polymers have been approved by FDA for use as surfactants, for dispersion of polyacrylamide retention and draining aids, employed prior to the sheet forming operation in the manufacture of paper and paperboard, that come in contact with aqueous and fatty foods. These block copolymers are produced by the reaction of polyethylene glycol (minimum molecular weight of 1500) with 12-Hydroxystearic Acid (FDA 1992b).

BIOLOGICAL PROPERTIES

Effect on Muscle Contraction

The effect of 12-Hydroxystearic Acid (solubilized in propylene glycol) on the twitch response of the coaxially stimulated guinea pig ileum preparation was evaluated. Each of six sections of terminal ileum was immersed in an organ bath that was filled with modified Kreb's solution. The organ baths were gassed with 95% oxygen-5% carbon dioxide and maintained at 37 to 38°C. Voltage from a stimulator was passed through a voltage divider to drive six tissues simultaneously. When added to the organ bath at concentrations of 10^{-7} to 10^{-5} M, 12-Hydroxystearic Acid caused a small, transient stimulation of the smooth muscle twitch contractile height. This stimulatory effect was not dose related and, therefore, was not quantified. Furthermore, this effect was not observed after the tissues were allowed a longer initial equilibration period in the bath solution. Control concentrations of propylene glycol did not alter the contractile activity of ileal smooth muscle (Stewart, Gaginella, and Bass 1975).

Effects on Proliferation of Cells in Culture

In a study of primary and secondary lipid peroxidation products as modulators of DNA synthesis, murine Lewis carcinoma cells were treated with Hydroxystearic Acid at physiologic levels (50 and 100 μM). The test substance was dissolved in 90% ethanol and then added to the culture medium. DNA profiles obtained from flow cytometry analysis of cell cycle showed a time- and dose-dependent accumulation of cells in the G2-M phase compared to untreated exponentially growing cells. To determine if this effect was mediated by interaction of Hydroxystearic Acid with cyclin-dependent kinases-cyclin complexes, histone H1 kinase activity in C 108 cell crude extracts was measured. It was determined that Hydroxystearic Acid inhibited histone H1 kinase activity up to 95% of that noted for mitotic cells (synchronized control Cl08 cells) (Casali et al. 1994).

In another study, the same research group sought to establish the role of Hydroxystearic Acid in human cell lines. Hydroxystearic Acid, found in murine carcinoma cells in the study above, was also present in lipid extracts from human colon carcinoma cells (HT29) and normal human embryonic intestine cells (1407). HT29 and 1407 cells were exposed in culture to Hydroxystearic Acid to determine the effect on cell proliferation. The test substance was dissolved in 90% ethanol and added to each culture medium, yielding final concentrations of 20, 50, and 100 μM. At all concentrations, there was significant inhibition of proliferation of HT29 cells. At the 20 and 50 μM levels, the effect was primarily cytostatic, but at the 100 μM level the HT29 cells were not viable after 4 days. Contrary to the results reported above with murine carcinoma cells, these human carcinoma cells accumulated in the GO-G1 phase of the cell cycle. 1407 cells treated with Hydroxystearic Acid showed a lesser and delayed cytostatic effect compared to HT29 cells, and flow cytometry analysis of cell cycle kinetics showed only a slight increase in the proportion of cells at GO-G1 (Gesmundo et al. 1994).

Metabolism and Distribution

The distribution and metabolism of 12-Hydroxystearic Acid were evaluated using 90 young male albino rats (Slonaker substrain of Wistar strain; weights = 43 to 83 g). The rats were divided into groups of six and fed the following diets over a period of 16 weeks: 20% corn oil (control diet); 1% hydrogenated castor oil and 19% corn oil; and 10% hydrogenated castor oil and 10% corn oil. Laboratory chow accounted for 80% of each diet. The fatty acid composition of hydrogenated castor oil that was added to the diet was as follows: 86.5% 12-Hydroxystearic Acid, 10.3% nonoxygenated acids, and 3.2% 12-ketostearic acid. Therefore, the actual dietary concentrations of 12-Hydroxystearic Acid that were fed to experimental animals were 0.87% (in 1% hydrogenated castor oil diet) and 8.7% (in 10% hydrogenated castor oil diet). At 8 weeks after the initiation of feeding, half of the groups were fed a corn oil diet for the remainder of the 16-week study. After 4 weeks of feeding, three rats on the 8.7% 12-Hydroxystearic Acid diet and three rats on the 0.87% 12-Hydroxystearic Acid diet were killed for necropsy and abdominal adipose tissue was excised. Tissue samples were pooled within each experimental group. At 8 and 12 weeks, sets of three rats on the corn oil (control) diet were also used. Adipose tissue samples were obtained (at 16 weeks) from rats on the following diets: corn oil; 0.87% 12-Hydroxystearic Acid; 8.7% 12-Hydroxystearic Acid; 0.87% 12-Hydroxystearic Acid, changed to corn oil diet at 8 weeks; and 8.7% 12-Hydroxystearic Acid, changed to corn oil diet at 8 weeks. Adipose tissue samples obtained at 16 weeks were not pooled within each experimental group. Lipids were extracted from adipose tissue samples and carcasses (three rats on each diet) after 8, 12, and 16 weeks. The number of rats on each experimental diet that were alive at 4, 8, 12, and 16 weeks was 33, 30, 12, and 6, respectively. The number of control rats that were alive at 4, 8, 12, and 16 weeks was 15, 15, 12, and 6, respectively (Binder et al. 1970).

The results of the preceding study indicate that 12-Hydroxystearic Acid was deposited in abdominal fat, as well as other body lipids, along with its metabolites (hydroxypalmitic acid,

hydroxymyristic acid, and hydroxylauric acid). The percent composition of hydrogenated castor oil-derived hydroxy fatty acids in rat lipids was 8 1% 12-Hydroxystearic Acid, 17% 10-hydroxypalmitic acid, 1.6% 8-hydroxymyristic acid, and 0.4% 6-hydroxylauric acid. The greatest content of hydroxy acids in lipids was 4.4% in abdominal fat obtained from rats after 4 weeks of feeding of the 8.7% 12-Hydroxystearic Acid diet. This concentration decreased during the following weeks, and, at 16 weeks, was less than 2% (approximately the same concentration that was detected in carcass lipids). Hydroxy acids (as % of dry carcass weight) increased during weeks 8 to 16 in rats on both diets, 0.87% and 8.7% 12-Hydroxystearic Acid. After the diet for half of the experimental rats was changed to corn oil (control diet) at 8 weeks, the tissue content of hydroxy fatty acids decreased rapidly (Binder et al. 1970).

When 12-Hydroxystearate was added to the diet of one dog (weight not stated) in the amount of 2.2 g/day, 12-Hydroxystearic Acid accounted for 46% of the total fecal fatty acids. When the amount added to the diet was increased to 8.8 g/day, 12-Hydroxystearic Acid accounted for 60.2% of the total fecal fatty acids (Kim and Spritz 1968).

Hydroxystearic Acid has been detected in the feces of 12 normal subjects who had been encouraged to eat a normal mixture of foods. Gas-liquid chromatography served as the method of detection, and the results of this analysis did not give any indication as to the position of the hydroxyl group (Wiggins et al. 1974).

TOXICOLOGY

Subchronic Oral Toxicity

The subchronic (90-day) oral toxicity of hydrogenated castor oil was evaluated using weanling female rats (weights not stated). This study represented a preliminary feeding trial for the metabolism study (Binder et al. 1970) that is summarized in Metabolism and Distribution. The fatty acid composition of the test substance was as follows: 86.5% 12-Hydroxystearic Acid, 10.3% nonoxygenated acids, and 3.2% 12-ketostearic acid. The rats were divided into groups of three and fed diets containing 5, 10, and 20% hydrogenated castor oil, respectively, for 90 days. Thus, the content of 12-Hydroxystearic Acid in each diet was as follows: 5% diet (4.3% 12-Hydroxystearic Acid), 10% diet (8.7% 12-Hydroxystearic Acid), and 20% diet (17.3% 12-Hydroxystearic Acid). The control group was fed a commercial rat diet. At necropsy, organ weights were recorded and numerous tissues were preserved for microscopic examination. Blood samples were obtained for hematologic evaluation prior to necropsy. The only abnormality noted in this study was a reduced growth rate in rats fed diets containing 8.7 and 17.3% 12-Hydroxystearic Acid (Binder et al. 1970).

The investigators in the preceding experiment noted that hydrogenated castor oil was probably poorly digested because of its high melting point, and suggested that the poor body weight gains noted in that experiment may have been due to the lower caloric density of diets that contained 10%, or more, hydrogenated castor oil. Thus, in a second 90-day experiment, hydrogenated castor oil was dissolved in corn oil prior to addition to the diet. Groups of three weanling female rats were fed diets containing hydrogenated castor oil at concentrations of 1% (0.87% 12-Hydroxystearic Acid), 5% (4.3% 12-Hydroxystearic Acid), and 10% (8.7% 12-Hydroxystearic Acid). The control group was fed a commercial rat diet. At the time of necropsy, the growth of rats fed the diet containing 8.7% 12-Hydroxystearic Acid seemed equivalent to rats on other diets. Based on hematologic and microscopic examinations and organ weights, there were no detectable adverse effects (Binder et al. 1970).

Hepatotoxicity

The disturbance of oxidative phosphorylation in the rat liver, induced by 12-Hydroxystearic Acid, was evaluated. The basic reaction mixture (volume = 3 ml) for measuring mitochondrial swelling, respiration, and ATPase had the following composition: 0.75 mg mitochondrial protein (prepared from male rat liver); 75 mM sucrose; 75 mM Tris chloride buffer, pH 7.4; 2 mM potassium chloride; and 2 mM Tris malate. Solutions of the test substance in 0.03 ml of acetone were added to the reaction mixture. Acetone was added to control mixtures. Mitochondrial swelling was indicated by a decrease in absorbance at 520 nm. The procedures for measuring mitochondrial swelling, respiration, and ATPase have been described (Falcone and Hadler 1968; Hadler et al. 1971). Oxidative phosphorylation was uncoupled and mitochondria were damaged by 30 μM 12-Hydroxystearic Acid; respiration was stimulated in a transitory manner. The mitochondrial damage that resulted was to the extent that after respiration decreased, there was no subsequent stimulation of respiration when dinitrophenol was added to the reaction mixture. 12-Hydroxystearic Acid (30 μM) also induced mitochondrial ATPase activity. ATPase activity was inhibited by rutamycin (blocks phosphorylation by ATP); this effect is expected for ATP-energized mitochondrial reactions. A small, but decided, mitochondrial volume change (swelling) was also induced by 30 μM 12-Hydroxystearic Acid without the aid of ATP. This effect was inhibited by the respiratory inhibitor antimycin or dinitrophenol, but not by rutamycin; therefore, this effect was dependent on oxidative phosphorylation. 12-Hydroxystearic Acid–induced mitochondrial swelling was enhanced when ATP was added to the reaction mixture. The investigators concluded that 12-Hydroxystearic Acid interferes with the machinery of oxidative phosphorylation in rat liver mitochondria (Hadler and Mueller 1977).

MUTAGENICITY

The mutagenicity of 12-Hydroxystearic Acid was evaluated according to a modification of the Ames test (Ames, McCann, and Yamasaki 1975) using strains TA1535, TA100, TA1537, TA1538, and TA98 of *Salmonella typhimurium*, with and without metabolic activation. The Ames test was modified in order to increase the sensitivity of this assay to weak mutagens.

Dimethyl sulfoxide (DMSO) and ethanol served as vehicle controls and the following substances served as positive controls: benzo(α)pyrene B[α]P), 2-aminoanthracene, 9-aminoacridine, and sodium azide. 12-Hydroxystearic Acid, tested at concentrations of 500, 1000, and 2500 μg/plate, was not mutagenic for any strain. Mutagenic effects were noted with all of the positive control substances (Scheutwinkel-Reich, Ingerowski, and Stan 1980).

In another study, the mutagenicity of 12-Hydroxystearic Acid was evaluated using strain Hs30 of *Escherichia coli*. Cultures were prepared and incubated with 12-Hydroxystearic Acid (700 μM) for 18 and 48 hours according to the method of Nakamura and Yamamoto (1982). Positive control cultures were incubated with gingerol (700 μM). The mutation frequency was calculated according to the method of Green and Muriel (1976). 12-Hydroxystearic Acid induced mutations at a low frequency; the mutation frequency was 3 x 10^2 revertants per 10^8 viable cells per 700 μg 12-Hydroxystearic Acid. The mutation frequency for the positive control, gingerol, was 1 x 10^7 revertants per 10^8 viable cells per 700 μg gingerol. 12-Hydroxystearic Acid was classified as mutagenic (Nakamura and Yamamoto 1983).

The mutagenicity of 86% pure Hydroxystearic Acid (in DMSO), with and without metabolic activation, was evaluated in the L5178Y TK+/– mouse lymphoma assay according to the method of Clive and Spector (1975). An analysis of the test material showed the following: Hydroxystearic Acid, 94.9%; Stearic Acid, 8%; Palmitic Acid, 1%; triglyceride (castor oil), 5%; and polyvinyl stearate, < 1% (CTFA 1995d).

Cultures without metabolic activation were treated with concentrations of Hydroxystearic Acid ranging from 40 to 250 μg/ml (in DMSO), and those with metabolic activation were treated with concentrations of 10 to 100 μg/ml (in DMSO). Ethyl methanesulfonate (in DMSO) and 7,12-dimethylbenz(a)anthracene (in DMSO) served as positive controls, and solvent control cultures were treated with DMSO. Neither the nonactivated cultures (with total growth of 10% or greater) nor the activated cultures that were cloned had a mutant frequency that was at least twice the mean mutant frequency of the solvent controls. The total growths of cultures ranged from 1% to 7 1% (nonactivated) and from 18% to 119% (activated). Additionally, a dose-dependent response was not observed in activated or nonactivated cultures. The solvent and positive controls fulfilled the requirements for a valid test. It was concluded that Hydroxystearic Acid (in DMSO) was not mutagenic in the absence or presence of exogenous metabolic activation (Microbiological Associates 1993).

Hydroxystearic Acid (85% pure, in DMSO) was tested in a chromosome aberrations study using Chinese Hamster ovary (CHO) cells. (See above for the composition of the Hydroxystearic Acid used.) Cultures of CHO cells were exposed to concentrations ranging from 4 to 213 μg/ml (in DMSO), with and without metabolic activation, using Aroclor 1254–induced rat liver S-9. Nonactivated cultures were exposed to the test substance for 6, 18, and 42 hours, whereas activated cultures were exposed for 6 hours. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2 μg/ml, served as the positive control for nonactivated cultures, and Benzopyrene (B[α]P) [BP], 30μg/ml, was the positive control for activated cultures. Colcemid was added to each culture to a final concentration of 0.1 μg/ml to arrest cell division at metaphase. Cultures representing the three highest doses at each exposure duration with 200 scorable metaphase cells (100 per duplicate flasks) were selected for microscopic analysis. The results are shown in Table 3 for the initial test and in Table 4 for a repeat test. Hydroxystearic Acid (in DMSO) did not induce a statistically significant increase ($p > 0.025$, Fisher's exact test) in structural or numerical chromosome aberrations, with or without metabolic activation, at either of the test concentrations. Solvent and positive controls fulfilled the requirements for a valid test. Hydroxystearic Acid (in DMSO) was negative in this CHO cytogenicity study (Microbiological Associates 1994).

TABLE 3
Chromosome aberrations in Chinese Hamster Ovary (CHO) Cells exposed to Hydroxystearic Acid-initial test

Exposure duration (hrs)	Dose (μg/ml)	Survival[a]	Chromosome aberrations per cell[b]
Nonactivated cultures			
6	DMSO only		0.02
	54		0.02
	107		0.025
	213	52%	0.06
	MNNG[c]		0.295
18	DMSO only		0.00
	54		0.02
	107		0.015
	213	48%	0.005
	MNNG		0.235
42	DMSO only		0.01
	9		0.005
	18		0.00
	36	44%	0.01
	MNNG		0.345
S-9 activated cultures			
6	DMSO only		0.02
	54		0.04
	107		0.035
	213	82%	0.015
	B(α)P[d]		1.03

[a] Mean cells per treated flask/mean cells per DMSO flask.
[b] Average of two flasks.
[c] *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, positive control.
[d] Benzo(a)pyrene, positive control.

TABLE 4
Chromosome aberrations in Chinese Hamster Ovary cells exposed to Hydroxystearic Acid-repeat test

Exposure duration (hrs)	Dose (μg/ml)	Survival[a]	Chromosome aberrations per cell[b]
Nonactivated cultures			
6	DMSO only		0.005
	54		0.025
	107		0.02
	213	57%	0.015
	MNNG[c]		0.84
18	DMSO only		0.00
	54		0.015
	107		0.00
	213	40%	0.005
	MNNG		0.785
42	DMSO only		0.005
	9		0.003
	18		0.01
	36	46%	0.01
	MNNG		0.69
S-9 activated cultures			
6	DMSO only		0.01
	54		0.035
	107		0.015
	213	75%	0.025
	B(α)P[d]		1.05

"Mean cells per treated flask/mean cells per DMSO flask.
'Average of two flasks.
[c] *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, positive control.
[d] Benzo(a)pyrene, positive control.

CARCINOGENICITY

The carcinogenicity of 12-Hydroxystearic Acid was evaluated using two groups of 15 female CFW (Swiss-Webster) mice; the mice were 2 months old. 12-Hydroxystearic Acid (1 mg/0.1 ml tricapryhn) was administered to one group (Group 1) of mice and 12-Hydroxystearic Acid (0.05 mg 11 ml tricaprylin) was administered to the second group (Group 2). In each group, the test substance was injected subcutaneously at approximately the same sites in the inguinal and axillary regions of each animal. Injections were made twice per week for a total dose of 80 mg delivered in a total of 8 ml of tricaprylin (Group 1) and a total dose of 4 mg delivered in a total of 8 ml of tricaprylin (Group 2). Vehicle and untreated control groups consisted of 104 and 202 mice, respectively. Only 6 of the 15 mice that received a total of 80 mg 12-Hydroxystearic Acid survived to the 18th month of the study, and there were no survivors (at 18 months) in the group that received a total of 4 mg 12-Hydroxystearic Acid. 12-Hydroxystearic Acid induced subcutaneous sarcomas at the site of injection in 9 of the 28 mice (14 per dose group) that were alive at 6 months, and was classified as tentatively carcinogenic. All of the sarcomas were observed at the lower dose (total dose of 4 mg in 8 ml tricaprylin for 80 weeks). One sarcoma was observed in both vehicle and untreated control groups. Two pulmonary tumors (one per experimental group) and two lymphomas (one per experimental group) were also reported. Compared to controls, there was no significant increase in the number of lung tumors. Five and 11 pulmonary tumors were observed in the vehicle and untreated control groups, respectively. Lymphomas were not observed in the vehicle control group and four lymphomas were observed in the untreated control group (Swem et al. 1970).

In another experiment, the carcinogenicity of 12-Hydroxystearic Acid was evaluated using the pulmonary tumor induction technique (Shimkin et al. 1969). The test substance was dissolved in 0.1 ml tricaprylin and injected intraperitoneally (i.p.) into each of nine strain A/He male mice (2 months old). Twelve i.p. injections were made three times per week for 4 weeks, and the total dose of 12-Hydroxystearic Acid that was administered was 60 mg. At 20 weeks after the last injection, the mice were killed, the lungs were excised and fixed, and the pulmonary nodules counted. A single pulmonary nodule was observed in one mouse and two mice had two pulmonary tumors each (mean = 0.6 lung tumor/animal). A comparison of these results with untreated mice indicated that the frequency of lung tumors was within the spontaneous occurrence (Swem et al. 1970).

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

The semiquantitative formula for two antiperspirant prototype formulations is shown in Table 5 (CTFA 1995e, 1995f). Included with that data was the following summary: "From chemical analysis and structure activity relationship analysis, it was concluded that Hydroxystearic Acid [HSA] (including major contaminants) will undergo oxidative metabolism endogenously and the metabolites will have no potential for reproductive or developmental toxicity. Based upon these considerations, a teratology study should not be considered a necessary element of

TABLE 5
Semiquantitative formula for antiperspirant formulations, BD007-36, BD0017-074, BD0014-184, BD0014-162, BD0047-064, and BD0047-058, containing Hydroxystearic Acid (CTFA 1995e, 1995f)

Aluminum Zirconium Trichlorohydrex Gly	
Cyclomethicone	40 to 70%
Octyldodecanol	8 to 20%
Hydroxystearic Acid	7%
Dibutyl lauroyl glutamide	1 to 8%
Minor ingredients	<2% total

safety support for HSA under current conditions of use in cosmetic products. Nevertheless, in the course of a safety program for some antiperspirant prototypes, two test materials (BD0047-064 and BD0047-058) containing HSA were evaluated. The results show that there were no indications of maternal or developmental toxicity in any treatment group (including BD0047-064 and BD0047-058) when compared to the control" (CTFA 1995e, 1995f).

The results of teratogenicity studies using two antiperspirant prototype formulations (off-white solids) containing 7% Hydroxystearic Acid were evaluated using two groups of 30 mated Charles River Crl:CD VAF/Plus female rats (12.5 weeks old; weights = 2 11 to 289 g) (International Research and Development Corporation 1994). The test substance was applied (with gloved finger) to dorsal skin (clipped free of hair) of each animal. Applications were made on gestation days 6 through 15, once daily, and left on for 6 hours each day. Test sites were covered (but not occluded) to prevent ingestion during the study, The control article was similarly applied to an additional group of 30 rats of the same strain. The control article was described as a clear liquid; details regarding its composition were not provided. For statistical analyses, experimental groups were compared to the control group; the levels of significance were at $p \leq 0.05$ and $p \leq 0.01$. All surviving animals were killed on day 20 of gestation, followed immediately by Cesarean section. Skin irritation reactions were observed during the treatment period. Compared to the control group, increases in the severity of erythema and desquamation at the application site were observed in experimental groups. In both experimental and control groups, the effect peaked at the 5th and 6th day of treatment, then diminished. In the control group, 10 rats had very slight erythema and one had well-defined erythema; desquamation was observed in 20 rats. In one experimental group, 10 rats had very slight erythema, 10 rats had well defined erythema, and one rat had moderate to severe erythema; desquamation was observed in 26 rats. In the other experimental group, 16 rats had very slight erythema, 6 had well-defined erythema, and three had moderate to severe erythema; desquamation was observed in 28 rats. Edema was not observed in experimental or control groups. No deaths were reported during the study. There were no significant differences in clinical or necropsy observations between experimental and control groups. There were also no significant differences between experimental and control groups with respect to implantations, postimplantation losses, corpora lutea, fetal sex ratio, mean fetal body weight, or uterine weight. The incidence of postimplantation losses/resorptions was 14 of 325 viable fetuses in the first experimental group and 20 of 316 viable fetuses in the second experimental group (International Research and Development Corporation 1994).

Regarding the incidence of fetal malformations, there were no test article-related or statistically significant differences between experimental and control groups. Additionally, the total number of litters with any malformation was comparable between experimental and control groups. The incidence of malformations was 2 of 325 viable fetuses in the first experimental group and 1 of 3 16 viable fetuses in the second group. Malformations were defined by the investigators as "those structural anomalies that alter general body conformity, disrupt or interfere with body function, or are thought to be incompatible with life." With respect to the incidence of fetal developmental variations, there were also no test article-related or statistically significant differences between experimental and control groups. Furthermore, the total number of litters with any developmental variation was comparable between experimental and control groups. The incidence of developmental variations was 88 of 325 viable fetuses in the first experimental group and 80 of 3 16 viable fetuses in the second group. The investigators defined developmental variations as "those alterations in anatomic structure that are considered to have no biological effect on animal health or body conformity, representing slight deviations from normal" (International Research and Development Corporation 1994).

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation

The skin irritation potential of four antiperspirant prototype formulations (see Table 5 for description) containing 7% Hydroxystearic Acid was evaluated using 35 healthy adult volunteers between the ages of 18 and 65 years old (33 females; 2 males). The four formulations were applied to the back of each subject (in vertical rows over the scapulae areas) in occlusive and semiocclusive patch tests. Prior to patch application, test sites on the back were wiped with a pad containing 70% isopropyl alcohol. Each formulation was applied to a patch by wiping the antiperspirant stick across the patch approximately five times. The quantity of test material applied per patch application (occlusive and semiocclusive) was in the range of 0.05 to 0.1 g. The semiocclusive patches were applied to the left side of the back three times, each for 48 hours (total of three 48-hour applications per test site). Occlusive patches were applied to the right side of the back (total of three 24-hour applications per test site). Both semiocclusive and occlusive patches were reinforced with strips of tape. Following patch removal, subjects were instructed to rinse the test sites with warm water. Patches were also removed by laboratory personnel, and were not reapplied to any site that was assigned a score of 2 or greater. Test sites were evaluated for skin irritation within 24 hours after patch removal according to the following scale: 0 (no visible erythema) to 3 (severe erythema, very intense redness). Reactions to semiocclusive patches were scored on days 4, 7, and 10; occlusive patch test reactions were scored on days 4, 6, and 8. Reactions to the four antiperspirant formulations are summarized in Table 6 and Table 7 (Hill Top Research, Inc. 1994).

SUMMARY

Hydroxystearic Acid is a fatty acid that is used as a surfactant–cleansing agent in cosmetic products. One method of production involves the catalytic hydrogenation of castor oil.

Product formulation data submitted to FDA in 1996 indicate that Hydroxystearic Acid was used in two cosmetic products

TABLE 6
Group reaction scores in human primary irritation tests-semioccluded application of Hydroxystearic Acid

Group	Grade	Day 4	Day 7	Day 10
Control	0	35	35	33
	1	0	0	2[a]
BD0007-36	0	32	28	31
	1	3	5	4[b]
	2	0	2"	0
BD0014-184	0	28	26	28
	1	7	8[d]	7'
	2	0	1	0
BDOO 17-74	0	26	28	26
	1	8	7[f]	9[g]
	2	1[h]	0	0
BDOO 14-162	0	28	32	34
	1	7	2	1
	2	0	1	0

"Both with vesicles; [b]one with vesicles; [c]one with papules; [d]one with vesicles; [e]one with vesicles; [f]one with papules; gone with vesicles; [h]one with papules.

categorized as body and hand skin care preparations (excluding shaving preparations).

In male rats fed a diet containing hydrogenated castor oil, Hydroxystearic Acid was deposited in abdominal fat, as well as other body lipids, along with its metabolites (hydroxypalmitic acid, hydroxymyristic acid, and hydroxylauric acid). Hydroxystearic Acid has also been detected in the feces of 12 subjects who presumably ate a normal mixture of foods. Reduced growth rate was noted in rats fed diets containing 8.7% and 17.3% 12-Hydroxystearic Acid, but not in rats fed 4.3% Hydroxystearic Acid, in a 90-day subchronic oral toxicity study. The results of a second 90-day experiment (no reduction in growth rate) confirmed that the reduction in growth rate previously observed was due to the lower caloric density of diets consisting of 8.7% and 17.3% Hydroxystearic Acid. In both experiments, the results of hematologic and microscopic evaluations were unremarkable.

TABLE 7
Group reaction scores in human primary irritation tests-occluded application of Hydroxystearic Acid

Group	Grade	Day 4	Day 6	Day 8
Control	0	34	34	35
	1	1	1	0
BD0007-36	0	30	22	22
	1	5	12"	12
	2	0	1	1
BDOO 14-184	0	28	27	24
	1	6	7	11
	2	1[b]	1	0
BDOO 17-074	0	30	25	22
	1	5	9	12
	2	0	1	1
BD0014-162	0	30	24	18
	1	5	10"	16
	2	0	1	1

"One with edema; [b]one with edema; [c]one with papules.

In an *in vitro* study, Hydroxystearic Acid interfered with oxidative phosphorylation in rat liver mitochondria. Oxidative phosphorylation was uncoupled and mitochondria were damaged. Hydroxystearic Acid was not mutagenic in strains TA1535, TA100, TA1537, TA1538, and TA98 of *S. typhimurium*. However, Hydroxystearic Acid was classified as mutagenic in strain Hs30 of *E. coli*. Hydroxystearic Acid was not mutagenic in the L5178Y TK+/– mouse lymphoma assay, with or without metabolic activation, nor did it produce chromosome aberrations in Chinese hamster ovary cells, with or without metabolic activation.

In an 18-month carcinogenicity study (subcutaneous study), Hydroxystearic Acid was classified as tentatively carcinogenic in Swiss-Webster mice. Subcutaneous sarcomas were observed at the site of injection in 9 of the 28 mice (14 per dose group) that were alive at 6 months. All of the sarcomas were observed in the low-dose group (total dose of 4 mg delivered in a total of 8 ml tricaprylin for 80 weeks). The high-dose group received a total dose of 80 mg delivered in a total of 8 ml of tricaprylin. In a second study in which nine A/He male mice received a total intraperitoneal dose of 60 mg Hydroxystearic Acid over a period of 4 weeks, the frequency of lung tumors was within the spontaneous occurrence.

The dermal teratogenicity of two antiperspirant prototype formulations containing 7% Hydroxystearic Acid was evaluated using two groups of 30 Charles River Crl:CD VAF/Plus female rats. There were no test article-related or statistically significant differences in the incidence of fetal malformations or fetal developmental variations between experimental and control groups. Skin irritation reactions, however, were observed in greater than 50% of the dams in both experimental groups. No deaths were reported during the study.

Skin irritation reactions to each of three antiperspirant prototype formulations, each containing 7% Hydroxystearic Acid, were observed in a human primary irritation patch test using 35 volunteers. Semioccluded patches produced reactions in as many as nine of the subjects, whereas occluded patches produced reactions in as many as 17 individuals. Only two reactions were noted in the semi-occluded patch controls and only one in the occluded patch controls. Although the formulations reportedly contained the same concentration of Hydroxystearic Acid, there were small differences in the numbers of individuals reacting to each.

DISCUSSION

Because of a paucity of information on Hydroxystearic Acid, the Expert Panel considered in its original assessment that the

available data on related compounds might be used (e.g., stearic acid). Findings on long-chain aliphatic acids were taken from the published CIR Report on Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid. Slight local edema and no deaths were observed among New Zealand white rabbits after 4 weeks of topical administration (dorsal skin) of product formulations containing 2.0% Stearic Acid. There were no significant gross or microscopic lesions that were considered treatment related. In 13-week dermal toxicity studies, two cosmetic product formulations containing, at most, 5% Stearic Acid produced moderate skin irritation (dorsal skin) in rats receiving 4.0 ml/kg and 227 mg/kg doses. All other physiologic parameters were normal. Low incidences of carcinomas, sarcomas, and lymphomas were observed in mice receiving single or repeated subcutaneous injections of 25 and 50 mg Palmitic Acid and up to 82 mg Stearic Acid. Stearic Acid was not carcinogenic in mice fed dietary doses up to 50 g/kg/day. In clinical primary and cumulative irritation studies, Oleic, Myristic, and Stearic Acids at concentrations of 100% or 40 to 50% in mineral oil were nonirritating. Mild to intense erythema in single insult occlusive patch tests, soap chamber tests, and 21-day cumulative irritation studies were produced by cosmetic product formulations containing 2-93% Oleic, Palmitic, Myristic, or Stearic Acid and were generally not related to the fatty acid concentrations in the formulations. In clinical repeated insult patch tests, maximization tests, and prophetic patch tests with cosmetic product formulations containing Oleic, Lauric, Palmitic, and Stearic Acids at concentrations ranging from less than 1 to 13%, no primary or cumulative irritation or sensitization was reported. Additionally, cosmetic product formulations containing 1 to 13% Oleic, Palmitic, or Stearic Acid did not induce photosensitization; however, there were slight reactions to some induction patches.

Because of the possible influence of the hydroxyl group on toxicity, however, the Expert Panel determined that these data are not pertinent to the safety assessment of Hydroxystearic Acid. Accordingly, the CIR Expert Panel issued a Final Report in March 1995 concluding that the available data were insufficient to support the safety of Hydroxystearic Acid. The following data were considered necessary to make a safety assessment: (1) concentration of use; (2) chemical characterization; (3) a dermal teratogenicity study; (4) one genotoxicity test using a mammalian system (if the results of the genotoxicity test are positive, a dermal carcinogenicity test by NTP standards will be requested); and (5) skin irritation data.

Subsequently, new data inclusive of all of the above data needs were received. The Expert Panel, with data now available on the use of the ingredient, received the reproduction and developmental toxicity and genotoxicity data that found no significant effects at exposures likely to exceed that seen from expected cosmetic use concentrations. The sarcomas produced by subcutaneous injection of Hydroxystearic Acid were considered to be a physical phenomenon unrelated to the specific material injected and not relevant to the use of this ingredient in cosmetics. Under semioccluded and occluded patch testing conditions, the Expert Panel recognized irritation was found with antiperspirant prototype formulations. It is the experience of the Expert Panel that such formulations under those exaggerated conditions do produce irritation, but are not generally irritating in actual use.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Hydroxystearic Acid is safe as a cosmetic ingredient in the present practices of use.

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