Final Report on the Safety Assessment of Hydrogenated Cottonseed Oil, Cottonseed (Gossypium) Oil, Cottonseed Acid, Cottonseed Glyceride, and Hydrogenated Cottonseed Glyceride[1]

Hydrogenated Cottonseed Oil, Cottonseed (Gossypium) Oil, Cottonseed Acid, Cottonseed Glyceride, and Hydrogenated Cottonseed Glyceride are cosmetic ingredients derived from Cottonseed Oil and used as skin-conditioning agents and surfactants. Non-oils known to be toxic that may be found in cottonseed oils include gossypol, aflatoxin, and cyclopropenoid fatty acids (CPFA). Toxic heavy metal and/or polychlorinated biphenyl (PCB) or other pesticide contamination is also possible. Cottonseed Oil was nontoxic in acute oral toxicity studies in rats. In a short-term study, rabbits that had been fed 2% Cottonseed Oil for 7 weeks had significantly lower blood chemistry parameters (compared to wheat bran controls) and significantly more stored hepatic vitamin A (compared to rabbits fed other fats). Cottonseed Oil controls used as vehicles in two parenteral studies produced negative results. Hydrogenated Cottonseed Oil tested in formulation did not produce dermal or ocular irritation in rabbits. An oral-dose reproductive study tested up to 30% Cottonseed Oil (with 1% CPFAs) and reported no adverse effects on sexual maturity and reproductive performance of the F_0 generation; changes were noted in the F_1 generation but reproductive capacity was not altered. Parenteral-dose reproductive studies reported no adverse effects. Cottonseed Oil was not mutagenic. Cottonseed Oil did not induce aberrant crypt foci when given orally to mice, but in other studies, it increased the incidence of spontaneous mammary tumors in rats and mice. Mice fed 20% Hydrogenated Cottonseed Oil during induction and promotion of photocarcinogenesis had significantly lower tumor incidence compared to mice fed 20% sunflower oil. Hydrogenated Cottonseed Oil in formulation (up to ~ 21%) was neither an irritant nor sensitizer in clinical studies. Limited clinical data indicated that Cottonseed Oil does not contain allergic protein. Based on the available data, it was concluded that these ingredients may be used safely in cosmetic formulations if established limits on gossypol, heavy metals, and pesticide concentrations are not exceeded.

INTRODUCTION

This report is a compilation of studies conducted on Hydrogenated Cottonseed Oil, Cottonseed (Gossypium) Oil, Cottonseed Acid, Cottonseed Glyceride, and Hydrogenated Cottonseed Glyceride. For convenience, the botanical name "Gossypium" is largely omitted from the text, but is included in the headings to remind the reader of the complete name.

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Bindu Nair Madhaven, former Scientific Analyst and Writer, prepared this report. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

CHEMISTRY

Definition

Hydrogenated Cottonseed Oil. Hydrogenated Cottonseed Oil (CAS No. 68334-00-9) is the end product of controlled hydrogenation of Cottonseed Oil (q.v.) (Wenninger and McEwen 1997).

Cottonseed (Gossypium) Oil. Cottonseed (Gossypium) Oil (CAS No. 8001-29-4) is the fixed oil expressed from the seeds of various species of cotton, *Gossypium*. This International Nomenclature Cosmetic Ingredient (INCI) name has been proposed to the Food and Drug Administration (FDA) as an interim step in harmonizing botanical nomenclature with international conventions (Wenninger and McEwen 1997).

Cottonseed Acid. Cottonseed Acid (CAS No. 68308-51-0) is the mixture of fatty acids derived from cottonseed oil (Wenninger and McEwen 1997).

Cottonseed Glyceride. Cottonseed Glyceride (CAS No. 8029-44-5) is the monoglyceride derived from cottonseed oil (Wenninger and McEwen 1997).

Hydrogenated Cottonseed Glyceride. Hydrogenated Cottonseed Glyceride (CAS No. 61789-07-9) is the end product of controlled hydrogenation of Cottonseed Glyceride (q.v.) (Wenninger and McEwen 1997).

Composition and Chemical/Physical Properties

Hydrogenated Cottonseed Oil. One company produces Hydrogenated Cottonseed Oil by refining crude oil through deacidification with alkali to remove free fatty acids. Steps are then taken to bleach, hydrogenate, and deodorize the oil. Lastly, the oil is sprayed into a powder (Karlshamns 1997).

The Cosmetics, Toiletry, and Fragrance Association (CTFA) description (not specification) of cosmetic grade Hydrogenated Cottonseed Oil is: a white, lard-like material that is insoluble in

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water and soluble in ether, chloroform, and hexane. It is obtained from controlled hydrogenation of pure cottonseed oil where the mono unsaturated acids formed during the process are essentially *cis* and *trans* 9-octadecenoic acids and their positional isomers (isooleic acids). It has a melting range of 108° to 112°F and a saponification value of 180 to 200. The free fatty acid content (as oleic acid) is 0.8% to 1.4%. The iodine value is specified by the buyer (Nikitakis and McEwen 1990a).

Cottonseed (Gossypium) Oil. One company produces Cottonseed Oil by refining crude oil through deacidification with alkali to remove free fatty acids. Steps are then taken to bleach and deodorize the oil. The raw material specifications call for 100% active component and 0.001% citric acid (Karlshamns 1997).

CTFA specifications for cosmetic grade Cottonseed Oil are: a refined, pale yellow, fixed oil obtained from the seeds of various cultivated species of *Gossypium hirsutum* Linné (Fam. *Malvaceae*). Its fatty acid composition is about 45% linoleic, 30% oleic, 21% palmitic, 2% myristic, and smaller amounts of stearic and arachidic acids. It is miscible with ether, chloroform, hexane, and carbon disulfide, and almost insoluble in alcohol. It has a specific gravity at 25°/25°C of 0.915 to 0.921, a saponification value of 189 to 198, and an iodine value of 99 to 113. It has a maximum acid value of 2.0 and a 1.5% maximum for unsaponifiable matter (Nikitakis and McEwen 1990b).

The free fatty acid content and general quality of cottonseed oils depend on the weather conditions during the time the cotton stands in the fields. The free fatty acid content usually ranges from 0.5% to 1.0%, although the range can be 1.5% to 3.0%; oil from wet or damaged seeds can be ≥5%. Cottonseed Oil is described as containing more saturated acids (primarily as palmitic acid) compared to other oils with a comparable iodine number. Oil obtained from the seeds of cotton grown in different areas, even within the U.S., have different iodine numbers and different concentrations of saturated acids (Swern 1979).

Cottonseed Acid. CTFA specifications for Cottonseed Acid (distilled) are: a light yellow solid with a bland fatty odor. It is composed of ~43% linoleic, 26% oleic, 25% palmitic, 3% stearic, and smaller amounts of myristic, palmitoleic, and linolenic acids. It is soluble in chloroform, ether, and the "usual fat solvents," and is insoluble in water. It has an acid value of 195 to 206, a saponification value of 195 to 207, and an iodine value of 90 to 110 (Nikitakis and McEwen 1990b).

Light Absorption

Cottonseed (Gossypium) Oil. Refined but unbleached cottonseed oil had absorption maxima at 410, 430, 455, and 480 nm (Swern 1979).

Contaminants

Cottonseed (Gossypium) Oil. Crude cottonseed oil can contain up to 2% of a variety of nonoil substances. These nonglyceride substances (exclusive of free fatty acids) include phospholipids, sterols, resins, carbohydrates, gossypol, and other pigments. Alkali refining removes almost all of the phospholipids. Concentrations of the toxic gossypol, which Swern considered one of two "major problems" with cottonseeds, are reduced to ≤0.01% in refined oil. The gossypol that remains is responsible for the oil's characteristic color.

The second "major problem" with cottonseeds, according to Swern, is the occurrence of aflatoxins resulting from infection with the *Aspergillus flavus* mold. The International Agency for Research on Cancer (IARC) categorized aflatoxins as group 1 agents, "carcinogenic to humans" (IARC 1976, 1987). Epidemiological studies noted "positive correlation between estimated aflatoxin intake or level of aflatoxin contamination of market food samples and cooked food and incidence of hepatocellular cancer." The observations were supported by positive results in laboratory carcinogenicity and mutagenicity studies. Swern noted that with proper growing and storage conditions, and extraction and refining of the oil, the problems of aflatoxins and/or gossypol (the first "major problem") were not "especially serious with refined cottonseed oil" (Swern 1979).

Cyclopropenoid fatty acids (CPFAs) (malvalic and sterculic) have been detected at 0.1% to 0.3% or up to 1% in Cottonseed Oil. These CPFA acids are undesirable because studies have reported biological effects that ranged from slowed growth or genital system malfunctions in chickens, rats, and mice, and synergy with aflatoxins in inducing hepatic cancer in rainbow trout. Lipid, protein, and carbohydrate metabolism are also modified by CPFAs. They affect the mixed hepatic oxidase system in chickens, rabbits, and trout, but their metabolism has not been studied extensively (Andrianaivo-Rafehivola, Gaydou, and Rakotovao 1994). In a study that used food-grade Cottonseed Oil with a cyclopropenoid content of 0.35%, a nonsignificant cocarcinogenic effect was observed in rats orally dosed with CPFAs and either aflatoxin or diethylnitrosamine (Nixon et al. 1974). Methods for deactivation and/or removal of cyclopropenoid fatty acids include hydrogenation, deodorization at 450° to 455°F in the presence of free cottonseed fatty acids, and heating alone with cottonseed fatty acids. The process is thought to open the cyclopropenoid ring and form esters with the free fatty acids (Swern 1979).

Refined oil contains about 60% to 75% of the tocopherol content of the crude (~300 mg/kg of the antioxidant α-tocopherol). Despite the presence of the antioxidant, the oil is not "especially oxidation stable" and is not "responsive to conventional antioxidants such as butylated hydroxyanisole and propyl gallate" (Swern 1979).

Cottonseed Oil may also be contaminated with rice bran oil (Swern 1979).

One company's contaminant information for both Hydrogenated Cottonseed Oil and Cottonseed Oil is detailed in Table 1.

USE

Cosmetic

The chemical class and function of the various ingredients are detailed in Table 2.

TABLE 1
Contaminant and impurity limit for Hydrogenated Cottonseed Oil and Cottonseed Oil (Karlshamns 1997)

Contaminant/impurity	Limit
1,4-Dioxane, ethylene oxide, free amines, and nitrosamines	Not added and are probably not formed during processing
Solvent residue	Volatile compounds removed below detection limits (0.01 ppm) by deodorization
Arsenic (as As)	<0.1 ppm
Mercury (as Hg)	<0.01 ppm
Lead (as Pb)	<0.1 ppm
Pesticides	Organochlorine and organophosphorous pesticides not detectable
Polycyclic aromatic hydrocarbons	Reduced to <10 ppb by processing
Aflatoxins	Reduced below detection limits (0.5 ppb) by neutralization and bleaching

As of January 1998, Hydrogenated Cottonseed Oil was used in 272 formulations, Cottonseed Oil was used in 4 formulations, Cottonseed Glyceride was used in 1 formulation, and Hydrogenated Cottonseed Glyceride was used in 5 formulations. Cottonseed Acid was not reported in use (FDA 1998). See Table 3.

Concentration of use is no longer reported to the FDA (FDA 1992). Data supplied from one source indicated use of Hydrogenated Cottonseed Oil in an eyebrow pencil at 21.03%, an eyeliner at 14.5%, a lip liner at 13.96%, and an eye shadow at 11.09% (CTFA 1998).

Data from 1984 indicated Cottonseed Oil was used in 15 formulations, most at concentrations of 0.1% to 50%, with 2 uses at >50%. Cottonseed Glyceride was used in three formulations at 1% to 5% (FDA 1984).

International

The CTFA *International Cosmetic Ingredient Dictionary* notes that Cottonseed (Gossypium) Oil will be labeled "Gossypium" in the European Union when regulations for ingredient labeling under the 6th Amendment to the *European Community Cosmetics Directive* go into effect. The ingredients Cotton (Gossypium Herbaceum) and Cottonseed (Gossypium Herbaceum) Extract (that are not included in this report) will also be labeled "Gossypium" (Wenninger and McEwen 1997).

Cottonseed Oil and Cottonseed Glyceride are listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS).* Cottonseed Oil that conforms to the specifications of the *Japanese Standard of Cosmetic Ingredients* has precedent for use without restriction in all *CLS* categories. Cottonseed Glyceride that conforms to the specifications of the *Japanese Cosmetic Ingredients Codex* has precedent for use without restriction in all *CLS* categories except eyeliner, lip, oral, or bath preparations, for which it has no precedent (Rempe and Santucci 1997).

Noncosmetic

Food

Cottonseed Oil is used in foods as a coating agent, emulsifying agent, formulation aid, and texturizer (National Academy of Science 1996a). Cottonseed Oil and Hydrogenated Cottonseed Oil have been used in margarines, shortenings, and cooking oils (Applewhite 1985).

Clinical

Cottonseed (Gossypium) Oil. In the 1950s and 1960s, intravenous cottonseed oil-based emulsions designed to provide daily basal nutritional requirements to pregnant women were

TABLE 2
Cosmetic function of Cottonseed Oil-derived ingredients (Wenninger and McEwen 1997)

Ingredient	Chemical class	Function in cosmetics
Hydrogenated Cottonseed Oil	Fats and oils	Skin-conditioning agent—occlusive; viscosity increasing agent—nonaqueous
Cottonseed (Gossypium) Oil	Fats and oils	Skin-conditioning agent—occlusive
Cottonseed Acid	Fatty acids	Surfactant—cleansing agent
Cottonseed Glyceride	Glyceryl esters and derivatives	Skin-conditioning agent—emollient; surfactant—emulsifying agent
Hydrogenated Cottonseed Glyceride	Glyceryl esters and derivatives	Surfactant—emulsifying agent

TABLE 3
Frequency of use (FDA 1998)

Product category	No. of formulations in category	No. containing ingredient
	Hydrogenated Cottonseed Oil	
Eyebrow pencil	91	33
Eyeliner	514	53
Eye shadow	506	30
Lipsticks	790	151
Other makeup preparations	135	5
1998 Total for Hydrogenated Cottonseed Oil		272
	Cottonseed Oil	
Tonics, dressings, and other Hair-grooming aids	549	1
Shaving cream	139	1
Cleansing	653	1
Other skin care preparations	692	1
1998 Total for Cottonseed Oil		4
	Cottonseed Glyceride	
Hair conditioners	636	1
1998 Total for Cottonseed Glyceride		**1**
	Hydrogenated Cottonseed Glyceride	
Eyeliner	514	1
Other fragrance preparations	148	2
Foundations	287	1
Moisturizing	769	1
1998 Total for Hydrogenated Cottonseed Glyceride		**5**

found to cause fever and jaundice and to interfere with blood coagulation. The response was attributed to the instability of the emulsion as evidenced by precipitation of the emulsifying agent and fat embolization. Subsequent clinical studies demonstrated that the cottonseed oil emulsion induced labor by affecting the myometrium through unknown mechanisms. Lipid emulsions in general were contraindicated during pregnancy because they would result in increased serum triglyceride concentrations, often with simultaneous temporary ketonemia that is harmful to the fetus. Further, studies in rats and dogs noted fat deposits in the placenta of animals that had been infused with lipid emulsions; the deposits could have caused infarction of the placenta. The two commercial products were removed from the market (Amato and Quercia 1991).

GENERAL BIOLOGY

Cell Growth

Hydrogenated Cottonseed Oil. Using pubescent BALB/c mice, Miyamoto-Tiaven, Hillyard, and Abraham (1981) demonstrated that normal mammary gland ductal epithelium grew more rapidly when the dietary fat contained linoleate (corn oil) than when it did not (Hydrogenated Cottonseed Oil).

Rao and Abraham (1976) reported that the growth rate of transplanted mammary adenocarcinoma in female C3H mice was enhanced by diets containing linoleate versus fat-free or saturated fat (15% Hydrogenated Cottonseed Oil) diets. The fatty acid composition of the Hydrogenated Cottonseed Oil was 26% palmitic acid and 74% stearic acid. Tumor weights were: 0.9 g for the Hydrogenated Cottonseed Oil group, 1.1 g for the fat-free group, and 3.1 to 3.6 g for the groups that received 1%, 3%, 5%, or 15% corn oil. The data were not analyzed for statistical significance. The fatty acid content of the transplanted adenocarcinomas was comparable between the Hydrogenated Cottonseed Oil and fat-free groups.

A subsequent study reported significantly greater growth of transplantable mammary adenocarcinoma in BALB/c mice fed diets containing 10% corn oil compared to mice fed either 10% Hydrogenated Cottonseed Oil or 10% corn oil plus indomethacin. Linoleate contained in the corn oil was considered crucial for tumor cell growth (Gabor, Hillyard, and Abraham 1985).

Abraham et al. (1984) reported greater duct tissue growth in BALB/c mice that were fed 10% corn oil diets versus 10% Hydrogenated Cottonseed Oil, but dietary fat had no effect on the growth rate of HAN, a transplanted preneoplastic cell line.

Incidence and tumor growth rate were greater in mice of the corn oil group compared to mice of the Hydrogenated Cottonseed Oil group. The investigators considered the smaller number of tumors noted in the Hydrogenated Cottonseed Oil group resulted from the slower growth rate of neoplasms in mice fed diets that lacked linoleate.

ANIMAL TOXICOLOGY

Acute

Cottonseed (Gossypium) Oil. Boyd and Boulanger (1969) reported an intragastric LD_{50} of 275 ± 22 ml/kg in young male albino rats. The dose represented a 4-day cumulative dose, as an earlier study noted that amounts >70 ml/kg were immediately evacuated through the anus. Estimates for the maximal LD_0 and the minimal LD_{100} were 203 and 347 ml/kg, respectively.

A reduction in the oral LD_{50} was noted in rats maintained on protein-deficient diets. The LD_{50} values were 281, 56, 33, and 53 ml/kg in rats fed the deficient diet for 0, 4, 8, 12, and 16 weeks, respectively. The investigators speculated that cannibalism observed in rats of the 16-week group could have added protein to the diet, thereby increasing resistance to Cottonseed Oil in that group (Boyd and Krijnen 1971).

Short-Term

Oral

Cottonseed (Gossypium) Oil. Rabbits fed 2% Cottonseed Oil for seven weeks (as opposed to controls that received an additional 2% wheat bran) had significantly lowered ($p \leq 0.05$) blood concentrations of glucose, inorganic phosphorus, and cholesterol, and reduced serum glutamate oxaloacetate transaminase activity. Rabbits of this group had significantly more ($p \leq 0.01$) stored hepatic vitamin A compared to rabbits that had received starch, tallow, or hydrogenated palm oil. The investigators considered the findings favorable and recommended further examination to support use of Cottonseed Oil (and tallow) in rabbit diet (Abdelhamid 1989).

Rabbits fed a diet of 1% cholesterol and 2% Cottonseed Oil developed alopecia in 3 months and atheroma of the aorta and cutaneous lesions resembling xanthoma in 6 to 7 months. The diet was considered atherogenic (Berberian, Ziboh, and Hsia 1976).

Parenteral

Cottonseed (Gossypium) Oil. A 0.2-ml dose of the appropriate test article, extracted in 0.9% sodium chloride United States Pharmacopeia (USP) solution, alcohol in saline, polyethylene glycol, and cottonseed oil National Formulary (NF), was injected by intracutaneous route into five separate sites on the right side of the backs of rabbits. The corresponding blank vehicle was injected on the left side of the back of each rabbit. At 24, 48, and 72 hours, observations for erythema and edema were conducted. There was no significant evidence of irritation or toxicity from any of the control blanks injected intracutaneously (NAMSA 1998).

In a similar study using mice, a single dose of the appropriate test article was injected into five mice per extract by either intravenous or intraperitoneal route. Again the test article was extracted in a 0.9% sodium chloride USP solution, alcohol in saline, polyethylene glycol, and cottonseed oil, NF. Observations were made immediately and at 4, 24, 48, and 72 hours after injection. There was no mortality or evidence of systematic toxicity from any of the extracts or the controls (NAMSA 1998).

Dermal Irritation

Hydrogenated Cottonseed Oil. Three primary irritation studies tested Hydrogenated Cottonseed Oil in formulation. In each study, a single occlusive patch was applied to nine rabbits. Sites were evaluated for erythema and edema at 2 and 24 hours after exposure. The maximum possible irritation index (PII) score was 8. An eye shadow containing 8.97% Hydrogenated Cottonseed Oil had PII of 0.06, an eyeliner containing 8.0% Hydrogenated Cottonseed Oil had a PII of 0.00, and an eyebrow pencil containing 3.4% Hydrogenated Cottonseed Oil had a PII of 0.22 (CTFA 1980, 1983).

Ocular Irritation

Hydrogenated Cottonseed Oil. Four ocular irritation studies each using six rabbits tested Hydrogenated Cottonseed Oil at 12.3%, 10.0%, 8.0%, and 3.4% in various eye formulations (eye shadow, eyeliner, eyebrow pencil). In each study the cornea, iris, and conjunctiva were evaluated at days 1, 2, 3, 4, and 7 after instillation. The formulations were classified as mildly irritating (CTFA 1981, 1982, 1983).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral

Cottonseed (Gossypium) Oil. Sheehan et al. (1967) reported that feeding of Sprague-Dawley rats with 5%, 10%, 15%, 20%, or 30% Cottonseed Oil (containing 1% CPFAs) did not significantly affect the sexual maturity and reproductive performance of the F_0 generation. Significant changes in sexual maturity and length of estrus cycle were noted in the F_1 generation, but reproductive capacity was not altered. The 20% mortality in F_1 newborns was contrasted with 100% mortality following dosing with 1% *Sterculia foetida* oil that could contain 50% CPFAs.

Parenteral

Cottonseed (Gossypium) Oil. Singh, Lawrence, and Autian (1972) investigated the teratogenicity of phthalate esters in rats. Test groups were injected with one of eight phthalate esters, one group received distilled water, and two groups (five rats each) received Cottonseed Oil (5 or 10 ml/kg). All were injected on gestation days 5, 10, and 15. The number of corpora lutea,

resorptions, dead fetuses, live fetuses, average weight of fetuses, and the number of gross and skeletal abnormalities in the Cottonseed Oil group were not significantly different from the untreated control group.

Other parenteral (Marks, Fisher, and Staples 1980) and oral dose (Marks, Kimmel, and Staples 1981; Marks, Ledoux, and Moore 1982) studies that investigated the teratogenic potential of other chemicals or compounds had Cottonseed Oil–vehicle control groups. These studies did not have untreated controls and the investigators did not comment upon the findings in the Cottonseed Oil–dosed groups.

GENOTOXICITY

Cottonseed (Gossypium) Oil. Graf et al. (1989) investigated 30 chemicals in the *Drosophila* wing somatic mutation and recombination test (SMART). One compound was given in a solvent mixture of 15% Cottonseed Oil, 1.2% lecithin, and 0.3% pluronic F-68. This mixture was also tested as a solvent control. Larvae of a mwh-flr^3 cross were fed the test substance until pupation (72 hours). Flies were collected in 70% ethanol, wings were mounted and examined microscopically for spots. Spot patterns indicated somatic point mutations, deletions, and/or mitotic recombination. The results of the solvent control were not remarkable (two other control groups were fed water or Tween 80 and ethanol).

CARCINOGENICITY

Oral

Cottonseed (Gossypium) Oil. Lam and Zhang (1991) investigated the effect of inhibitors on 1,2-dimethylhydrazine (DMH)-induced aberrant crypts in the colon of female CF1 mice. A vehicle control group of 13 mice (total from three experiments) received 0.3 ml Cottonseed Oil by gavage daily for 8 days. During these 8 days, mice of the treated groups received DMH either with or without one of three inhibitors or inhibitor alone. The inhibitors were dissolved in Cottonseed Oil. An untreated control group was not used. Mice were killed 21 days after the last DMH dose and the colons were removed. No aberrant crypt foci were observed in mice of the Cottonseed Oil or inhibitor-only groups.

The incidence of spontaneous mammary tumors at 35 weeks was greater in C3H mice fed diets containing 10% Cottonseed Oil compared to mice fed comparable fatty acid diets (Tinsley, Wilson, and Lowry 1982). Palpable tumors were noted in 19% of mice of the Cottonseed Oil group at 35 weeks and in 30% at 45 weeks. The incidences (%) for other groups were (35 weeks, 45 weeks): safflower (10, 21), corn (9, 21), olive (0, 12), and butter (8, 21). The time to 50% tumor incidence was also shorter in the Cottonseed Oil group. The data were not analyzed for statistical significance and no untreated control group was used. The fatty acid composition of mammary gland and hepatic tissues was consistent with the presence of cyclopropene fatty acids in the Cottonseed Oil (at nondetectable concentrations of $<1\%$). The investigators considered cyclopropene fatty acids as possibly responsible for the increased tumor incidence.

A study using Wistar and Fischer rats investigated the cocarcinogenicity of CPFAs with the known carcinogens aflatoxin and diethylnitrosoamine. A control group (19 females, 18 males) received 10% food-grade Cottonseed Oil ($\sim$0.35% CPFAs) without carcinogen for 2 years. No hepatic neoplasms were noted in either the Cottonseed Oil control group or in the untreated control group. However, six females of the Cottonseed Oil group developed mammary tumors compared to one untreated female (Nixon et al. 1974).

PHOTOCARCINOGENICITY

Oral

Hydrogenated Cottonseed Oil. Reeve, Bosnic, and Boehm-Wilcox (1996) investigated whether polyunsaturated fats act via immunosuppression to exacerbate photocarcinogenic responses in mice. Groups of 15 hairless female Skh:HR-1 mice were fed a diet containing 20% fat. Dietary fat concentrations were composed of 0%, 5%, 10%, 15%, or 20% Hydrogenated Cottonseed Oil (saturated fat source) and complementary amounts of sunflower oil (unsaturated fat source) to achieve a 20% fat diet.

Photocarcinogenesis was induced beginning on week 4 by irradiating (290–400 nm) mice on the dorsum, 5 days per week. Initial irradiation was 67% of the previously determined minimal erythemal dose (MED). The exposure time was increased by 2 minutes every week, until an exposure time of 20 minutes was achieved and maintained. The response of minimal erythema of the dorsal skin continued for 10 weeks for cumulative doses of 111 kJ/m^2 UVB and 2106 kJ/m^2 UVA. Mice continued to receive feed for 232 days from initial UV radiation.

To monitor immunosuppression, some mice were sensitized on the unirradiated abdomen with oxazolone/ethanol on either the last day of the study (chronic irradiation reaction assay) or on each of 3 consecutive days 1 week after exposure to 1 MED of unfiltered UVB radiation (acute reaction assay). Mice were challenged a week later by application of oxazolone/ethanol to the pinnae and the contact hypersensitivity (CHS) response was measured as pineal thickness prior to and 18 to 24 hours after challenge.

Tumors first appeared on day 113 in mice of the 20% and 15% Hydrogenated Cottonseed Oil group, as opposed to day 84 in mice fed $\leq$10% Hydrogenated Cottonseed Oil (and consequently, $\geq$10% sunflower oil). Final tumor incidence on day 232 was 79% in mice of the 20% Hydrogenated Cottonseed Oil (0% sunflower oil) group, 93% in mice of the 15% Hydrogenated Cottonseed Oil (5% sunflower oil) group, and 100% in mice of the $\leq$10% Hydrogenated Oil (and consequently, $\geq$10% sunflower oil) group. Tumor incidence was significantly less ($p < 0.05$) in mice fed only Hydrogenated Cottonseed Oil, compared with mice fed only sunflower oil; the differences among other groups were not significant. A "highly" significant decrease ($p < 0.01$)

in final tumor multiplicities was observed in mice fed only Hydrogenated Cottonseed Oil, compared with mice fed only sunflower oil. The difference was not significant between mice of the 20% and 15% Hydrogenated Cottonseed Oil groups. Most of the lesions were papillomas; a single squamous cell carcinoma developed in mice of the 20% Hydrogenated Cottonseed Oil Group, whereas four or five were noted in mice from the other groups.

In the chronic immunosuppression study, irradiated mice of groups that received ≥10% Hydrogenated Cottonseed Oil had "normal" CHS responses comparable to responses of non-irradiated mice fed stock diet. Significant immunosuppression was noted in mice that received either 5% or no Hydrogenated Cottonseed Oil (consequently, 15% or 20% sunflower oil).

The CHS response after acute irradiation was measured in mice that received either 20% Hydrogenated Cottonseed Oil or 20% sunflower oil and compared to stock-diet fed controls. Hydrogenated Cottonseed Oil had a protective effect: UVB-irradiated mice of the control group had 45% immunosuppression, mice of the sunflower oil group had 57% immunosuppression, and mice of the Hydrogenated Cottonseed Oil group had 10% immunosuppression. The investigators commented, "saturated fat-fed mice exposed in the short-term to an acute immunosuppressive dose of UVB radiation were spared . . . (from) UVR-impaired CHS responses." No difference in CHS response was noted in mice that received either 20% Hydrogenated Cottonseed Oil or sunflower oil without radiation, "indicating that the persistent immunosuppression was likely to have been induced by the carcinogenic irradiation regime."

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation

Hydrogenated Cottonseed Oil. A cumulative irritation study tested an eyebrow pencil containing 3.4% Hydrogenated Cottonseed Oil using 10 panelists (9 female, 1 male). Patches containing the test material were applied to the back. Panelists were instructed to remove the patch after 23 hours, shower, and then report for site evaluation and patch reapplication. The material was applied to the same site 21 consecutive times. The eyebrow pencil had a total score of 4; the maximum score was 630. It was classified as mild (Hill Top Research 1984).

A group of approximately 65 panelists were instructed to apply an eye shadow pencil containing 10.4% Hydrogenated Cottonseed Oil over the eyelid until the "desired color intensity is achieved." No details were provided as to duration or frequency of exposure, but panelists were examined by a physician at the start of the study and after 2 and 5 weeks of use. The study was "double-blind" and a control group received an eye shadow crayon. The incidence and severity of adverse reactions was considered "acceptably low." No further details were given (CTFA 1977).

Cottonseed Glyceride. A patch containing 2.7% Cottonseed Glyceride in petrolatum was applied (0.05 ml) to the forearm of 55 panelists. The patch was removed after 24 hours and the sites were scored on a scale of 0 to 5. No reactions were observed (CTFA No date).

Dermal Sensitization

Hydrogenated Cottonseed Oil. An eyebrow pencil containing 20.86% Hydrogenated Cottonseed Oil and a lip liner containing 11.3% Hydrogenated Cottonseed Oil were both tested in the same repeated-insult patch test (RIPT) using 101 panelists. Nine induction patches were applied, followed by a challenge. No further details were provided regarding the protocol. Neither test material produced reactions during induction or at challenge (TKL Research 1996).

An eye lining pencil containing 14.5% Hydrogenated Cottonseed Oil was tested in an RIPT using 103 panelists. A total of nine 24-hour induction patches were applied to the back within a 3-week period. Following a 2-week nontreatment period panelists were challenged. Twelve panelists had a single instance of a "barely perceptible" reaction during induction. No reactions were observed at challenge (AMA Laboratories 1989).

A foundation stick containing 10.6% Hydrogenated Cottonseed Oil was tested in a maximization assay using 26 panelists. During induction panelists were pretreated with a 24-hour patch containing sodium lauryl sulfate (SLS) followed by a 48-hour occlusive patch containing the test material (0.1 ml). If no irritation was noted at the time of patch removal the protocol was repeated for a total of five exposures. Following a 10-day nontreatment period, panelists received a 1-hour SLS patch applied to an unexposed site and were then challenged with the test material. Challenge sites were evaluated 1 hour after patch removal and 24 hours later. No reactions were noted at challenge (Ivy Laboratories 1996).

Cottonseed (Gossypium) Oil. A patch testing reference book by DeGroot (1994) noted that testing with pure Cottonseed Oil is recommended.

Atkins, Wilson, and Bock (1988) reported that seven subjects with demonstrated hypersensitivity to cottonseed protein were not sensitive to Cottonseed Oil under skin prick test conditions. The investigators cited earlier studies and noted that the similar findings "clearly demonstrated the absence of the water-soluble allergens of Cottonseed protein . . . in Cottonseed oil and conclusively demonstrated that cottonseed-protein-sensitive individuals could ingest Cottonseed oil without difficulty."

SUMMARY

Hydrogenated Cottonseed Oil, Cottonseed (Gossypium) Oil, Cottonseed Acid, Cottonseed Glyceride, and Hydrogenated Cottonseed Glyceride are cosmetic ingredients derived from Cottonseed Oil. Possible contaminants include gossypol, aflatoxin, and CPFAs. These ingredients are used as skin-conditioning agents and surfactants and were used collectively in 282 formulations in 1998.

Cottonseed Oil has an LD_{50} of 275 ml/kg in young male albino rats. In a short-term study, rabbits that had been fed 2% Cottonseed Oil for 7 weeks had significantly lower blood chemistry parameters (compared to wheat bran controls) and significantly more stored hepatic vitamin A (compared to rabbits fed other fats). Cottonseed Oil controls, used as vehicles in two parenteral studies, produced negative results.

Hydrogenated Cottonseed Oil tested in formulation did not produce dermal or ocular irritation in rabbits.

An oral-dose reproductive study tested up to 30% Cottonseed Oil (with 1% CPFAs) and reported no adverse effects on sexual maturity and reproductive performance of the F_0 generation; changes were noted in the F_1 generation but reproductive capacity was not altered. Parenteral dose reproductive studies reported no adverse effects.

Cottonseed Oil was tested as a solvent control in a SMART mutagenicity assay. Cottonseed Oil did not induce aberrant crypt foci when given orally to mice. In other studies, it increased the incidence of spontaneous mammary tumors in rats and mice.

Mice fed 20% Hydrogenated Cottonseed Oil during induction and promotion of photocarcinogenesis had significantly lower tumor incidence compared to mice fed 20% sunflower oil. In general, tumor incidence was lower and CHS responses were more normal in irradiated mice that were fed Hydrogenated Cottonseed Oil versus sunflower oil. Saturated fats were considered responsible for the protective effects of Hydrogenated Cottonseed Oil.

Hydrogenated Cottonseed Oil in formulation (up to almost 21%) was neither an irritant nor sensitizer in clinical studies. Limited clinical data indicated that Cottonseed Oil does not contain allergic protein.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel was of the opinion that Hydrogenated Cottonseed Oil, Cottonseed (Gossypium) Oil, Cottonseed Acid, Cottonseed Glyceride, and Hydrogenated Cottonseed Glyceride may be used safely in cosmetic formulations. However, the Panel recognized the need to limit the presence of gossypol, heavy metals, and/or polychlorinated biphenyl (PCB) or other pesticide contamination.

Gossypol is to be limited to a concentration of <450 ppm according to 21 CFR 172.894. This value was adopted from the Code of Federal Regulations (CFR) limit on modified cottonseed products intended for human consumption.

The values for lead have been adopted from the CIR final report on Lard Glyceride, Hydrogenated Lard Glyceride, Lard Glycerides, Hydrogenated Lard Glycerides, Lard and Hydrogenated Lard (CIR 1998a). Within that report, the limitation of lead is adopted specifically from the *Food Chemicals Codex* (National Academy of Sciences 1996b). The limitations for arsenic and mercury are adopted from the CIR final report on Acid Violet 43 (CIR 1998b). Collectively, those limits are: lead, $\leq$0.1 mg/kg; arsenic, $\leq$3 ppm (as As); and mercury, $\leq$1 ppm (as Hg).

The Panel limited the total PCB/pesticide contamination to not more than 3 ppm, with not more 1 ppm for any specific residue. These limits are also found within the CIR final report on Lard Glyceride, Hydrogenated Lard Glyceride, Lard Glycerides, Hydrogenated Lard Glycerides, Lard and Hydrogenated Lard, in which these limits are modeled after the USP standards for modified lanolin (Committee of Revision of the United States Pharmacopeial Convention 1995). The Panel recognizes that these limits were developed for uses other than cosmetics, but considers that such limits would assure that any cosmetic product with these ingredients can be used safely.

CONCLUSION

Based on the available data, the CIR Expert Panel concludes that Hydrogenated Cottonseed (Gypossium Oil), Cottonseed Acid, Cottonseed Glyceride, and Hydrogenated Cottonseed Glyceride are safe as used in cosmetic products, provided that established and imposed limits on gossypol, heavy metals, and pesticide concentrations are not exceeded.

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