RE-REVIEW

Supplement Book 2

Alkyl Esters

CIR EXPERT PANEL MEETING

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ALKYL ESTERS RE-REVIEW – SUPPLEMENTAL BOOK 2

Relevant Acids

Also, see Supplemental Book 1 for additional information on Coconut Acid (p 299)

Final Report

Dicarboxylic Acids and Their Salts as Used in Cosmetics
Esters of Dicarboxylic Acids as Used in Cosmetics

December 14, 2010

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ABSTRACT

The CIR Expert Panel assessed the safety of dicarboxylic acids and their salts and esters as used in cosmetics. Most dicarboxylic acids function in cosmetics as pH adjusters or fragrance ingredients. The functions of most of the salts are not reported. Some of the esters function as skin conditioning or fragrance ingredients, plasticizers, solvents, or emollients. The Expert Panel noted gaps in the available safety data for some of the dicarboxylic acid and their salts and esters in this safety assessment. The available data on many of the ingredients are sufficient, however, and similar structural activity relationships, biologic functions, and cosmetic product usage, suggest that the available data may be extrapolated to support the safety of the entire group. The Panel concluded that the ingredients named in this report are safe in the present practices of use and concentration.

INTRODUCTION

This safety assessment includes sebacic acid and other alkyl α,ω-dicarboxylic acids, and their salts, monoesters and diesters as used in cosmetics. The dicarboxylic acids are terminally functionalized straight alkyl chains characterized by a separation between the acid functional groups of one to 10 carbons (1 carbon = malonic acid; 2 carbons = succinic acid; 3 carbons = glutaric acid; 4 carbons = adipic acid; 5-6 carbons = no representative cosmetic ingredients; 7 carbons = azelaic acid; 8 carbons = sebacic acid; 9 carbons = no representative cosmetic ingredients; and 10 carbons = dodecanedioic acid). The simple alkyl di-esters are the result of the condensation of alkyl dicarboxylic acids and two equivalents of alkyl alcohols. These ingredients can be metabolized via hydrolysis back to the parent alcohol, the mono-ester, and the parent dicarboxylic acid (Figure 1). The simple alkyl esters (mono- and di-) of these dicarboxylic acids have straight or branched side chains ranging in length from one to 18 carbons. Throughout this report, the data are presented by order of acid chain length (i.e., beginning with malonic acid and ending with dodecanedioic acid; beginning with dimethyl malate and ending with diisocetyl dodecanedioate).

This report presents available information pertinent to the safety of 56 cosmetic ingredients in two groups, first, the 12 alkyl dicarboxylic acids/salts and, second, the 44 corresponding esters (mono- and di-). The alkyl dicarboxylic acids and salts include:

malonic acid
succinic acid
sodium succinate
disodium succinate
glutaric acid
adipic acid
azelaic acid
dipotassium azelate
disodium azelate
sebacic acid
disodium sebacate
dodecanedioic acid.

The esters include:

diethyl malonate
decyl succinate
dimethyl succinate
diethyl succinate
dicapryl succinate
dicetearyl succinate
diisobutyl succinate
diethylhexyl succinate
dimethyl glutarate
diisobutyl glutarate
diisostearyl glutarate
dimethyl adipate
diethyl adipate
dipropyl adipate
dibutyl adipate
dihexyl adipate
dicapryl adipate
di-C12-15 alkyl adipate
ditridecyl adipate
dicetyl adipate
diisopropyl adipate
diisobutyl adipate
diethylhexyl adipate
diisooctyl adipate
diisononyl adipate
diisodecyl adipate
dihexyldecyl adipate
diheptylundecyl adipate
dioctyldodecyl adipate
diisocetyl adipate
diisostearyl adipate
isostearyl sebacate
diethyl sebacate
dibutyl sebacate
dicaprylyl/capryl sebacate
diisopropyl sebacate
diethylhexyl sebacate
dibutyloctyl sebacate
diisooctyl sebacate
dihexyldecyl sebacate
dioctyldodecyl sebacate
diisostearyl sebacate
dioctyldodecyl dodecanedioate
diisocetyl dodecanedioate

The structures and functions of these ingredients are presented in Table 1.

A safety assessment of diethylhexyl adipate (often inaccurately named dioctyl adipate)[1] and diisopropyl adipate was published in 1984 with the conclusion that these ingredients are safe as used in cosmetics.[2] The safety of these ingredients was reviewed and confirmed in 2005[3] and 2006.[4] Additionally, dibutyl adipate was originally reviewed in 1996, and at that time the available data were found insufficient to support the safety of dibutyl adipate in cosmetic formulations. When re-reviewed in 2006, additional data were made available to address the data needs identified by the CIR Expert Panel, and an amended conclusion was issued stating that dibutyl adipate is safe for use in cosmetic formulations.[5]

The acids and their salts included in this report function in cosmetics as pH-adjusters, and the esters function as fragrance ingredients, plasticizers, skin-conditioning agents or solvents and corrosion inhibitors.

CHEMISTRY

Definition, Structure and Manufacture

The CAS numbers, definitions, structures and functions for the alkyl dicarboxylic acid, salt and ester ingredients included in this report are given in Table 1.

Alkyl Dicarboxylic Acids

While many of the alkyl dicarboxylic acids are present in natural products, commercial production of these acids has historically occurred via alkali pyrolysis of lipids.[6] For example, when castor oil (a lipid which is comprised of approximately 84% ricinoleic acid-sidechain bearing triglycerides) is pyrolyzed with sodium hydroxide, some of the major products are sebacic acid and 2-octanol (Figure 2).[6] Sodium and potassium salts of the alkyl dicarboxylic acids are readily prepared via addition to the appropriate stoichiometric equivalent(s) of sodium hydroxide or potassium hydroxide, respectively.

Some of the ingredients in this assessment are tallow derivatives. The CIR accepts the Food and Drug Administration (FDA) determination (21 CFR 700.27(a)), that tallow derivatives are not prohibited cattle materials and may be used in cosmetics. .

Malonic Acid (C3)

Malonic acid, first prepared by malic acid oxidation, is commonly manufactured by more recent methods including the ozonolysis of cyclopentadiene or the air oxidation of 1,3-propanediol.[7]

Succinic Acid (C4)

Succinic acid is an intermediate of the citric acid cycle and is found in almost all plant and animals cells, although at very low concentrations.[8] Succinic acid is commonly produced synthetically by catalytic (e.g., nickel or palladium catalyst) hydrogenation of maleic anhydride.

Glutaric (C5) and Adipic(C6) Acids

Although glutaric acid is often encountered in nature, adipic acid is not commonly encountered in nature. Glutaric and adipic acids were first synthesized by oxidation of castor oil with nitric acid. However, adipic acid is now more commonly manufactured by oxidation of cyclohexane, cyclohexanol, or cyclohexanone, and glutaric acid may be manufactured by ozonolysis of cyclopentene.[9]

Azelaic Acid (C9)

Azelaic acid, first detected in rancid fats, was originally produced via nitric acid oxidation of oleic acid. [10] Azelaic acid is a naturally-occurring dicarboxylic acid that can be found in dietary sources, such as whole grains.[11] Azelaic acid is commonly manufactured by oxidative cleavage of oleic acid (obtained from grease or tallow) with chromic acid, nitric acid or

by ozonolysis.[10,7]

Sebacic Acid (C10)

Sebacic acid was originally isolated from distillation products of beef tallow. More recently, however, sebacic acid has been manufactured via alkali pyrolysis of castor oil, as mentioned above and drawn in Figure 2, or by alkali pyrolysis of ricinoleic acid.[12,7]

Dodecanedioic Acid (C12)

Dodecanedioic acid can be manufactured by fermentation of long-chain alkanes with a specific strain of *Candida tropicalis*.[13] Another method of manufacture involves the nitric acid oxidation of a mixture of cyclododecanone and cyclododecanol.[7]

Alkyl Dicarboxylic Acid Esters

The alkyl dicarboxylic acids are easily esterified with the appropriate alcohol, with our without acid or metal catalyst (Fischer esterification).[9] For example, diethylhexyl adipate can be manufactured from adipic acid and ethylhexanol with an acid catalyst (Figure 3).

Diethyl Malonate

Malonic acid esters can be produced either by cobalt-catalyzed alkoxycarbonylation of chloroacetates with carbon monoxide in the presence of the appropriate alcohol, or by hydrolysis of cyanoacetic acid followed by esterification with the respective alcohol.[14] Diethyl malonate is prepared from chloroacetic acid and sodium cyanide followed by esterification with ethanol and sulfuric acid.[15]

Diisopropyl Adipate

Diisopropyl adipate is produced by esterification of adipic acid with an excess of isopropanol. The excess alcohol is removed by vacuum stripping and the ester is then alkali-refined and filtered.[2]

Dibutyl Adipate

Adipic acid is esterified with butyl alcohol by a continuous distillation process.[16]

Diethylhexyl Adipate

Diethylhexyl adipate can be prepared by the reaction of adipic acid and 2-ethylhexanol in the presence of an esterification catalyst such as sulfuric acid or *para*-toluenesulfonic acid (Figure 3).[17] Purification of the reaction product includes removal of the catalyst, alkali refining, and stripping.[2]

Alkyl Succinates

Succinic anhydride reacts readily with alcohols to give monoesters of succinic acid (e.g., decyl succinate from decanol), which are readily further esterified to the diesters by Fischer methods.[7] Dimethyl succinate can be produced from methanol and succinic anhydride or succinic acid, or by hydrogenation of dimethyl maleate. Diethyl succinate can be prepared by the same methods (from ethanol or diethyl maleate).

Physical and Chemical Properties

Tables 2a lists physical and chemical properties of the dicarboxylic acids and salts and Table 2b lists the properties of the esters. Charts 1, 2, and 3 demonstrate the relationship between molecular weight and the log octanol – water partitioning coefficient.

Dicarboxylic Acids - General

The alkyl dicarboxylic acids vary considerably in their physical properties. The shorter chain (malonic, succinic, and glutaric) members are crystalline solids, very water-soluble, and have limited solubility in organic solvents. As the chain

length increases through adipic to dodecanedioic, water solubility decreases sharply (although still soluble in hot water). In other words, the water solubility of these acids is inversely proportional to their chain length. There is a marked alternation in melting point with changes in carbon number from even to odd.[7] Odd members (e.g., malonic acid and glutaric acid) exhibit lower melting points and higher solubility than even carbon number alkyl dicarboxylic acids (e.g., succinic acid and adipic acid). These alternating effects are believed to be the result of the inability of odd carbon number compounds to assume an in-plane orientation of both carboxyl groups with respect to the hydrocarbon chain.

Dicarboxylic acids react with Brønsted-Lowry bases (e.g., sodium hydroxide) to form carboxylate salts (e.g., sodium succinate or disodium succinate). Dicarboxylic acids also react with alcohols to give mono- and di-esters, such as those in this report.

Esters

The diesters, in contrast to the free acids, are much more lipid soluble and more difficult to dissolve in water. The mono-esters, by definition, are hybrids of the acids and diesters, but their physical properties are much more closely related to the diesters.

The short-chain alkyl (i.e., methyl, isopropyl, and butyl) mono- and diesters are more soluble in water, less lipophilic, and relatively more volatile than the corresponding longer-chain alkyl (i.e., C8-C13 alcohol) esters.[18] Most esters with molecular weights greater than 340 have boiling points greater than 300°C and are relatively non-volatile and lipophilic (log K_{ow} >7).

Analytical Methods

Succinic Acid

Methods used to analyze succinic acid include acidimetric titration for acidity; comparison with platinum-cobalt (Pt-Co) standard calibrated solutions for color; oxidation with potassium permanganate for detection of unsaturated compounds; atomic absorption or plasma spectroscopy for metals; and titration with silver nitrate or barium chloride for chloride or sulfate detection, respectively.[7] Small concentrations of succinic acid can be detected by common instrumentation such as gas/liquid chromatography and polarography.

Adipic Acid

Adipic acid can be extracted from a water sample and analyzed by gas chromatography/mass spectrometry.[17]

Sebacic Acid

Gas chromatography can be used to identify sebacic acid in air.[19]

Diisopropyl Adipate and Diethylhexyl AdipateDiisopropyl adipate and diethylhexyl adipate can be identified through standard infrared (IR) spectroscopy. Gas-liquid chromatography (GLC), liquid-liquid extraction, mass spectrometry, and high-pressure liquid chromatography (HPLC) are also methods of analysis for the adipates.[2]

Impurities

Diethyl Malonate

Diethyl malonate is a colorless organic liquid with an ester like odor.[14] The purity is typically > 99 %. Impurities from the production process include ethanol (ca. 0.1 % w/w), ethyl acetate (ca. 0.05 % w/w), and ethyl methyl malonate (ca. 0.05 % w/w).

Dibutyl Adipate

Impurities are generally not found due to the manufacturing process, but available data demonstrate that arsenic levels are below a detection limit of 1 ppm, heavy metals (as lead) are below a detection limit of 10 ppm, and sulfated ash is

below a detection limit of 0.1%.[16]

Diisopropyl Adipate and Diethylhexyl Adipate

Diisopropyl adipate and diethylhexyl adipate are considered stable; however, hydrolysis of the ester groupings may occur in the presence of aqueous acids or bases. No known impurities occur in either diisopropyl adipate or diethylhexyl adipate, although the acid values imply the presence of adipic acid or of the monoester in both.[2]

Diethylhexyl adipate is commercially available with the following specifications: purity – 99 to 99.9%; acidity – 0.25 µg/100g max; moisture – 0.05 to 0.10% max.[17]

Diisopropyl Sebacate

A supplier reported that the expected impurities in diisopropyl sebacate are the starting material sebacic acid, <0.3%, and isopropyl alcohol, <0.2%.[20]

Ultraviolet Absorption

The ingredients included in this review would not be expected to have any meaningful ultraviolet (UV) absorption. Except for the acid and ester functional groups, these ingredients do not possess any conjugated π-bonds or non-bonding electrons. The π-bonds and non-bonding electrons in the acid and ester functional groups are not part of any conjugated systems. Accordingly, the likelihood of any of these ingredients to absorb light within the UVA-UVB spectrum, at a detectable molar absorptivity, is extremely low. As such, no UVA-UVB absorption data were found.

USE

Cosmetic

The ingredients included in this safety assessment have a variety of functions in cosmetics.[21] The majority of the dicarboxylic acids function in cosmetics as pH adjusters or fragrance ingredients. The functions of most of the salts are not reported, but it is stated that sodium succinate functions as a buffering agent or pH adjuster. For the esters, some of the common functions include skin conditioning agents, fragrance ingredients, plasticizers, solvents, and emollients. The functions of all ingredients are listed in Table 1.

Six of the 12 dicarboxylic acids and their salts and 24 of the 44 esters included in this safety assessment are reported to be used in cosmetic formulations. The frequency of use of the acids and salts, as supplied to the FDA by industry in 2010 as part of the Voluntary Cosmetic Registration Program (VCRP),[22] and the concentration of use, as supplied by industry in response to Personal Care Products Council (Council) surveys in 2009[23] and 2010,[24,25] are found in Table 3a. The frequency and concentration of use of the esters, with the exception of dibutyl, diisopropyl, and diethylhexyl adipate, which have previously been reviewed, are found in Table 3b. The 2010 and historical use data for the 3 previously reviewed esters are found in Table 3c. The 6 acids and salts and 20 esters not currently reported to be used are listed in Table 3d.

For the dicarboxylic acids and their salts, disodium succinate has the greatest number of reported uses, with a total of 45. The acid with the highest concentration of use is succinic acid, with a use concentration up to 26%; use at this concentration is in a bath product that will be diluted for use. The highest leave-on concentration is 0.4% disodium succinate, with dermal contact exposure.

For the esters, diisopropyl adipate has the greatest number of uses, with 70 reported. The highest concentration of use is for dimethyl glutarate, 15% in a dermal rinse-off product. The ingredients with the highest leave-on use concentrations, which are all dermal contact exposures, are diethylhexyl adipate, 14%, diisostearyl adipate, 10%, and diisopropyl sebacate, 10%.

Some of the ingredients are applied around the eye, can possibly be ingested, or involve mucous membrane expo-

sure, and some are used in underarm deodorants. None are reported to be used in baby products.

Dicapryl and diethylhexyl succinate, dibutyl, dicapryl, diisopropyl, diisobutyl, and diethylhexyl adipate, diisopropyl, diethylhexyl, and dioctyldodecyl sebacate, and dioctyldodecyl and diisocetyl dodecanedioate are used in hair sprays, and effects on the lungs that may be induced by aerosolized products containing this ingredient, are of concern.

The aerosol properties that determine deposition in the respiratory system are particle size and density. The parameter most closely associated with deposition is the aerodynamic diameter, d_a, defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. In humans, particles with an aerodynamic diameter of $\leq 10\mu m$ are respirable. Particles with a d_a from 0.1 – 10 µm settle in the upper respiratory tract and particles with a $d_a < 0.1$ µm settle in the lower respiratory tract.[26,27]

Particle diameters of 60-80 µm and ≥80 µm have been reported for anhydrous hair sprays and pump hairsprays, respectively.[28] In practice, aerosols should have at least 99% of their particle diameters in the 10 – 110 µm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 µm.[29] Therefore, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

The dicarboxylic acids and their salts and esters are in the European Union (EU) inventory of cosmetic ingredients.[30]

Non-Cosmetic

Many of the dicarboxylic acids, their salts, and their esters are used in foods as direct or indirect food additives. The alkyl dicarboxylic acids are unusually versatile because of their two carboxyl groups.[9] This enables many additional types of useful reactions, particularly the manufacture of polymers (e.g., nylon). The most common uses include functions as plasticizers, lubricants and building blocks in the manufacture of polyesters, polyamides and other plastics. The alkyl dicarboxylic acid salts are used to synthesize cyclic ketones, including commercially used macrocyclic musk compounds.[31] The diesters have widespread use as lubricants, plasticizers, and solvents.[32]

Malonic Acid

Malonic acid is a useful intermediate in the manufacture of barbiturates.[33]

Succinic Acid

Succinic acid is listed by the FDA as a food additive that is Generally Recognized as Safe (GRAS).[34] Succinic acid is also utilized in detergents, pigments, toners, cement additives, soldering fluxes and as an intermediate in the synthesis of a number of pharmaceutical products.[7]

Adipic Acid

Adipic acid is listed as a GRAS food additive by the FDA.[35] Adipic acid has several industrial uses in the production of adhesives, plasticizers, gelatinizing agents, hydraulic fluids, lubricants, emollients, polyurethane foams, leather tanning, and urethane.[7] However, the bulk of the industrial production of adipic acid is driven by its usefulness in the manufacture of nylon-6,6 (in combination with 1,6-hexanediamine).

Azelaic Acid

FDA has approved azelaic acid for use in treating acne and rosacea. A skin cream containing 20% (w/w) azelaic acid is indicated for the topical treatment of mild-to-moderate inflammatory acne vulgaris,[36] and a gel containing 15% azelaic acid is approved for treating rosacea.[37] These drugs are available by prescription only. (As a reference point, azelaic acid is reported to be used in cosmetics at 0.3% in leave-on and 10% in rinse-off formulations that have dermal exposure.[25])

Azelaic acid is used in the manufacture of plasticizers, lubricants, and greases. Azelaic acid was identified as a molecule that accumulated at elevated levels in some parts of plants and was shown to be able to enhance the resistance of

plants to infections.[38]

Sebacic Acid

Sebacic acid was widely used in the U.S. as an aromatic in food before 1973.[39]

Sebacic acid is used in resorbable polymer systems that deliver chemotherapeutic agents (e.g. cisplatin, carboplatin) that are implanted at the site of tumors to provide for sustained release of the drugs.[40] Sebacic acid and its derivatives have a variety of industrial uses as plasticizers, lubricants, diffusion pump oils, candles and as intermediates in the synthesis of polyamides and various alkyd resins.[7]

Dodecanedioic Acid

Dodecanedioic acid is used in the production of nylon (nylon-6,12), polyamides, coatings, adhesives, greases, polyesters, dyestuffs, detergents, flame retardants, and fragrances.[41]

Diethyl Malonate

Diethyl malonate finds great utility as the starting material in Malonic Ester Synthesis, a classic organic chemistry reaction wherein a very wide variety of esters can be synthesized.[31]

Diisobutyl Adipate

Diisobutyl adipate is considered by FDA to be a Prior-Sanctioned Food Ingredients, Plasticizer.(21 CFR § 181.27).

Diethylhexyl Adipate

Diethylhexyl adipate is used as a plasticizer for polyvinyl chloride (PVC) plastics.[42]

Diethyl Sebacate

Diethyl sebacate was widely used in the U.S. as an aromatic in food before 1973.[39]

Dibutyl Sebacate

Dibutyl sebacate is a component of PVC.[43]

DICARBOXYLIC ACIDS AND THEIR SALTS

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Dicarboxylic acids are natural metabolic products of the ω-oxidation of monocarboxylic acids when the β-oxidation of free fatty acids is impaired.[44] Under normal physiological conditions, dicarboxylic acids are rapidly β-oxidized, resulting in very low cellular concentrations and practically non-detectable concentrations in the plasma.[45] Medium-chain dicarboxylic acids (up to 12 carbon atoms) are β-oxidized in mitochondria and peroxisomes. Oxidation of odd- and even-numbered chains proceeds to different end points. Odd-chain dicarboxylic acids are β-oxidized, giving acetyl Co-A and malonic acid (C3). Oxidation can then go no further, and malonic acid is the starter of fatty acid synthesis. Even-chain carboxylic acids are completely oxidized and produce succinyl-CoA, a gluconeogenic substrate, as an intermediate metabolite. Dicarboxylic acids are more polar than their esters, therefore they will diffuse less readily through normal cell membranes.[46]

Malonic Acid

Malonic acid can be activated to malonyl-CoA and undergoes decarboxylation to acetyl-CoA by various mammalian tissues.[14]

Adipic Acid

(The studies on adipic acid summarized in this section were performed between 1919 and 1960 and were described in a summary document.[47])

Non-Human

Adipic acid metabolism was studied using fasted male albino rats.[47] In one study, in which the rats were given a single oral dose, by gavage, with 50 mg radioactive adipic acid (labeled on C1 or C2), 70% of the radioactivity was exhaled as carbon dioxide in 24 h. Adipic acid and the metabolites urea, glutamic acid, lactic acid, β-ketoadipic acid, and citric acid, were recovered in the urine. Very little radioactivity was found in the tissues. Fasted male rats were also given a single dose of a solution containing 50 mg radioactive adipic acid (labeled on C1), by gavage, in conjunction with the intraperitoneal (i.p.) injection of 2 ml of 0.5 M sodium malonate. After 24 h, both radioactive adipic acid and succinic acid were found in the urine, which the researchers stated was an indication that adipic acid underwent β-oxidation. In a study in which rats were fed 25 mg radioactive adipic acid (labeled on C1) and 100 mg γ-phenyl-α-aminobutyric acid, followed by a 48-h urine collection, it was determined that acetate is a metabolite of adipic acid. Finally, rats were given radioactive sodium bicarbonate with non-radioactive adipic acid. Radioactive citric acid was formed, which suggested that carbon dioxide interacted with a metabolite of adipic acid. (Details not specified.)

Two rats were dosed orally by gavage with 2.43 g/kg partially neutralized adipic acid for 28 days. In the urine, 67% of the dose was recovered unchanged. There was no change in excretion pattern over time during the study.

Rabbits were dosed orally by gavage (n=4) or by intravenous (i.v.) administration (n=2) with 2.43 g/kg partially neutralized adipic acid for 2 days. Following oral administration, 53-61% of the dose was recovered unchanged in the urine. With i.v. administration, 59-71% was recovered unchanged in the urine. In another study using rabbits, animals were given a subcutaneous (s.c.) dose of 2000 mg adipic acid; 3 rabbits were given a single dose, one was dosed on days 1 and 5, and one was dosed on days 1, 5, 9, 13, and 15. On average, 61% of the dose was recovered unchanged in the urine. There was an increase in urinary oxalic acid concentrations.

A female dog was fed either 150 mg/kg bw adipic acid (in 2 feedings) for 5 days or 750 mg/kg bw (in 2 feedings) for 7 days. In the urine, 18% and 63.6% of the low and high doses, respectively, were recovered unchanged.

Rabbits (number not stated) were given up to 4 s.c. injections of ≤2000 mg sodium adipate.[48] An average of 61% of the dose was recovered unchanged in the urine. Oxalic acid was increased in the urine.

Human

In a study in which one subject was given 33 mg/kg bw sodium adipate, orally, for 5 days (10 g total), 6.76% of the dose was recovered in the urine. In another study in which one person was given 100 mg/kg bw adipic acid for 10 days (70 g total), 61% of the dose was recovered in the urine. Administration of 19.0 g adipic acid over 5 days or 23.4 g over 6 or 9 days (1 subject per dose) resulted in 53% of the administered dose recovered in the urine.

C9 to C12 Dicarboxylic Acids

Non-Human

Groups of 30 male Wistar rats were dosed orally, by gavage, with azelaic (C9), sebacic (C10), undecanedioic (C11), or dodecanedioic (C12) acid.[49] Ten rats in each group were dosed with 20, 50, or 100 mg of the respective acid. Blood, urine, and feces from the treated rats were analyzed and compared to the blank control obtained from untreated rats. (None of the C9-C12 acids were found in the blank controls.) In urine, approximately 2.5% of azelaic, 2.1% of sebacic, 1.8% of undecanedioic, and 1.6% of dodecanedioic acid was recovered after 5 days; the amount recovered was not affected by dosage. The dicarboxylic acids were not excreted in conjugated form. None of the C9-C12 dicarboxylic acids were recovered in the feces. In the plasma, dicarboxylic acid catabolites that were 2-, 4-, or 6-carbons shorter than the corresponding dicarboxylic acid were detected.

Human

Groups of 3 male and 2 female subjects were also dosed with azelaic, sebacic, undecanedioic, or dodecanedioic acid orally, in gelatin capsules, once a wk for 5 wks.[49] The dose administered increased each week, from 0.5 g at wk 1 to 5.0 g at wk 5. None of the C9-C12 acids were found in the blank control samples of blood, urine, and feces obtained from non-treated humans. In urine, approximately 60% of azelaic, 17% of sebacic, 5% of undecanedioic, and 0.1% of dodecanedioic acid was recovered after 12 h; the amount recovered was not affected by dosage. At 24 h, the amounts recovered were not much increased. Initially, undecanedioic and dodecanedioic acid administration raised the urinary pH to a value of 7.4-8.5; the pH returned to normal within 3-6 h. The dicarboxylic acids were not excreted in conjugated form. None of the C9-C12 dicarboxylic acids were recovered in the feces. In the plasma, dicarboxylic acid catabolites that were 2-, 4-, or 6-carbons shorter than the corresponding dicarboxylic acid were detected. Plasma levels of azelaic acid peaked at 2 h, while the levels of the other three acids peaked at 3 h. Recovery in the plasma was greatest for azelaic acid, 74.6 µg/ml with the 5 g dose, and the amount detected decreased with increasing chain length.

Azelaic Acid

Azelaic acid is a dietary constituent found in whole grain cereals and animal products.[50] It can be formed endogenously from longer-chain dicarboxylic acids, metabolism of oleic acid, and ψ-oxidation of monocarboxylic acids.[51] Endogenous plasma concentration and daily urinary excretion of azelaic acid are highly dependent on dietary intake. Azelaic acid crosses the blood-brain barrier.[52]

A group of 25 male Wistar rats were dosed orally, by gavage, with 100 µCi of [1,9-^{14}C]azelaic acid, and the animals were killed at various intervals 1-96 h after dosing.[49] After 12 and 48 h, 13 and 14.5% of the radioactivity was found in expired carbon dioxide, respectively. Approximately 40% of the radioactivity was recovered in the urine over 5 days. The C7 and C5 dicarboxylic acid metabolites were found in the urine up to 72 h after dosing. Very little was recovered in the feces. Labeled dicarboxylic acids were present in the blood for up to 72 h, and consisted mainly of dicarboxylic acid metabolites. Radioactivity was found in all tissues, with the highest levels present in the liver, lungs, and kidneys after 12 h. Tissue radioactivity levels then decreased slowly in all organs except adipose tissue, in which case increasing levels were still seen at 96 h. Approximately 90% of the radioactivity found in the tissues was present in the lipids, and it was essentially localized in the fatty acid portion of the triglycerides and of the phospholipids. Traces of C9, C5, and C7 dicarboxylic acids were detected in the first 24 h.

Sebacic Acid

Sebacic acid is oxidized to water and carbon dioxide, passing through acetyl-CoA and succinyl-CoA formation.[53]

Disodium Sebacate

Non-Human

Disodium sebacate, 80 and 160 mg with 25 µCi of (1,10) [^{14}C]sebacic acid tracer, was administered by a single i.v. injection to 14 male Wistar rats, and blood samples were obtained at various intervals 5-320 min after dosing.[53] The plasma half-life of radioactive disodium sebacate was 37.86 and 39.82 min for the 80 and 160 mg dose groups, respectively. The apparent volume of distribution was 2.65 ml/100 g body wt.

In a second experiment, a group of 4 male Wistar rats were given a single dose 160 mg disodium sebacate with 25 µCi sebacic acid tracer by i.v. injection, and expired carbon dioxide, urine, and feces were collected. The carbon dioxide half-life for radioactive sebacate was 93.64 min; 25% of the administered dose was expired in carbon dioxide. A total of 34.6% of sebacate was recovered in the urine in 24 h, while 5.08% suberic acid (C8) was recovered in the same time frame.

Most of the excretion occurred in the first 4 h. Radioactivity was not found in the feces.

In the third experiment, groups of 10 male Wistar rats were also given 160 mg disodium sebacate with 25 µCi sebacic acid tracer by i.v. injection, and the animals were sacrificed at various intervals from 30-360 min after dosing. The amount of radioactivity in various organs was analyzed. No appreciable radioactivity was found in the body. Sebacate appeared to be in an absorption phase in fat 1 h after dosing, but no radioactivity was found in the body after 24 h.

The pharmacokinetics of disodium sebacate was studied in male and female Wistar rats.[54] Sebacate was administered either i.p., 6 doses of 10-320 mg, or orally, 2 doses of 80 or 60 mg. Plasma concentrations of sebacate and urinary concentrations of sebacate and its products of β-oxidation (suberic and adipic acids) were measured using GLC/mass spectrometry. Both renal and non-renal elimination parameters were obtained. The sebacate half-life was 31.5 min. The tissue elimination rate was 0.0122 min^{-1}, and the overall volume of distribution was 26.817 ml/100 g. The renal clearance was 0.291 ml/min/100 g, which was much less than the value of the glomerular filtration rate (GFR) of approximately 1 ml/min/100g reported elsewhere, suggesting the presence of sebacate reabsorption from the ultrafiltrate. Sebacate renal clearance was found to be a concentration-independent function, suggesting the presence of a passive back-diffusion. The relative bioavailability of the oral route compared to the i.p. route was 69.09%, showing an extensive absorption of the compound.

Human

The metabolism and excretion of disodium sebacate was studied in 7 fasting male subjects that were given a continuous steady infusion of 20 g unlabeled disodium sebacate over 480 min.[55] At 240 min into the infusion, (1,10)[C^{14}]sebacic acid was infused simultaneously as a tracer (sp. act. 0.416 µCi/min). The was a gradual increase in the amount of radioactivity expired in carbon dioxide for the first 300 min; the value remained elevated for an additional 120 min before declining. At 24 h, 11.38 mmol sebacate was recovered in the urine, as well as 2.04 mmol suberic acid and 1.11 mmol adipic acid, which was less than 15% of the dose administered. The serum concentration of unlabeled sebacate reached a plateau after 270 min of infusion. Ten to 15% of serum radioactivity was found in the aq. fraction of serum extracts. The renal clearance rate was 5.67 ml/min. The overall tissue uptake of unlabeled sebacate was 180 µmol/min, and the apparent distribution volume was 12.46 l. The percent oxidation of sebacate was 6.14%.

The pharmacokinetic profile of disodium sebacate during a short-time infusion (5 h at 10 g/h) was also studied in 7 male subjects.[56] Sebacate in serum and urine was measured by HPLC. The apparent volume of distribution of sebacate was 8.39 l, and the plasma fractional removal rate constant was 0.0086 min^{-1}.

Six male subjects were given a single i.v. bolus of 1 g disodium sebacate, while another 6 received 10 g of sebacate in 500 ml of distilled water, i.v., at a rate of 3.33 g/h over 3 h.[57] For the group given a bolus dose, the distribution phase had a short half-life, 0.34 h, and a rapid elimination, 2.045 h^{-1}. For the group given the 3 h infusion, 12% of the dose was excreted as sebacic acid in 24 h; suberic acid (C8) and adipic acid were also present in the urine.

Dodecanedioic Acid

A group of 25 male Wistar rats were dosed orally, by gavage, with 100 µCi of [10,11-^{3}H]dodecanedioic acid, and the animals were killed at various intervals 1-96 h after dosing.[49] Approximately 50% of the radioactivity was recovered in the urine over 5 days. The C10, C8, and C6 dicarboxylic acid metabolites were found up to 72 h after dosing. Only 2% of the radioactivity was recovered in the feces. Labeled dicarboxylic acids were present in the blood for up to 72 h, and consisted mainly of dicarboxylic acid metabolites. Radioactivity was found in all tissues, with the highest levels present in the liver, lungs, and kidneys after 24 h. Tissue radioactivity levels then decreased slowly in all organs except adipose tissue, in

which case an increase in radioactivity was still seen at 96 h. Radioactivity levels were 20-40% lower in the lipid extracts of the tissues than in the residual matter. ^{3}H was distributed in the whole molecule, not only the fatty acid portion, of the phospholipid and triglyceride fractions. Traces of C12, C10, C8, and C6 dicarboxylic acids were detected in the first 24 h.

Male Wistar rats were given an i.v. bolus of 800 µmol/kg disodium dodecanedioic acid.[58] The apparent volume of distribution was 0.248 l/kg, and the plasma half-life was 12.47 min. The renal clearance was 0.00051 l/kg/min, while systemic clearance was 0.0138 l/kg/min. Only 3-5% of the dose was recovered in the urine.

Percutaneous Absorption

Azelaic Acid

Vehicle affects the absorption of azelaic acid.[46] After a 12 h period, absorption from a 15% azelaic gel was 8%, while absorption from a water-soluble polyethylene glycol ointment base was only 3%. (Species and details not given.)

The *in vitro* percutaneous absorption of a 15% azelaic acid gel through human skin, prior to or after the application of three different moisturizer formulations, was determined.[59] All doses were applied as 5 µl/cm^2. The second dose was applied 15 min after the first. [^{14}C]Azelaic acid had a finite dose absorption profile, with a rise to peak penetration followed by a slow but steady decline. *In vitro*, 70% of the azelaic acid diffused into the reservoir solution over 48 h. The application of a moisturizer, and whether it was applied prior to or following azelaic acid administration, did not have a statistically significant effect on the penetration of azelaic acid. However, there was a trend toward greater percutaneous penetration and mass distribution with the application of a moisturizer lotion prior to the azelaic acid gel.

The percutaneous absorption of azelaic acid was determined using 6 male subjects. A total of 5 g of a cream containing 20% azelaic acid was applied to the face (1 g), chest (2 g) and upper back (2 g) of each subject, giving an area dose of approx 5 mg cream/cm^2 skin. The test areas were covered 1 h after dosing with cotton tissues, and washed 24 h after dosing. After 1 wk, 100 ml of an aq. microcrystalline suspension containing 1 g azelaic acid was given orally to each subject. Urinary excretion of unchanged azelaic acid was measured after each dose. Following dermal application, 1.29% of the dose was recovered unchanged in the urine in 24 h, and a total of 2.2% was recovered by day 3. Following oral administration, 61.2% of the dose was recovered within 4 h; excretion was complete at this point. Assuming similar rates and pathways in biotransformation following both routes of exposure, percutaneous absorption of azelaic acid was determined to be 3.6% of the dermally applied dose.[60]

Peroxisome Proliferation

Adipic Acid

The effect of adipic acid on hepatic peroxisome proliferation was evaluated in an *in vivo* study in which 4 male F344 rats were fed chow containing 2% adipic acid dissolved in alcohol.[61] After 3 wks of dosing, the animals were killed. Adipic acid did not induce peroxisome proliferation and did not affect relative liver to body weights.

Cellular Effects

Dicarboxylic acids have a cytotoxic effect on the abnormally hyperactive and malignant epidermal melanocytes. Dicarboxylic acids, C8to C13, have been shown to inhibit mitochondrial oxidoreductases,[62] and they have been shown to reversibly inhibit microsomal NADPH and cytochrome P450 reductase.[63] Medium chain length dicarboxylic acids are also competitive inhibitors of tyrosinase *in vitro*.

Adipic Acid

The effect of adipic acid on primary keratinocyte cultures was evaluated using epidermal cells from neonatal NMRI mice.[64] Concentrations of ≤30 mM did not inhibit ^{3}H-thymidine incorporation or affect DNA synthesis, while 40 and 50 mM

inhibited both of these parameters. No effect on labeling indices was observed with 1-30 mM adipic acid.

Azelaic Acid

Azelaic acid, a naturally occurring competitive inhibitor of tyrosinase, has a cytotoxic effect on malignant melanocytes .[65] Azelaic acid is also a competitive inhibitor of a number of oxidoreductive enzymes, enzymes involved in DNA synthesis, and of oxidoreductases of the respiratory chain.[66] It has been reported that, *in vitro*, azelaic acid has time- and dose-dependent, reversible, and anti-proliferative and cytotoxic effects on a number of tumoral cell lines. Azelaic acid had no effect on normal cell lines.

Disodium Azelate

Disodium azelate inhibited cell proliferation and affected viability of Cloudman and Harding-Passey murine melanomata at concentrations $\geq 10^{-2}$ M when incubated over a 3 day period.[62] The mitochondria were the prime target of action.

The effect of disodium azelate on primary keratinocyte cultures was evaluated using epidermal cells from neonatal NMRI mice.[64] A dose-dependent inhibition of ^{3}H-thymidine incorporation into DNA, ranging from 50% inhibition with 20 mM to 90% inhibition with 50 mM disodium azelate, was observed following a 12 h incubation period. Concentrations of 1 and 10 mM did not affect DNA synthesis, but a marked reduction was seen with 20-50 mM. The effects on DNA synthesis were time-dependent, with the maximum inhibitory effect observed at 4 h; this effect was reversible. RNA and protein synthesis were also inhibited during the first 4 h of incubation with 50 mM disodium azelate. Cellular structure was altered upon incubation with disodium azelate, primarily affecting mitochondria, and the rough endoplasmic reticulum. These effects were also reversible.

Dodecanedioic Acid

The disodium salt of dodecanedioic acid inhibited cell proliferation and affected viability of Cloudman and Harding-Passey murine melanomata at concentrations $\geq 10^{-2}$ M, when incubated over a 3 day period.[62] The mitochondria were the prime target of action.

ANIMAL TOXICOLOGY

Acute Toxicity

The acute oral, dermal, inhalation and parenteral toxicity of the dicarboxylic acids and some of the salts are summarized in Table 4.[47,67-72] The oral LD_{50} values of the dicarboxylic acids had a wide range, for example, adipic acid had values for rats ranging from 940 mg/kg to greater than the highest dose tested (11,000 mg/kg). Most reported values for the acids were >200 mg/kg. The reported dermal LD_{50} values ranged from >6000 mg/kg dodecanedioic acid to >10,000 mg/kg glutaric acid.

Short-Term Oral Toxicity

Adipic Acid

Groups of 6 male Sprague-Dawley rats were dosed orally (method not specified) with 3600-5600 mg/kg bw adipic acid as an 18.6-24.9% solution in saline for 14 days.[47] Three animals of the 3600 mg/kg bw group, 5 of the 4000 mg/kg bw group, and all of the 4500-5600 mg/kg bw groups died prior to study termination. Signs of toxicity included depressed activity, labored respiration, ataxia, and convulsions. No gross findings were noted at necropsy at study termination.

Groups of 5 rats were dosed with 0 or 3000 mg/kg bw of a neutralized 20% adipic acid solution orally, by gavage, for 4 wks. A non-significant decrease in body weight gain was observed. In a 4 wk study in which a group of 3 rats was dosed orally, by gavage, with 2400 mg/kg bw adipic acid, no significant toxicological effects were noted.

In a 4-wk dietary study in which groups of 17-20 female rats were fed 0-40 mg/day (0-435 mg/kg bw/day) adipic

acid, no effects were reported. The no-observable adverse effect level (NOAEL) was >435 mg/kg bw/day. In a 5-wk dietary study in which groups of 15-18 male rats were fed 0-800 mg/day (0-13,333 mg/kg bw bw/day) decreased body weight gains, an unkempt appearance, and diarrhea were observed for the animals fed 800 mg/day the first 3 wks. In another 5-wk dietary study in which groups of 4 rats, gender not specified, were fed 100 or 200 mg/day (310-922 mg/kg bw/day) of a 20% adipic acid solution in ethanol, 5 days/wk, no signs of toxicity were observed.

Ten rats were dosed orally, method not specified, with 199 mg/day (638-1332 mg/kg bw/day) sodium adipate, 5 days/wk for 9 wks. No toxicological effects were observed.

A group of 5 guinea pigs, gender not specified, were dosed orally using capsules with 400 mg/day (682-942 mg/kg bw/day) adipic acid for 5 days, followed by dosing with 600 mg/day (1032-1739 mg/kg bw/day), 5 days/wk for 5 wks. No signs of toxicity were observed.

No toxicity was observed in a study in which pigs were fed 1% adipic acid in the diet for 7 days.

Short-Term Inhalation Toxicity

Adipic Acid

Mice, gender and number per group not specified, were exposed to 460 mg/m^3 adipic acid dust for 1.5 mos.[47] (Details of exposure were not specified.) Decreased weight gain, altered oxidase activity, and upper respiratory tract, liver, kidney, and central nervous system effects were observed. (Details were not given.)

Two male and 2 female rats were exposed to 126 mg/m^3 adipic acid dust for 15 days, 6 h/day. No signs of toxicity were observed, and no gross or microscopic findings were noted at necropsy.

Subchronic Oral Toxicity

Sodium Succinate

The oral toxicity of sodium succinate was evaluated using F344 rats.[71] Groups of 10 males and 10 females were given 0, 0.3, 0.6, 1.25, 2.5, 5 or 10% sodium succinate in the drinking water for 13 wks. All animals were killed at the termination of dosing. Body weight gains of animals of the 10% group were significantly decreased, and all animals of this group died by wk 4. These animals were extremely emaciated; however, no compound-related microscopic lesions were found. Body weight gains were decreased in animals given ≥2.5% sodium succinate, as compared to controls. No toxicological treatment-related effects were observed.

Glutaric Acid

Groups of 15 male and 15 female Sprague Dawley rats were fed a diet containing 0-2% glutaric acid for 90-days.[68] Body weight gains were decreased for males and statistically significantly decreased for females of the 2% group. No differences were noted between test and control animals in hematology, clinical chemistry, or urinalysis. There were no microscopic findings or organ weight changes attributable to the test substance. There was no treatment-related mortality. The NOAEL was ≥1%, and the LOAEL was 2% glutaric acid.

Four male and 4 female Beagle dogs were fed a diet containing 0-5% glutaric acid for 90 days. Decreased body weights, accompanied by reduced feed consumption, were observed for the males and females of the 5% group and females of the 3% group. No other treatment-related effects were observed. The NOAEL was ≥2% and the LOAEL was 3%.

Adipic Acid

Groups of 8-10 male rats were given 0, 420, 840, 1700, or 3400 mg/kg bw/day sodium adipate for 19 wks in a protein deficient diet.[70] Animals were killed after either 7 wks or at study termination. For unexplained reasons, only 5-7 animals/group survived until study termination. Rats of the 3400 mg/kg bw/day group had decreased body weight gains and

decreased body weights. (Statistical significance not stated.) Slight effects were seen in the liver, and the NOAEL was 3333 mg/kg bw.

Adipic/Glutaric/Succinic Acid Mixture

Groups of 15 male and 15 female rats were dosed orally, by gavage, for 90 days with 0-30% of a mixture that contained 4% adipic, 16% glutaric, and 5% succinic acid.[68] The vehicle was deionized water, and the dosing volume was 10 ml/kg. Two males and 1 female of the 30% group died, and the deaths were considered dose-related. Also in this group, body weights were reduced for males and females, and feed consumption was statistically significantly reduced in males. An increased incidence of labored breathing and rales was noted. The urine pH was statistically significantly reduced in both males and females dosed with 30% of the mixture. In the 10% group, body weight gains were slightly, but not statistically significantly, reduced in females and feed consumption was statistically significantly reduced in males. The NOAEL was 3% and the LOAEL was 10%.

Subchronic Inhalation Toxicity

Adipic Acid

Mice, gender and number per group not specified, were exposed to 13 or 129 mg/m^3 adipic acid for 4 mos.[47] (Details of exposure were not specified.) Decreased weight gain, altered oxidase activity, and upper respiratory tract, liver, kidney, and central nervous system effects were observed.

Chronic Oral Toxicity

Adipic Acid

Groups of 13-15 male and female rats were fed a diet containing 0, 1600, or 3200 mg/kg bw/day adipic acid for 33 wks.[47] Rats were killed at various intervals throughout the study. Ten of 14 rats fed 3200 mg/kg bw/day died during wks 0-4; surviving rats had decreased weight gains during this time. However, at study termination, body weights were for surviving animals of this group were similar to controls. Slight effects were seen in the liver. (Statistical significance not stated.)

In a 2-yr study, groups of 20 male rats were fed a diet containing 0, 0.1, 1, 3, and 5% adipic acid (equiv. to 0, 75, 750, 2250, and 3750 mg/kg bw/day), and groups of 10 and 19 females were fed 0 and 1% adipic acid, respectively. Weight gains of male rats fed 3 and 5% adipic acid were significantly less than controls. There were no significant toxicological findings upon gross or microscopic observation. The NOAEL was 1% adipic acid for male and female rats.

Azelaic Acid

Groups of 15 male and 15 female Wistar rats were fed a diet containing 140 or 280 mg/kg bw azelaic acid for 180 days, and a control group of 10 males and 10 females was given untreated feed.[72] No significant toxicological effects were observed. Growth was similar between test and control groups, as were the microscopic examinations and clinical chemistry parameters. The researchers found similar, negative, results when groups of 10 male and 10 female New Zealand rabbits were fed diets containing 0, 200, or 400 mg/kg bw azelaic acid for 180 days.

Disodium Sebacate

Groups of 10 male and 10 female Wistar rats were fed a diet containing 0, 500, or 1000 mg/kg bw disodium sebacate for 6 mos, after which time they were killed and necropsied.[67] Growth was similar between test and control groups, as were the microscopic examinations and clinical chemistry parameters. The researchers found similar, negative, results when groups of 10 male and 10 female New Zealand rabbits were fed diets containing 0, 750, or 1000 mg/kg bw disodium sebacate for 6 mos.

Ocular Irritation

Ocular irritation studies are summarized in Table 5.

Succinic Acid

The ocular irritation potential of succinic acid was evaluated using albino rabbits.[68] Undiluted test material, 0.005 ml, was applied to the center of the cornea. The eyes were not rinsed. Succinic acid was a severe eye irritant, with necrosis visible upon staining. The score for ocular irritation, on a scale of 1-10, was 8.

Glutaric Acid

A Draize ocular irritation study was performed in which 100 mg of glutaric acid was instilled in the eyes of 3 rabbits and the eyes were rinsed 24 h after application.[68] Glutaric acid was irritating to rabbit eyes, with a primary irritation index (PII) of 35.2/110. Mild erythema, slight edema, and slight dullness were still present after 7 days.

Adipic Acid

The ocular irritation of adipic acid was evaluated using groups of 2 albino rabbits.[68] Ten or 57.1 mg of adipic acid was placed in the eye of each rabbit, and the eye of 1 animal in each group was rinsed. With 10 mg followed by rinsing, mild conjunctival irritation was observed; and the eye was normal within 3 days. In the unrinsed eye, mild conjunctival irritation and a minimal iritic effect were observed; minimal conjunctival irritation was still observed after 7 days and the eye was normal after 14 days. With instillation of 57.1 mg adipic acid followed by rinsing, moderate to mild conjunctival irritation and transient mild opacity were observed; the eye was normal in 3 days. In the unrinsed eye, moderate to mild conjunctival irritation, mild opacity of the cornea, and a minimal iritic effect were observed; the eye was normal at day 7. However, other studies have reported that adipic acid produced severe irritation in rabbit eyes, and the signs of irritation were still present after 8 days.[47]

Adipic/Glutaric/Succinic Acid Mixture

The ocular irritation potential of a mixture of adipic, glutaric, and succinic acid, percentages not specified, was evaluated using 2 male albino rabbits.[68] One-tenth ml of the test substance was instilled in the conjunctival sac of each animal, and the eye of one animal, but not the other, was rinsed. The contralateral eye served as the negative control. Mild to severe conjunctivitis was observed in on both the rinsed and unrinsed rabbit eyes. Both eyes were normal within 21 days.

Dodecanedioic Acid

In studies using rabbits that evaluated the ocular irritation of dodecanedioic acid, slight irritation was reported in one study, with a PII of 11.96/110, and small areas of corneal opacity and mild conjunctival irritation were seen in the other study.[69] Details were not provided.

Dermal Irritation/Sensitization

Dermal irritation and sensitization studies are summarized in Table 6.

Succinic Acid

Succinic acid was a slight irritant to rabbit skin.[68] Details were not provided.

Glutaric Acid

The dermal irritation potential of glutaric acid was determined using 2 male and 4 female New Zealand white rabbits.[68] A 0.5 g aliquot of glutaric acid was applied to the clipped skin on the back of the rabbits. The test site was scored for irritation after 3 min, and the site was then washed. The test material was then applied to two other test sites, which were covered with a rubber wrap. The sites were examined at 1 and 4 h, and the site was washed after both examinations. The sites were then evaluated at 24 and 48 h after application. Slight erythema was seen in one rabbit throughout the study.

Irritation was not observed in the other rabbits.

Adipic Acid

A dermal irritation study was performed in which 500 mg of 50% aq. adipic acid was applied under an occlusive patch to a 5 cm x 5 cm area of intact and abraded skin of 6 rabbits for 24 h.[47] With intact skin, an erythema score of 2-3/4 was reported, with clearing by day 3. With abraded skin, mild to severe erythema and edema were reported, which cleared by day 7.

Adipic acid, undiluted or as an 80% aq. paste, was applied occlusively to the backs of ears of rabbits for 24 h. Two rabbits were used per group. No irritation was observed on the backs of animals. Erythema was observed on the ear, with clearing by 72 h. In another study in which adipic acid was applied occlusively for 24 h, irritation was not observed. Details were not provided.

A semi-occlusive application of 500 mg of a paste of 50% adipic acid in propylene glycol to 6 rabbits produced slight to mild irritation in 3 of the rabbits. A semi-occlusive application of undiluted adipic acid was not corrosive. Adipic acid, 50% in propylene glycol, was not irritating to a group of 10 guinea pigs.

The sensitization potential of adipic acid was evaluated using groups of 10 guinea pigs. For induction, 0.1 ml of 1% aq. adipic acid was given as a sacral intradermal injection, once a week for 4 wks. After a 2-wk non-treatment period, the dermal challenge was performed with 0.05 ml of 50 and 25% adipic acid in propylene glycol. Adipic acid produced very mild or no irritation and it was not a sensitizer.

Adipic/Glutaric/Succinic Acid Mixture

A mixture of adipic, glutaric, and succinic acid (percentages not specified) was evaluated for irritation and for sensitization using groups of 10 male guinea pigs.[68] The primary irritation potential was evaluated by applying 0.05 ml of an 8 or 80% suspension in dimethyl phthalate to the shaved, intact skin on the shoulder of the animals. The sensitization potential was also evaluated, using 4 sacral intradermal injections of 0.1 ml of a 1% suspension for induction. After a 13-day non-treatment period, a dermal challenge was performed with 0.05 ml of an 8% and 80% suspension of the mixture. Ten previously untreated guinea pigs were exposed to the same challenge applications as the test animals. In the test for primary irritation, the 8% suspension produced no irritation, and no to mild irritation was observed 24 h after exposure to the 80% suspension. No sensitization was observed at either dose.

Dodecanedioic Acid

Dodecanedioic acid was not an irritant to rabbit skin in a 4-h exposure study or upon application of 0.5 g.[69] In a maximization study using female guinea pigs, 0.5% dodecanedioic acid was injected intracutaneously at induction and 25 and 50% was used for the dermal challenge. Dodecanedioic acid was not a sensitizer.

Mucosal Irritation

Succinic Acid

Succinic acid has been considered to be an exacerbating factor in ulcerative colitis, therefore its influence on rat colonic mucosa in terms of mucosal blood flow and superoxide generation was investigated.[73] The left side of the colon of 5 male and 5 female rats was exposed, and 0.9-5% succinic acid in physiological saline was instilled into the colonic lumen. A segment of the colon was then ligated as to not include the mesenteric blood vessel. Mucosal blood flow decreased with all dose levels. Microscopically, the higher the concentration of succinic acid, the greater was the erosion formation in the colonic mucosa. Significant polymorphonuclear cell infiltration superoxide generation from colon tissue was observed with 0.01% succinic acid, as compared to higher or lower concentrations. Succinic acid, at fecal concentrations found in active stage

ulcerative colitis, appears to be implicated in mucosal injury, mediated by a decrease in colonic mucosal blood flow and infiltration of superoxide-generating polymorphonuclear cells into the mucosa.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Malonic Acid

Malonic acid, 0.1%, reduced the pH of sperm suspensions from 7.5 to 4.5-5.5 and it rendered human spermatozoa immotile within 30 min.[74] A concentration of 1.0% reduced the pH to 1.5-3.0 and was almost instantaneously spermicidal.

Succinic Acid

Thirty ovariectomized female rats were given daily subcutaneous injections of 5.0 mg/day succinic acid for 3 wks.[68] Ten females were used as controls. Daily vaginal smears were similar for test and control animals. Microscopically, no changes were seen in the uterine horn, cervix, or vagina of the animals.

Glutaric Acid

The reproductive toxicity of glutaric acid was evaluated using groups of 25 female rats.[68] The animals were dosed orally, by gavage, with 0, 125, 400, or 1300 mg/kg glutaric acid on days 6-15 of gestation, and the animals were killed on day 20 of gestation. No toxicological or reproductive effects were observed for the 125 mg/kg group. In the 400 mg/kg group, salivation, rales, and nasal discharge were observed. One dam of the 1300 mg/kg group died on day 10 of gestation, and one was killed due to moribund condition on day 13 of gestation. Mean body weight gains were decreased in the 1300 mg/kg group during dosing, but body weight gains in this group were normal post-dosing. Clinical signs of toxicity in the 1300 mg/kg group included salivation, rales, nasal discharge, and staining around the mouth, nares, and anogenital area. No adverse effects on pregnancy and no teratogenic effects were reported at any of the dose levels. There was a significant increase in resorptions in the 1300 mg/kg group compared to controls, but the value was within normal expected limits and, therefore, not considered biologically meaningful.

Groups of 18 gravid female New Zealand white rabbits were dosed orally, by gavage, on days 6-18 of gestation with 0, 50, 160, or 500 mg/kg glutaric acid, and the animals were killed on day 29 of gestation.[68] No test-article related mortality occurred. There were no clinical signs of toxicity, and body weights were not affected. No embryotoxic, teratogenic, or adverse reproductive effects were reported.

Adipic Acid

Groups of 20-24 gravid albino CD-1 mice were dosed orally, by gavage, with 0, 2.6, 12, 56, or 263 mg/kg bw adipic acid on days 6-15 of gestation.[47] All animals were killed on day 17 of gestation. No reproductive, developmental, or maternal effects were observed, and the NOAEL for maternal and developmental toxicity was 263 mg/kg bw. Similar results were obtained in a study in which gravid Wistar rats were dosed orally, by gavage, with 0, 2.9, 13, 62, or 288 mg/kg bw adipic acid on days 6-15 of gestation. The NOAEL for maternal and developmental toxicity was 288 mg/kg bw.

Groups of 21-24 gravid hamsters were dosed orally, by gavage, with 0, 2.9, 5, 44, or 205 mg/kg bw adipic acid on days 6-10 of gestation. A significant increase in resorption per implant site was observed with 205 mg/kg bw adipic acid, resulting in a decreased number of live fetuses. (This decrease was not evaluated statistically.) No other effects were reported.

Groups of 10-14 gravid Dutch-belted rabbits were dosed by oral intubation with 0, 2.5, 12, 54, or 250 mg/kg bw adipic acid on days 6-18 of gestation. No reproductive, developmental, or maternal effects were observed. The NOAEL for maternal toxicity was ≥250 mg/kg bw and for developmental toxicity was 250 mg/kg bw.

Azelaic Acid

Reproductive and teratogenic effects of azelaic acid were evaluated using Wistar rats and New Zealand rabbits.[72] A group of 20 gravid rats was fed a diet containing 140 mg/kg bw/day azelaic acid, and a control group of 10 gravid rats was given untreated feed. Half of each group was killed and necropsied on day 19 of gestation, and the remaining animals continued dosing for 3 mos. The day of gestation that dosing started is not clear. No gross or microscopic lesions were observed for the uteri, placentas, or ovaries. There were no differences in reproductive, teratogenic, or developmental effects between treated and control groups, nor were there any differences in fetal weights of the live fetuses. Similar results were seen using groups of 20 gravid rabbits fed 200 mg/kg bw/day azelaic acid; 10 untreated gravid rabbits were used as a negative control group.

Embryotoxic effects were observed in oral studies with rats receiving 2500 mg/kg bw/day of azelaic acid.[51] Similar effects were observed in studies in rabbits given 150 to 500 mg/kg bw/day and in monkeys given 500 mg/kg bw/day. The doses at which these effects were noted were all within toxic dose ranges for the dams. No teratogenic effects were observed. (Details were not provided.)

Disodium Sebacate

Reproductive, teratogenic, and developmental effects of disodium sebacate were evaluated using Wistar rats and New Zealand rabbits.[72] Groups of 20 gravid rats were fed a diet containing 0 or 500 mg/kg bw/day disodium sebacate, and groups of 20 gravid rabbits were fed 0 or 1000 mg/kg bw. Half of each group was killed and necropsied on day 19 of gestation, and the remaining animals continued dosing for 3 mos. The day of gestation that dosing started is not clear. No gross or microscopic lesions were observed for the uteri, placentas, or ovaries. There were no differences in reproductive or developmental effects between treated and control groups, nor were there any differences in fetal weights of the live fetuses.

Dodecanedioic Acid

The reproductive toxicity of 0-1000 mg/kg bw dodecanedioic acid was evaluated in an OECD combined repeated doe and reproductive/developmental toxicity screening test using male and female Crl:CD:BR rats.[69] The no-observable effect level (NOEL) for reproductive and developmental toxicity was 1000 mg/kg bw.

Sodium Salt of Adipic, Azelaic, Sebacic, and Dodecanedioic Acids

The influence of the sodium salt of some dicarboxylic acids (adipic acid, azelaic acid, sebacic acid, dodecanedioic acid) on both spontaneous and evoked muscle activity of the uterine horns of 35 female Wistar rats (250-300g) has been studied in vitro.[75] Spontaneous activity of uterine muscle was inhibited by dicarboxylic salts causing the total abolition of mechanical events at concentrations of 24, 32, 40, and 64 x 10^{-3} M. Dicarboxylic salts antagonized the maximal isometric contraction of the uterine horn induced by administration of acetylcholine, oxytocin or prostaglandins (PGF_2-α). The amount of antagonism was dependent upon the concentration of dicarboxylic salt used. Dicarboxylic salts had an specific inhibitory effect on the uterine horn which progressively increased with their chain length. The results suggested that the inhibitory effects of dicarboxylic salts on smooth muscle could be due to a cellular membrane hyperpolarization.

GENOTOXICITY

Available genotoxicity studies are summarized in Table 7.

In Vitro

Malonic Acid

Malonic acid, 3333 µg/plate, was not mutagenic in a National Toxicology Program (NTP) preincubation assay, with or without metabolic activation.[76]

Succinic Acid

The genotoxic potential of succinic acid was evaluated in an Ames test and in a chromosomal aberration study using a Chinese hamster fibroblast cell line.[77] Succinic acid, at a concentration of ≤5.0 mg/plate in phosphate buffer, was not mutagenic in the Ames test. (Whether metabolic activation was used is not stated.) Concentrations of ≤1.0 mg/ml in saline were not genotoxic in the chromosomal aberration assay.

Disodium Succinate

The genotoxic potential of disodium succinate was evaluated in an Ames test and in a chromosomal aberration study using a Chinese hamster fibroblast cell line.[77] In the Ames test, disodium succinate was not mutagenic at concentration up to 5.0 mg/plate in phosphate buffer. (Whether metabolic activation was used is not stated.) Equivocal genotoxic results were obtained in the chromosome aberration assay of ≤15.0 mg/ml disodium succinate in saline using Chinese hamster fibroblast cells.

Disodium succinate, ≤10 mg/plate, was negative in another Ames test, with and without metabolic activation.[78]

Glutaric Acid

Glutaric acid was evaluated *in vitro* in a standard Ames assay, the L5178Y/TK ± mouse lymphoma assay with and without metabolic activation, and the mammalian *in vitro* Balb/c-3T3 cell transformation assay with and without metabolic activation.[79] The Ames tests were negative. However, the cell transformation assay was positive both in the presence and absence of metabolic activation and the results in the mouse lymphoma assay were dependent upon pH of the culture medium. The researchers stated that the variable response in the mouse lymphoma assay and the positive effect in the cell transformation assay may have been an indirect effect of other factors (such as the pH or osmolarity of the media in which the cells were exposed), rather than a direct effect of glutaric acid.

Adipic Acid

Adipic acid was evaluated in a number of Ames assays using *Salmonella typhimurium* and *Escherichia coli*; results were negative, with or without metabolic activation, at concentrations as high as 10,000 mg/plate.[47,80,81] Negative results were also obtained in an Ames test with 0-200 mg/l adipic acid using *S. typhimurium* TA1530 and G-46 without metabolic activation[47]. Results were negative in a yeast gene mutation assay using *Saccharomyces cerevisiae* without metabolic activation at concentrations ≤200 mg/l. A mouse lymphoma assay using L5178Y/TK ± cells was negative with and without metabolic activation at concentrations of ≤2000 µg/plate,[81] as was a cytogenetic assay using human embryonic lung fibroblast cells with ≤200 mg/l adipic acid.[47] In a viral enhanced cell transformation assay using Syrian hamster embryo cells at doses of 62-1000 µg/ml adipic acid, results were negative.

Adipic/Glutaric/Succinic Acid Mixture

A mixture of adipic, glutaric, and succinic acid, percentages not specified, tested as a 50% aq. solution, was not mutagenic in an Ames assay using *S. typhimurium*, with or without metabolic activation, at concentrations of ≤300 µg/plate.[68] Negative results were also obtained in an unscheduled DNA synthesis assay at concentrations of ≤5000 µg/plate using rat hepatocytes and in an HGPRT assay at concentrations of ≤2500 µg/plate, without, and of ≤3500 µg/plate, with, metabolic activation. In an *in* vitro transformation assay using Chinese hamster ovary (CHO) cells at concentrations of ≤1500 µg/ml without and ≤2500 µg/ml with metabolic activation, positive results were obtained with, but not without, metabolic activation at 2000 µg/plate.

Azelaic Acid

Azelaic acid, 20%, was not mutagenic or genotoxic in an Ames assay, HGPRT test in CHO cells, or human

lymphocyte test.[51] Details were not provided.

Dodecanedioic Acid

Dodecanedioic acid was not mutagenic in an Ames assay at concentrations of ≤5000 µg/plate, with and without metabolic activation.[69] Toxicity occurred at ≥500 µg/plate.

In Vivo

Glutaric Acid

Glutaric acid was evaluated in a mammalian micronucleus cytogenetic assay in mice.[79] Glutaric acid was not genotoxic in this assay. (Details not specified.)

Adipic Acid

Adipic acid was not genotoxic in *in vivo* cytogenetic assays using chromosomes from rats dosed orally, by gavage, with a single dose of 5000 mg/kg bw or daily for 5 days with 2500 mg/kg bw.[47] Adipic acid was also not genotoxic in dominant lethal studies with doses up to 5000 mg/kg bw.

Adipic/Glutaric/Succinic Acid Mixture

A mixture of adipic, glutaric, and succinic acid, percentages to specified, was not genotoxic *in vivo* using male and female Sprague Dawley rats dosed orally by gavage with 2750 and 1375 mg/kg of the mixture, respectively.[68]

Azelaic Acid

Azelaic acid was not genotoxic in a dominant lethal assay in mice.[51] (Details not specified.)

Dodecanedioic Acid

Dodecanedioic acid, ≤5000 mg/kg bw, was not mutagenic in a micronucleus assay using mice.[69]

CARCINOGENICITY

Sodium Succinate

Groups of 50 male and 50 female F344 rats were given drinking water containing 0, 1, or 2% sodium succinate for 2 yrs, and the carcinogenic potential was determined.[71] Dosing was discontinued after 104 wks, and, after a 9-wk recovery period, the rats were killed. Body weights of the high dose animals were decreased by 10% as compared to controls. There were no statistically significant differences in overall tumor incidence or mean survival time between treated and control animals. An increase in the incidence of C-cell adenoma/carcinoma of the thyroid in females of the 2% group, and a positive trend in the occurrence of this tumor, was considered a function of experimental variability and not related to dosing. Sodium succinate was not toxic or carcinogenic to male or female F344 rats when given in the drinking water for 2 yrs.

Adipic Acid

Adipic acid was not carcinogenic in the 2-yr chronic oral toxicity study (described previously) in which groups of 20 male rats were fed diets containing 0, 0.1, 1, 3, and 5% adipic acid, and groups of 10 and 19 females were fed 0 and 1% adipic acid, respectively.[70]

Tumor Promotion

Succinic Acid, Sodium Succinate, Disodium Succinate

The promotion of urinary bladder carcinogenesis by sodium succinate was evaluated using male F344 rats.[82] Groups of 16 male F344 rats were given 5% succinic acid, sodium succinate, or disodium succinate with 0.05% *N*-butyl-*N*- (4-hydroxybutyl)nitrosamine (BBN) in the drinking water for 4 wks, followed by dietary administration of 5% of the respective test article without BBN for 32 wks. Negative controls were given water with BBN only and untreated feed. Groups of 8 male F344 rats followed the same protocol without the addition of BBN to the drinking water, as did a group of non-BBN-

treated negative controls. The animals were killed at wk 37.

In the BBN-pretreated groups, many rats given sodium or disodium succinate developed hematuria towards the end of the study. There were no statistically significant differences in body or organ weights between the control and test groups. (Information on organ and body weights was not provided for the non-BBN groups.) Large tumors were found on the urinary bladders of the BBN-pretreated animals given sodium and disodium succinate; tiny lesions were found in the control or succinic acid BBN-pretreated animals. The incidence and number of urinary bladder carcinomas and papillomas and of papillary or nodular hyperplasia (preneoplastic lesions) were statistically significantly increased in the sodium and disodium succinate BBN-pretreated groups as compared to the succinic acid and control BBN-pretreated groups. The incidence and numbers observed in the sodium and disodium succinate groups were not statistically significantly different from each other. An association between tumor area and sodium intake was noted. Urinary bladder lesions were not observed in any of the animals that were not pretreated with BBN. Urinary pH and electrolyte concentrations were affected by dosing with sodium or disodium succinate with BBN, as compared to the control and succinic acid groups, and statistically significant differences between these two groups were observed as well.

The researchers also evaluated cell proliferation and DNA synthesis in the urinary bladder epithelium. Groups of 20 male F344 rats were given 5% succinic acid, sodium succinate, or disodium succinate in the feed, without BBN pretreatment for 8 wks. Negative controls were given basal diet. Five rats per group were given an i.p. injection of 50 mg/kg bw 5-bromo-2'-deoxyuridine (BrdU) 1 h prior to being killed. Compared to control values, BrdU uptake was statistically significantly increased by increased disodium succinate and was increased, but not in a statistically significant manner, by sodium succinate. Succinic acid did not have any effect on DNA synthesis. Microscopically, simple hyperplasia was observed in the urinary bladders of animals given sodium and disodium succinate. The appearance of the urinary bladder epithelial surface was altered by sodium and disodium succinate. Spermidine/spermine N^1-acetyltransferase activity in the urinary bladder epithelium was increased for disodium succinate, but not sodium succinate, when compared to controls. Urinary pH and electrolyte concentrations were affected as described previously.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation

Azelaic Acid

The cumulative irritation potential of a 15% azelaic acid gel (prescription formulation; vehicle not identified) was determined in a study using 31 female and 2 male subjects.[83] (During the study, 1 subject withdrew for personal reasons.) White petrolatum was used as a negative control. Azelaic acid and petrolatum, 0.2 g of each, were applied under occlusion to 2 cm x 2 cm sites on the back of each subject 3 times per week for 3 wks. Weekday patches were removed after 24 h, while the patches applied on Fridays were removed after 72 h. The test sites were evaluated 15-30 min after removal of the patch, and then a new patch was applied. Application was discontinued if severe irritation, which was designated by a maximum erythema score of 3, was observed. A 15% azelaic acid gel was statistically significantly more irritating than the negative control, with a mean cumulative irritancy index of 1.05/3. Individual reaction scores for the test article ranged from 0 to 3, and 5 subjects discontinued patching with azelaic acid due to an irritation score $\geq$3. Cumulative irritancy increased with successive patching. The researchers noted that since the vehicle used for azelaic acid was not tested, there was uncertainty as to whether the vehicle components affected the irritation scores.

Twice daily application of a cream containing 20% azelaic acid has been reported to cause erythema, irritation, pruritus, dryness, scaling, and burning.[84]

Case Reports

Adipic Acid

In two case reports with industrial exposure to adipic acid, positive sensitization reactions were reported with follow-up testing.[47]

ESTERS OF DICARBOXYLIC ACIDS

Much of the information on the esters of dicarboxylic acids was obtained from summary documents that mostly contained unpublished data. Data on esterase metabolites other than the parent dicarboxylic acid (i.e. parent alcohol and monoester) are summarized in Appendix I, immediately following the reference section.

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Metabolism of diesters in animals is expected to occur, initially, via enzymatic hydrolysis, leading to the corresponding dicarboxylic acids and the corresponding linear or branched alcohol.[85] These dicarboxylic acids and alcohols can be further metabolized or conjugated to polar products that are excreted in urine. However, other studies have shown that enzymatic hydrolysis of at least some diesters may be incomplete and result, instead, in the production of monoesters.[86]

Diethyl Malonate

Diethyl malonate is hydrolyzed via a two-step reaction to malonic acid and the corresponding alcohol, ethanol.[14]

Dimethyl malonate, which is not listed in the *International Cosmetic Ingredient Dictionary*, has similar physicochemical properties and hydrolyzes in the same manner to malonic acid and methanol. Because of this similarity, data on dimethyl malonate are included in this safety assessment to provide read-across data.

Distribution of diethyl malonate (and dimethyl malonate) is likely to occur in the water compartments, and accumulation in fat is unlikely based on physical and chemical properties. Both esters are likely to be metabolized by unspecific (serine-) esterases of different tissues, in particular, in the liver to the mono- esters and then to malonic acid and ethanol (or methanol). The hydrolysis product is likely to be metabolized via physiological pathways, such as the tricarboxylic acid cycle, as they are part of the normal intermediate metabolism. Both are assumed to readily absorb via mucous membranes.

In Vitro - Non-Human

The percutaneous absorption of radiolabeled diethyl malonate was determined *in vitro* using skin from Yorkshire pigs.[14] [2-^{14}C]Diethyl malonate was applied either undiluted (100 µg/cm^2) or diluted in ethanol at 12.5 mg/ml with an applied dose of 100 µg/cm^2 or as 0.5 mg/ml with an applied dose of 4 µg/cm^2. At 50 h, with undiluted diethyl malonate, 8.8% of the radioactivity was found in the skin and 3% was in the receptor fluid. With 100 µg in ethanol, 13% of the radioactivity was found in the skin and 6% in the receptor fluid and with 4 µg in ethanol, 30% was found in the skin and 10% in the receptor fluid. Absorption appeared to be enhanced with ethanol.

The percutaneous absorption of 1 mg/cm^2 [2-^{14}C]diethyl malonate in 10 µl acetone was determined *in vitro* also using skin from Yorkshire pigs. At 24 h, 0.2-1.6% of the diethyl malonate was found in the receptor fluid, 0.2-0.9% was found in the skin, and 0.6-0.7% was found on the skin surface. Skin mediated hydrolysis amounted to 15-35% of the applied dose. In the receptor fluid, 20-21% of the applied dose was present as hydrolysis products. In the skin and on the skin surface, 3-5% and 2-4%, respectively, of the applied dose was present as hydrolysis products.

In Vivo - Non-Human

The percutaneous penetration of radiolabeled diethyl malonate was studied *in vivo* in the following animal models:

athymic nude mouse, human, and pig skin grafted to athymic nude mice, in weanling pigs, and in hairless dogs.[14] [$^{2\text{-}14}$C]Diethyl malonate was applied at a dose of 0.1 mg/cm^2 for 24 h to a 1.27 cm^2 area of mouse skin, or for 48 h to a 25 cm^2 area of pigs and hairless dogs using non-occluded applications. According to the authors, the percutaneous absorption, was estimated from the recovery of radioactivity in urine and feces and corrected for the recovery observed after parenteral (s.c.) administration. Absorption was 15% in nude mice, 4 % in human skin grafted to nude mice, 6% in pig skin grafted to nude mice, 2.5% in pigs, and 4% in dogs.

In Vitro - Human

An *in vitro* skin absorption study was performed using diethyl malonate, no vehicle given.[14] Human cadaver split thickness skin was used in flow through cells. Diethyl malonate (4 µl) was applied to the skin samples. After 24 h, 16% of the applied dose had penetrated through the skin. The maximum flux rate was reached after 5 h and amounted to 280 µg/h (350 µg/cm^2/h); the mean penetration rate was 99 µg/h (120 µg/cm2/h). Much of the test substance, 45 to 50%, evaporated from the skin, and 34 to 39% remained on the skin.

Ditridecyl Adipate

The percutaneous absorption of [^{14}C]ditridecyl adipate was determined using groups of 10 male and 10 female Sprague-Dawley rats that were untreated or that had previously been exposed to unoccluded dermal applications of 0 or 2000 mg/kg bw ditridecyl adipate, 5 days/wk for 13 wks.[87] (This study is described in the section on 'Subchronic Dermal Toxicity'). A single 58 µl dose of 2000 mg/kg bw [^{14}C]ditridecyl adipate was applied topically (size of test site not specified), and urine and feces were collected for 4 days. In the previously untreated rats, a total of 11.6 and 10.6% of the [^{14}C] solution was absorbed by male and female rats, respectively, over 4 days. Approximately 63 and 52% of the absorbed dose (7.4 and 5.5% of the applied dose, respectively) was found in the tissues of males and females, respectively. A total of 3.5-4.7% of the applied dose was recovered in the urine and 0.4-0.7% in the feces of previously untreated rats. The values for the animals previously dosed with 2000 mg/kg bw ditridecyl adipate were not statistically significantly different from the controls. In the previously dosed animals, a total of 10.8 and 9.1% of the dose was absorbed by males and females, respectively, over the 4 days, with approximately 87 and 81% of the absorbed dose (9.4 and 7.4% of the applied dose, respectively) found in the tissues of the male and female rats, respectively. A total of 0.7-1.3% of the [^{14}C] was recovered in the urine and 0.4-0.6% in the feces. Based on the radioactivity recovered in the urine, the bioavailability of ditridecyl adipate was 2-6%, and previous dosing did not significantly affect absorption.

Diethylhexyl Adipate

In Vitro

The *in vitro* hydrolysis of diethylhexyl adipate (and mono-(2-ethylheyxl) adipate [MEHA]) using tissue preparations from the liver, pancreas, and small intestine of 2 rats was examined, as were the effects of diethylhexyl adipate on serum and hepatic enzymatic activities *in vitro*.[88] Diethylhexyl adipate was readily hydrolyzed to MEHA or adipic acid by each tissue preparation. The formation of adipic acid was rapid and approximately the same for all three tissues, while the formation of MEHA was rapid only in pancreatic tissue and was negligible in the intestine. The rate of hydrolysis from MEHA to adipic acid was greater that than from diethylhexyl adipate and the highest activity was found in intestinal tissue. In examining the effects on serum and hepatic enzymes, only N-demethylase activity was considerably inhibited by diethylhexyl adipate.

In Vivo – Non-Human

The elimination, distribution, and metabolism of diethylhexyl adipate was investigated using male Wistar rats.[88] In these studies, diethylhexyl adipate was labeled at the carbonyl carbon. In elimination studies, 2 rats were dosed by gavage

with 500 mg/kg bw [^{14}C]diethylhexyl adipate (1.26 µCi/rat) as a saturated solution in dimethyl sulfoxide (DMSO), and respired carbon dioxide, urine, and feces were collected for 2 days. At 24 h after dosing, 86% of the administered dose was excreted, and at 48 h, more than 98% of the dose was excreted. In one animal, 44.8% of the dose was excreted in expired carbon dioxide and 33.9% in the urine at the 48 h measurement, while in the other rat, 21.1% and of the dose was excreted in expired carbon dioxide and 52.2% in the urine. Little (1.4 or 5% of the dose) was excreted in the feces.

In the distribution study, 3 rats per group were given a single dose as described above. The animals were killed at various intervals, and blood, organ, and tissue samples were collected. Not taking into account the stomach and intestines, the greatest levels of radioactivity, as a percent of dose administered, were found in the liver, kidney, blood, muscle, and adipose tissue. These values ranged from 0.34-8.21% at 6 h, with the greatest percentage found in the adipose tissue, and from 0.54-3.44% at 12 h, with the greatest percentage found in the muscle. In most tissues, the amount of residual radioactivity reached a peak by 6 h, except for the liver, kidneys, testicles, and muscle, which reached a peak at 12 h. The researchers stated that the elimination of radioactivity from the tissues and organs was very rapid, and there was no specific organ affinity.

The metabolism of diethylhexyl adipate was examined in rats dosed orally, by gavage, with 100 mg of non-labeled diethylhexyl adipate as a 5% solution in DMSO. A control group was dosed with vehicle only. The rats were killed 1, 3, or 6 h after dosing. The metabolites were determined using GLC. Diethylhexyl adipate was rapidly hydrolyzed to adipic acid, the main intermediate metabolite, and MEHA. In the urine, adipic acid was detected at 1 h, and excretion as adipic acid in the urine reached 20-30% at 6 h. Diethylhexyl adipate and MEHA were not detected in the urine. Adipic acid only also was detected in the blood and the liver, with constant excretion of 0.5-0.7% of the dose in the blood and excretion in the liver increasing with time, with 2-3.3% excreted in the liver at 6 h. In the stomach, diethylhexyl adipate, adipic acid, and MEHA were found. The concentrations of diethylhexyl adipate declined rapidly, while the levels of adipic acid (9-10%) and MEHA (6-11.5%) peaked at 3 h. Adipic acid, but not MEHA, was found in the intestine and increased with time, reaching 19% at 6 h.

The absorption, distribution, and elimination of diethylhexyl adipate was examined using radioactive labeling on the acid [carbonyl-^{14}C] (specific activity 39.5 mCi/mmol) or the alcohol [2-ethylhexyl-1-^{14}C] (44.1 mCi/mmol).[42] The researchers used both DMSO and commercial corn oil as vehicles for all tests, since the were of the opinion that DMSO is an active penetrant and carrier of other substances through tissue membranes. It was also their opinion that a fat-soluble substance, such as diethylhexyl adipate, is more realistically studied dissolved in corn oil. The following groups of animals were dosed with 84.3 µg (9 µCi) [carbonyl ^{14}C]diethylhexyl adipate or 84.3 µg (10 µCi) [2-ethylhexyl-1-^{14}C]diethylhexyl adipate in both vehicles: 12 male NMRI mice were dosed i.v. and killed at intervals from 5 min to 4 days after dosing; 10 male NMRI mice were dosed intragastrically (i.g.) and killed at intervals from 20 min to 4 days after dosing; 12 gravid NMRI mice were dosed i.v. or i.g. on day 17 of gestation and killed at intervals from 20 min to 24 h. Six male rats were dosed i.g. with 843 µg (90 µCi) [carbonyl ^{14}C]diethylhexyl adipate or 843 µg (100 µCi) [2-ethylhexyl-1-^{14}C]diethylhexyl adipate and killed at intervals from 20 min to 4 h. Whole body autoradiography was used to determine tissue distribution.

Following dosing with [carbonyl-^{14}C]diethylhexyl adipate, distribution was similar in male mice, male rats, and gravid mice. The amount of radioactivity in the tissues peaked at a later time following i.g. dosing as compared to i.v. dosing. The presence of radioactivity in the gastrointestinal tract following i.v dosing indicated biliary excretion. Four h following both i.v. and i.g. dosing, the greatest uptake of radioactivity was found in the liver, bone marrow, brown fat, adrenal cortex, kidneys, and a few other tissues. At 24 h after i.g. dosing, significant levels of radioactivity remained in several tissues, including the liver, of both rats and mice. In gravid mice, a “remarkable strong uptake” of radioactivity in the corpora lutea of

the ovary was observed at all time intervals with both i.v. and i.g. dosing, and some radioactivity was found in the fetal intestine, liver, and bone marrow.

Similar distribution patterns were seen following dosing with [2-ethylhexyl-1-^{14}C]diethylhexyl adipate as were seen with [carbonyl-^{14}C]diethylhexyl adipate. Following i.g. dosing, the appearance of radioactivity was lessened and not as great as it was with i.v. dosing. Very high radioactivity levels were seen in the liver and kidney at 5 min-1 h after i.v. dosing and at 20 min-4 h after i.g. dosing. The radioactivity in the liver was still high at 24 h after i.g. dosing in mice and rats. Radioactivity was also seen in the intestinal contents at 1-4 h after i.v. dosing, again indicating biliary excretion. At longer intervals after i.v. injection, 4 h-4 days, radioactivity was detected in the bronchi of mice. While radioactivity was observed in the ovaries of gravid mice and some fetal tissues following dosing with [carbonyl-^{14}C]diethylhexyl adipate, none was detected in the ovaries of gravid mice after dosing with [2-ethylhexyl-1-^{14}C]diethylhexyl adipate, and very little radioactivity was seen in some fetal tissues.

The effect of vehicle on the absorption and biliary and urinary excretion of diethylhexyl adipate was also examined using rats in a gavage study with [^{14}C]diethylhexyl adipate. Radioactivity was measured every 30 min for 7.5 h. The times and extent of absorption were different for all four preparations of [^{14}C]diethylhexyl adipate. Radioactivity levels in the blood increased faster and were greater with DMSO as the vehicle, as compared to corn oil. The highest blood radioactivity levels were found with [carbonyl-^{14}C]diethylhexyl adipate in DMSO. Biliary excretion of [^{14}C]diethylhexyl adipate was greatly affected by vehicle; with DMSO, 41% of the dose was detected in the bile, while only 10% of the dose was found with the corn oil vehicle. This difference was not seen with [carbonyl-^{14}C]diethylhexyl adipate. Finally, vehicle did not have much influence on urinary excretion. However, unlike the results reported by the previous researchers, little radioactivity was excreted in the urine. The researchers hypothesized that since the study duration was only 7.5 h, urinary excretion may not have been complete.

The metabolism of diethylhexyl adipate was examined *in vivo* using male Wistar rats and compared to *in vitro* metabolism using hepatocytes.[89] *In vivo*, rats were dosed with 0.665 or 1.5 g/kg diethylhexyl adipate in corn oil by gavage for 5 days, and the controls were given vehicle only. Urine was collected daily. Diethylhexyl adipate was not recovered in the urine after 24 h. Adipic acid was the main metabolite of diethylhexyl adipate. *In vitro*, the first hydrolysis of diethylhexyl adipate appears to be a rate-limiting step. *In vivo*, it was thought that this hydrolysis probably occurs in the gastrointestinal tract. Metabolic pathways (ω and ω-1oxidations, glucuronidation) seemed to prove that transformations of diethylhexyl adipate are localized mainly in the liver.

Oral administration of diethylhexyl adipate to cynomolgus monkeys results in rapid elimination, with 47-57% of the dose excreted in the urine.[17] Unchanged diethylhexyl adipate is absorbed from the gastrointestinal tract, and the glucuronide of MEHA and traces of unchanged diethylhexyl adipate were found in the urine. (Details were not provided).

<u>In Vivo - Human</u>

The pharmacokinetics of [$^{2}H_{10}$]diethylhexyl adipate, labeled on the ethyl side-chains, were examined using 6 male subjects.[90] A dose of 46 mg [$^{2}H_{10}$]diethylhexyl adipate in corn oil, for a total volume of 0.5 cm^3, was administered orally in a gelatin capsule. Blood samples were taken for up to 31 h after dosing, and urine samples were taken at intervals for up to 96 h after dosing. In the plasma, unconjugated [$^{2}H_{5}$]2-ethylhexanoic acid was the only measurable diethylhexyl adipate-related compound. This compound appeared rapidly in the plasma, and the peak concentrations (1.6 ±0.5 µg/cm^3) occurred between 1 and 2 h. [$^{2}H_{5}$]2-Ethylhexanol was detected, but it was below the limit of quantification. The rate of metabolite formation was calculated, since there was no evidence of diethylhexyl adipate absorption, as 1.63 ± 1.19 hr^{-1}. The rate of elimination

from the plasma was also rapid and estimated to be 0.42 ± 0.15 h^{-1}., which corresponded to an elimination half-life of 1.65 h. Although there were inter-individual differences in the rate and extent of [$^2H_5$]2-ethylhexanoic acid formation, it was below the limit of detection in all subjects by 31 h.

In the urine, [$^2H_5$]2-ethylhexanoic acid was again the principal metabolite, and it was probably eliminated as a conjugated product. This conjugated form, most likely the glucuronide, accounted for up to 99% of the total [$^2H_5$]2-ethyl-hexanoic acid measured. Conjugation of the other urinary metabolites was minimal. Peak urinary elimination of the measured metabolites occurred within 8 h of dosing, and no metabolites were detected in the urine after 36 h. The rates of elimination were similar for all metabolites, with a mean elimination half-life of 1.5 h. The measured urinary metabolites accounted for 12.1% of the dose, with the majority being eliminated in 24 h. Fecal analysis determined that a minor portion of the dose was present as diethylhexyl adipate (0.43%) and [$^2H_5$]MEHA (0.27%). The researchers noted that recovery of the administered dose was incomplete and hypothesized that it was most probably due to further systemic metabolism.

Diethylhexyl Sebacate

Diethylhexyl sebacate is not readily absorbed through the skin of guinea pigs (no further details were provided).[1] It was noted that the metabolism of diethylhexyl sebacate in rodents and humans may follow partially common pathways,

Peroxisome Proliferation

Diethylhexyl adipate is a peroxisome proliferator requiring extensive phase I metabolism to produce the proximate peroxisome proliferator, which in both mice and rats, appears to be 2-ethylhexanoic acid.[91,92] Diethylhexyl adipate has been studied because it is structurally related to diethylhexyl phthalate, although diethylhexyl adipate is not as potent a proliferator as diethylhexyl phthalate.[93,94]

Peroxisome proliferation causes an increase in liver weights and can induce hepatocarcinogenicity in rats and mice. Peroxisome proliferation is not believed to pose the risk of inducing hepatocarcinogenesis in humans, as a species difference in response to peroxisome proliferators exists.[95] *In* vitro and *in* vivo studies examining the induction of peroxisome proliferation by diethylhexyl adipate and diethylhexyl sebacate are summarized in Table 8.[61,91-94,96,97] While proliferation was observed, these ingredients have much weaker activity than diethylhexyl phthalate and ciprofibrate, which are very effective peroxisome proliferators.

Humans do not react to peroxisome proliferators in the manner that rodents do.[95] There is no effect on organelle proliferation and induction of peroxisomal and microsomal fatty acid-oxidizing enzymes in species other than rats and mice, including humans. Consequently, these results have no relevance to humans.

DNA Binding/DNA Synthesis

Diethylhexyl Adipate

The potential of diethylhexyl adipate to bind to liver DNA of female NMRI mice was evaluated by administering a solution of 119 mg diethylhexyl adipate/ml with 3.85 mCi/ml of [^{14}C]diethylhexyl adipate (labelled at C1 of the alcohol moiety) and 27.7 mCi/ml of [^3H]diethylhexyl adipate (tritiated at positions 2 and 3 of the alcohol moiety) in olive oil.[98] The animals were dosed by gavage, and the livers were excised 16 h after dosing. Some animals were pretreated with 10 g/kg of unlabeled dietary diethylhexyl adipate for 4 wks. Diethylhexyl adipate did not covalently bind to hepatic DNA in mice. Pretreatment with diethylhexyl adipate caused an increase in liver weight, but no increase in DNA binding. The researchers stated that tumorigenicity of diethylhexyl adipate must be due to an activity other than DNA binding.

The ability of diethylhexyl adipate to stimulate liver DNA synthesis in male F344 rats was investigated using radiolabeled thymidine.[99] Contrary to expected results, diethylhexyl adipate did stimulate DNA synthesis. The stimulation factor,

which is indicated by the ratio of the thymidine incorporation in treated animals compared to controls, was 10.5 and the doubling dose, which is the dose that produced a doubling of the control level DNA synthesis, was 0.7 mmol/kg.

The effect of dosing with diethylhexyl adipate on 8-hydroxydeoxyguanosine (8-OH-dG) in liver and kidney DNA of rats was examined.[100] Groups of 10 male F344 rats were fed a diet containing 0 or 2.5 diethylhexyl adipate. Five animals per group were killed after 1 wk, and the other 5 after 2 wks of dosing. Relative liver to body weights were statistically significantly increased after 1 and 2 weeks of dosing, and the relative kidney to body weights were statistically significantly increased only after 2 wks. A statistically significant increase in 8-OH-dG was observed in the liver DNA, but not the kidney DNA, at wk 1 and 2.

The IARC remarked that the weight of evidence for diethylhexyl adipate, and other rodent peroxisome proliferators in general, demonstrated that rodent peroxisome proliferators do not act as direct DNA-damaging agents.[17]

Hepatic Lipid Metabolism

Diethylhexyl Adipate

Dietary administration of diethylhexyl adipate affects hepatic lipid metabolism.[17] Hepatic fatty acid-binding protein and microsomal stearoyl-CoA desaturation were increased in Wistar rats fed 2% diethylhexyl adipate for 7 days.[101,102] When fed to rats for 14 days, an increase in hepatic phospholipid levels and a decrease in phosphatidylcholine:phosphatidylethanolamine ratio was reported.[103] In male NZB mice fed 2% diethylhexyl adipate for 5 days, an induction of fatty acid translocase, fatty acid transporter protein, and fatty acid binding protein in the liver was reported.[104]

Cellular Effects

Dibutyl Adipate

Dibutyl adipate was tested for cytotoxicity in the metabolic inhibition test. A dilution series of dibutyl adipate was suspended in HeLa cells. Dibutyl adipate had no acute toxicity to the cells, which was attributed to its insolubility in water.[5]

ANIMAL TOXICOLOGY

Acute Toxicity

Acute toxicity data on esters of dicarboxylic acids are presented in Table 9.

The acute toxicity of esterase metabolites are also summarized in this table.

Short-Term Oral Toxicity

Dibutyl Adipate

Male and female Crj:CD(SD) rats, number per group not specified, were dosed orally, by gavage, with 0, 20, 140, or 1000 mg/kg bw dibutyl adipate in olive oil daily for 28 days.[105] No clinical, hematological, or microscopic test-article related changes were observed.

Diethylhexyl Adipate

In a 14-day dietary study, groups of 5 male rats and mice were given ≤50,000 ppm and groups of 5 female rats and mice were given ≤100,000 ppm diethylhexyl adipate. Male rats and mice fed 50,000 ppm and female rats and mice fed ≥25,000 ppm had decreased weight gains or weight loss. (It is not specified whether the results were statistically significant.) One female rat and all female mice of the 100,000 ppm group died.[2]

In a 14-day study in which 5 male and 5 female Wistar and F344 rats and Swiss and B6C3F$_1$ mice were dosed with 0-2.5 g/kg diethylhexyl adipate in corn oil for 14 days, diethylhexyl adipate was toxic to female B6C3F$_1$ mice, causing mortality, at a dose level of 2.5 g/kg.[92] The toxicity of two metabolites of diethylhexyl adipate, 2-ethylhexanol and 2-ethyl-

hexanoic acid, was also examined using Wistar rats and Swiss mice. 2-Ethylhexanol was toxic to male and female rats, with mortality reported at doses >1.05 g/kg in male and female rats. 2-Ethylhexanoic acid was toxic to female rats, with mortality reported at doses ≥1.9 g/kg; mortality was not reported for male rats. These effects were not reported in mice.

In a 1 and 4-wk dietary study in which groups of 5-8 rats and mice were fed diets containing 0-4.0% and 0-2.5% di-ethylhexyl adipate, respectively, feed consumption by rats was decreased in the 4.0% group at 1 wk and in the 2.5 and 4.0% dose groups at 4 wks.[96] Body weights were significantly decreased in these groups. Feed consumption by mice was not affected, but a significant decrease in body weights was seen in the 1.2 and 2.5% dose groups at 4 wks.

Toxicity was evaluated in a study in which groups of 10 female Crl:CD(SD) rats were dosed, by gavage, with 5 ml/kg of 0, 200, 1000, or 2000 mg/kg bw diethylhexyl adipate in corn oil for 2 or 4 wks.[120] All animals survived until study termination. In the 2-wk study, no statistically significant findings were observed for the animals dosed with 200 mg/kg bw, and the only statistically significant finding in the 1000 mg/kg bw dose group was an increase in relative liver to body weight. In the 2000 mg/kg bw dose group, there was staining around the perineum, statistically significant increases in relative liver and kidney to body weights, and a statistically significant decrease in the relative weight of the left ovary. Microscopically, abnormal findings were reported for both the ovary and kidney. In the ovary, an increase in atresia of the large follicle and a decrease in currently formed corpora lutea were seen in animals dosed with 1000 and 2000 mg/kg bw, and in the 2000 mg/kg bw group, an increase in follicular cysts was observed. In the kidney, an eosinophilic change of the proximal tubule was observed for the 2000 mg/kg bw dose group. The NOAEL was 200 mg/kg bw.

In the rats dosed for 4 wks, similar observations were made. There was staining around the perineum of animals dosed with 1000 and 2000 mg/kg bw diethylhexyl adipate, and final body weights of animals dosed with 2000 mg/kg bw were statistically significantly decreased. The relative kidney to body weights were statistically significantly increased in animals at all dose levels, and liver weights were statistically significantly increased in animals of the 1000 and 2000 mg/kg bw dose groups. The mean estrous cycle length was statistically significantly decreased in the 200 mg/kg bw dose group, but this was not considered treatment-related since a dose-response was not seen. The same microscopic abnormalities reported in the 2 wk study were seen in the ovaries and kidneys of the animals dosed with 1000 and 2000 mg/kg bw in the 4-wk study. As in the 2-wk study, the NOAEL for ovarian toxicity was 200 mg/kg bw.

Short-Term Dermal Toxicity

Dibutyl Adipate

Groups of 10 rabbits were dosed dermally with 0.5 or 1.0 ml/kg/day of a 20% dispersion of dibutyl adipate, 5x/wk for 6 wks. A significant reduction in body weight gain was seen for animals of the 1.0 ml/kg/day group, and renal lesions were seen in one animal of each group. [5]

Diisopropyl Adipate

An immersion study was performed using guinea pigs in which a product containing 20.75% diisopropyl adipate was diluted, giving an actual adipate concentration of 0.10%. The animals were immersed 4 h/day for 3 days. There were no signs of systemic toxicity, and the degree of dermal irritation was considered minimal.[2]

Diethylhexyl Adipate

Diethylhexyl adipate, 410 or 2060 mg/kg bw, was applied to the shaved abdomen of male rabbits, 4 per group, 5 days per wk for 2 wks.[116] Mineral oil was applied in the same manner to a group of 4 rabbits as a negative control. A collar was used to restrict ingestion. One animal in the 410 mg/kg bw group died during wk 2 of the study. All other animals in this group appeared normal. Slight to moderate erythema was observed at the test site. No animals of the 2060 mg/kg bw group

died, but 3 of the 4 did not gain weight, and they had labored breathing and were lethargic during wk 2. Moderate erythema was observed in this group. Microscopically, one animal of the 2060 mg/kg bw group had altered cytology of the liver parenchymal cells. No other microscopic lesions were noted.

Short-Term Inhalation Toxicity

Diethylhexyl Sebacate

No deaths occurred when 4 rats, 2 guinea pigs, 2 rabbits and 1 cat were exposed to 400 mg diethylhexyl sebacate/m^3, 7 hrs/day, for 10 days.[1] Details were not provided.

Subchronic Oral Toxicity

Diethyl Malonate

Groups of 10-16 male and female CD rats were fed diets containing either 0, 36 (males) or 41 mg/kg bw/day (females) diethyl malonate for 90 days.[14] No treatment related effects were observed.

Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid

Groups of 15 male and 15 female Sprague-Dawley rats were fed a diet containing 0, 0.1, 0.5, or 2.5% di-C7-9 branched and linear alkyl esters of adipic acid for 90 days, corresponding to approximately 1500 mg/kg bw/day for high dose males and 1900 mg/kg bw/day for high dose females.[87] All rats were killed for necropsy at study termination. No systemic toxicity was reported. Small, but significant, increases in absolute and relative kidney to body weights reported for females of the 2.5% dose group were not considered treatment-related. The NOAELs for male and female rats were 1500 and 1950 mg/kg bw/day, respectively.

Diethylhexyl Adipate

In a 13-wk dietary study, groups of 10 rats and 10 mice were fed ≤25,000 ppm diethylhexyl adipate. With the exception of decreased weight gain for some of the groups, no compound-related toxicologic effects were observed.[2]

In a 90-day dietary study, groups of 10 rats per group were fed 0-4740 mg/kg bw diethylhexyl adipate for 90 days.[116] Mortality occurred in the 4740 mg/kg bw group, but the number of deaths was not specified. Decreased growth and feed consumption was reported for animals fed 2920 mg/kg bw. Changes in kidney and liver weights were noted, but no details were given. The NOEL was 610 mg/kg bw, and the LOEL was 2920 mg/kg bw diethylhexyl adipate.

Groups of 15 male and 15 female Sprague Dawley rats were fed 0 or 2.5% diethylhexyl adipate for 90 days.[87] At study termination, all animals were killed for necropsy. Body weight gains were statistically significantly decreased for treated males and females, and relative kidney and liver to body weights were statistically significantly increased for treated females, when compared to controls.

In a 13-wk dietary study described in the section on "Peroxisome Proliferation" in which groups of 5-8 rats and mice were fed diets containing 0-4.0% and 0-2.5% diethylhexyl adipate, feed consumption by rats was decreased in the 2.5 and 4.0% dose groups, and body weights were significantly reduced in these groups.[96] Feed consumption by mice was not affected, but a significant decrease in body weights was seen in the 1.2 and 2.5% dose groups.

Diisononyl Adipate

Groups of 10 male and 10 female rats were fed 0, 50, 150, or 500 mg/kg bw diisononyl adipate for 13 wks.[87] A statistically significant increase in relative kidney to body weights was reported for males and females given 500 mg/kg bw, but absolute kidney weights were not affected and no significant microscopic effects were seen. Microscopic changes in any of the organs, including the testes and epididymis of males and ovaries of females, were not observed. There were no significant toxicological findings, and the NOAEL was 500 mg/kg bw/day.

In another 13-wk study, groups of 4 male and 4 female Beagle dogs were fed 0, 0.3, 1.0, or 3.0% diisononyl adipate; the high dose was increased to 6% during wks 9-13.[87] No significant findings were reported for the 0.3 or 1.0% groups. In the high dose group, decreased body weights, testes weight, and feed consumption, increased liver weights, elevated enzyme levels, liver and kidney discoloration, and microscopic changes in the liver, testes, spleen, and kidneys were reported. The dietary NOAEL for diisononyl adipate was 1.0%.

Subchronic Dermal Toxicity

Dibutyl Adipate

No adverse effects were reported in a study using 4 dogs in which entire-body applications of an emulsion containing 6.25% dibutyl adipate were made 2x/wk for 3 mos.[5]

Ditridecyl Adipate

Ditridecyl adipate, 0, 800, or 2000 mg/kg bw, was applied to the backs of groups of 10 male and 10 female Sprague-Dawley rats, 5 days/wk for 13 wks.[87] The test sites were not occluded, but the animals wore Elizabethan collars. Slight erythema and flaking of the skin was observed in the treated groups, with hyperplasia of the sebaceous glands in the dermis, but otherwise no significant differences were observed between test and control animals. Differences in relative organ to body weights were not statistically significant, and ditridecyl adipate did not appear to cause systemic toxicity.

Subchronic Inhalation Toxicity

Diethylhexyl Sebacate

Groups of 12 F344 rats, gender not specified, were exposed 4 h/day, 5 days/wk, to 25 or 250 mg/m^3 diethylhexyl sebacate for ≤13 wks.[87] No adverse systemic or lung effects were observed.

Chronic Oral Toxicity

Diethylhexyl Adipate

Intragastric doses of ≤2.0 g/kg diethylhexyl adipate to rats (number not stated) for 6 mos produced no enzymatic changes, but levels of sulphydryl compounds in the blood were increased. Hepatic detoxification appeared depressed at the onset of the study, but it was accelerated after 6 mos. Administration of 0.1 g/kg for 10 mos decreased central nervous system excitability.[2]

Dibutyl Sebacate

Groups of 5 male and 5 female Sprague-Dawley rats were fed a diet containing 0, 0.01, 0.05, 0.25, or 1.25% dibutyl sebacate for 1 yr.[115] Necropsies were performed whenever rats exhibited significant weight losses or other evidences of severe concurrent infection. Dibutyl sebacate had no effect on growth or well-being.

The researches then fed groups of 16 male Sprague Dawley rats a diet containing 0.01, 0.05, 0.25, 1.25, or 6.25% dibutyl sebacate for 2 yrs.[115] Two control groups were given untreated feed. Necropsies were performed on 3 rats from each group after 1yr, and the experiment was terminated at the end of the 2-year feeding period. Interim, animals were killed whenever they became moribund. In such instances the rats usually had incapacitating tumors or severe intercurrent infections. Dibutyl sebacate did not adversely affect growth or survival, and it did not produce significant hematological changes in peripheral blood. As the rats increased in age, slight changes in distribution of leukocytes were found, but these trends occurred in both the control and treatment groups.

Ocular Irritation

Ocular irritation data on esters of dicarboxylic acids are presented in Table 10. The available ocular irritation data on esterase metabolites are also summarized in this table.

Diethyl Malonate

The ocular irritation potential of diethyl malonate was evaluated using rabbits, number and gender not specified.[14] A volume of 0.1 ml was instilled into the conjunctival sac of one eye, which was not rinsed, and the contralateral eye was untreated and served as the negative control. Diethyl malonate produced slight to moderate irritation.

In a similar study, undiluted dimethyl malonate produced slight to moderate irritation in rabbit eyes.[14] All signs of irritation were cleared by day 8.

Dibutyl Adipate

Undiluted dibutyl adipate was minimally irritating to the eyes of rabbits, and 0.1% in olive oil was non-irritating.[5]

Diisopropyl Adipate

The ocular irritation potential of 2 lots of undiluted diisopropyl adipate was evaluated using rabbits. One caused negligible irritation, while the other was non-irritating. A formulation containing 0.7% diisopropyl adipate produced some corneal stippling in rabbit eyes, while a formulation containing 5.0% and one containing 20.75% were non-irritating to rabbit eyes. [2]

The ocular irritation of undiluted diisopropyl adipate was evaluated using 3 albino rabbits.[113] A volume of 0.1 ml was instilled into the conjunctival sac of one eye, which was not rinsed. The contralateral eye was untreated and served as the negative control. Diisopropyl adipate was not irritating.

Diethylhexyl Adipate

Undiluted diethylhexyl adipate was non-irritating to rabbit eyes and a formulation containing 0.0175 was, at most, a mild transient irritant.[2]

Diisopropyl Sebacate

A primary ocular irritation study was performed using 6 New Zealand white rabbits to determine the ocular irritation potential of diisopropyl sebacate.[121] A volume o f 0.1 ml was instilled into one eye of each animal, which was not rinsed, and the contralateral eye of each animal served as the control. The average Draize scores were 2.0 at 24 and 48 h, 0.3 at 72 h, and 0.0 at 4 days. Diisopropyl sebacate was a minimal ocular irritant.

Diethylhexyl Sebacate

The ocular irritation of a cream containing 1.2% diethylhexyl sebacate was evaluated using the *in vitro* EpiOcular MTT viability assay.[122] The tissue samples were exposed to undiluted test material for 64 min, 256 min, or 1200 min. Following treatment, the viability of those tissues were calculated. The ET_{50} (time for tissue viability to be reduced by 50%) was 484.9 min, and diethylhexyl sebacate was considered to be non-irritating.

Dioctyldodecyl Dodecanedioate

The primary eye irritation of dioctyldodecyl dodecanedioate was evaluated using 6 albino rabbits.[123] A volume of 0.1 ml was instilled into one eye of each animal, which was not rinsed. and the contralateral eye served as a negative control. They eyes were evaluated at 24, 48, and 72 h. At 24 h, the maximum mean total score (MMTS) was 0.00, and dioctyldodecyl dodecanedioate was considered not irritating.

Diisocetyl Dodecanedioate

The primary eye irritation of diisocetyl dodecanedioate was evaluated using the procedure described above.[124] The MMTS was 0.00, and diisocetyl dodecanedioate was considered not irritating to the eyes of rabbits.

Dermal Irritation

Dermal irritation and sensitization data on esters of dicarboxylic acids are presented in Table 11. The available

dermal irritation and sensitization data on esterase metabolites are also summarized in this table.

Diethyl Malonate

The dermal irritation potential of diethyl malonate was evaluated using a 24 h occlusive application.[14] Diethyl malonate was slightly irritating to rabbit skin.

Dimethyl Malonate

Dimethyl malonate was applied undiluted to rabbit skin for 4 h under a semi-occlusive patch.[14] Slight erythema was observed only at 30-60 min after patch removal, and dimethyl malonate was considered non-irritating to rabbit skin.

Dibutyl Adipate

Application of undiluted butyl adipate to rabbit skin resulted in a primary irritation score of 2/8. Undiluted dibutyl adipate caused moderate erythema in rabbits following repeated dermal exposure. However, material impregnated with dibutyl adipate was not irritating to the skin of rabbits. Application of dibutyl adipate at 10% in acetone produced no observable adverse effect when applied to rabbit ears, and no dermal reaction was observed following twice daily application for 14 days to the backs of hairless mice. Two perfume formulations containing 1.1% diisopropyl adipate were not primary dermal irritants using rabbits[5]

Diisopropyl Adipate

Draize tests of undiluted diisopropyl adipate resulted in, at most, mild irritation of rabbit skin. In Draize tests with formulations containing 5.0% or 20.75% diisopropyl adipate, minimal irritation was reported with both formulations. [2]

Diethylhexyl Adipate

Undiluted diethylhexyl adipate was a very mild irritant when applied under occlusion to intact and abraded rabbit skin. A formulation containing 0.175% diethylhexyl adipate had an irritation index of 1.6/4.[2]

Diisodecyl Adipate, Dioctyldodecyl Adipate, Diisocetyl Adipate

The dermal irritation potential of diisodecyl adipate, dioctyldodecyl adipate and diisocetyl adipate was determined using 3 albino rabbits.[110,111, 112,] Undiluted test material was applied to the skin for 4 h under a semi-occlusive patch. The erythema scores for each of the three materials were 0-1 during 1-72 h, and the edema scores were 0. Diisodecyl adipate, dioctyldodecyl adipate and diisocetyl adipate were considered non-irritating to rabbit skin.

Diethyl Sebacate

Undiluted diethyl sebacate and 30% diethyl sebacate in ethanol were tested on 8 male Japanese White strain rabbits (gender not specified).[125] The flank of the animals was clipped free of hair 1 day prior to application of test substance. The skin of 4 animals was abraded. The test substance, 0.3 ml, was applied occlusively to the back of all animals for 24 h. The skin reactions were evaluated at 24 h and 72 h. The primary irritation score was 0.0 (none to weak irritant) in undiluted diethyl sebacate and 0.3 (none to weak irritant) in 30% diethyl sebacate. These results suggest that 100% diethyl sebacate has no primary skin irritation under these test conditions.

Diisopropyl Sebacate

A primary dermal irritation study on diisopropyl sebacate was performed using 6 New Zealand white rabbits.[121] A dermal application of 0.5 ml of undiluted test material was applied to an abraded and an intact site on each animal. The test sites were occluded for 24 h and observed individually for erythema, edema, and other effects 24 and 72 h after application. Mean scores from the 24 and 72 h reading were averaged to give a primary irritation index (PII) of 2.88. Diisopropyl sebacate was not considered a primary dermal irritant.

The dermal irritation potential of diisopropyl sebacate was determined using 3 albino rabbits.[126] Undiluted test

material was applied to the skin for 4 h under a semi-occlusive patch. The erythema scores were 1 during 1-72 h, and the edema scores were 0-1. Diisopropyl sebacate was considered non-irritating to rabbit skin.

Diethylhexyl Sebacate

The dermal irritation potential of diethylhexyl sebacate was evaluated using the same procedure.[109] The erythema scores were 1 during 1-72 h, and the edema scores were 0. Diethylhexyl sebacate was considered non-irritating to rabbit skin.

Patch tests with diethylhexyl sebacate (neat; 48-hr occluded) did not irritate the skin of 2-4 rabbits.[1] It was also reported that diethylhexyl sebacate was non-irritating to the skin of guinea pigs. No further study details were provided.

Dermal Sensitization

Dimethyl Malonate

Dimethyl malonate was not a sensitizer in a Buehler guinea pig sensitization test according to OECD TG 406.[14]

Dibutyl Adipate

Dibutyl adipate was not a dermal sensitizer in guinea pigs when tested at 25% in a maximization test.[5]

Diethylhexyl Adipate

Diethylhexyl adipate, 0.1%, was not a sensitizer in a maximization study using guinea pigs.[2]

Diethylhexyl Sebacate

A limited attempt was made to sensitize a group of 2-4 rabbits by applying diethylhexyl sebacate using occlusive patches.[1] No reactions were seen in an occlusive challenge with the undiluted test article 2 weeks later. Details were not provided.

Dioctyldodecyl Dodecanedioate

A maximization test was performed to evaluate the sensitization potential of dioctyldodecyl dodecanedioate.[127] Ten female guinea pigs were used. The dose used at intradermal injection was 0.1 ml, and 0.5 ml was used for the topical challenge. Slight erythema was observed at induction, but a sensitization reaction was not observed.

Phototoxicity

Diisopropyl Adipate

Two perfume formulations containing 1.1% diisopropyl adipate were not phototoxic in rabbits.[2]

Mucous Membrane Irritation

Diethylhexyl Adipate

A product containing 0.175% diethylhexyl adipate did not produce mucous membrane irritation in rabbits.[2]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Dimethyl Malonate

The reproductive and developmental toxicity of dimethyl malonate was evaluated using groups of 10 male and 10 female Wistar rats.[14] The animals were dosed with 0, 100, 300, or 1000 mg/kg bw dimethyl malonate orally, by gavage. Males were dosed for 2 wks prior to mating, during mating, and 2 wks after mating, for a total of 39 doses. Females were dosed 2 weeks prior to mating, during mating, and through day 4 of lactation. A recovery group of 5 male and 5 female high dose animals were observed for 14 days after the termination of dosing. Microscopically, the incidence of treatment-related hepatocellular hypertrophy of the liver was observed for males and females given 1000 mg/kg bw dimethyl malonate. This effect was not observed in the recovery animals or in the other test groups. No other significant toxicological effects were observed. Performance in a functional observation battery was similar for test and control animals. There was no effect on

fertility. In the 100 mg/kg bw group, a statistically significant decrease in the number of live pups was due to an increase in post-implantation loss. This effect was not considered treatment related, and no developmental toxicity was reported. The NOAEL was 300 mg/kg bw for repeated doses and maternal toxicity and 1000 mg/kg bw for fertility and developmental toxicity.

Dimethyl Adipate

The fetotoxic and teratogenic effects of dimethyl adipate were evaluated in a study in which groups of 5 gravid Sprague Dawley rats were dosed i.p. with 0.0603-0.6028 ml/kg (1/30, 1/10, 1/5, and 1/3 of the i.p. LD_{50} value) on days 5, 10, and 15 of gestation.[114] A pooled volume control consisted of animals dosed with 10 ml/kg distilled water, saline, or cotton-seed oil. A positive control group was not used. All animals were killed and examined on day 20 of gestation. The mean fetal weights and the numbers of live fetuses were not statistically significantly different between treated and blunt-needle control groups. Resorptions in animals dosed with 0.1809 ml/kg were statistically significantly increased when compared to the pooled controls, but not the blunt-needle controls. Gross and skeletal abnormalities, but not visceral, were statistically significantly increased in fetuses of the 0.3617 and 0.6028 ml/kg groups.

Diethyl Adipate

The fetotoxic and teratogenic effects of diethyl adipate were evaluated following the same procedure described above.[114] These rats were dosed i.p. with 0.0837-0.8373 ml/kg diethyl adipate. The mean fetal weight and the number of live fetuses were not statistically significantly different between treated and blunt-needle control groups, and the number of resorptions was similar between treated animals and both the blunt needle and pooled controls. There were no differences in the incidences of gross, skeletal, or visceral abnormalities in fetuses of the treated groups compared to pooled controls.

Dipropyl Adipate

The fetotoxic and teratogenic effects of dipropyl adipate were evaluated following the same procedure described earlier.[114] These rats were dosed i.p. with 0.1262-1.2619 ml/kg dipropyl adipate. The numbers of live and dead fetuses were not statistically significantly different between treated and blunt-needle control groups, but there was a statistically significant decrease in the mean fetal weight of the 0.7572 ml/kg group. Resorptions in animals dosed with 1.2619 ml/kg were statistically significantly increased when compared to the pooled controls, but not the blunt-needle controls. Gross abnormalities, but not skeletal or visceral, were statistically significantly increased in fetuses of the 1.2619 ml/kg group.

Dibutyl Adipate

A reproductive toxicity study was performed in which groups of 5 gravid Sprague Dawley rats were dosed i.p. with 0.1748-1.7480 ml/kg dibutyl adipate on days 5, 10, and 15 of gestation. The incidence of gross abnormalities was only statistically significantly increased in the high dose group when compared to pooled controls.[5]

The reproductive toxicity of dibutyl adipate was evaluated in a study Sprague-Dawley rats.[105] Groups of 13 male and 13 female rats were dosed with 0, 100, 300, or 1000 mg/kg bw dibutyl adipate orally, by gavage, for 14 days prior to mating through parturition; males were dosed for a total of 42 days and female dams were dosed until day 3 of lactation. The test article had no effect on fertility. Body weight gains of males of the 1000 mg/kg bw group were slightly decreased. Kidney weights of the high dose males and females sere increased compared to controls. No gross or microscopic effects were noted at necropsy, and the internal genitalia were normal. Dosing with dibutyl adipate did not produce any reproductive effects. The only effect on the offspring was a decrease in pup weight on post-natal days 0 and 4 and in viability on post-natal day 4 in the 1000 mg/kg group. The NOEL for parental and offspring toxicity was 300 mg/kg bw/day. The reproductive NOEL was 1000 mg/kg bw/day.

Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid

Groups of 24 gravid Sprague Dawley rats were dosed orally by gavage with 0, 1000, 4000, or 7000 mg/kg bw/day di-C7-9 branched and linear alkyl esters of adipic acid on days 6-19 of gestation, and all animals were killed and examined on day 20.[87] All dams survived until study termination. Body weights were significantly decreased for dams of the 7000 mg/kg bw group. Weights of male and female fetuses of the 7000 mg/kg bw group were slightly, but not statistically significantly, decreased compared to the other groups. A greater incidence of rudimentary structures was observed for high dose fetuses as compared to the other groups in this study, but the incidence was within the range of historical controls. There was no evidence of developmental toxicity at any dose tested.

Ditridecyl Adipate

The reproductive and developmental toxicity of ditridecyl adipate was evaluated using groups of 15 mated female Sprague-Dawley rats.[87] Doses of 0, 800, and 2000 mg/kg bw were applied dermally without occlusion on days 0-19 of gestation, and the dams were killed on day 20. Mild skin irritation consisting of erythema and flaking were observed at the test sites of the treated animals. No maternal mortality was reported. Weight gains were statistically significantly decreased for the 2000 mg/kg bw group during days 0-3 and 16-20 of gestation. Weight gains were statistically significantly decreased in the 800 mg/kg bw group during days 0-3 of gestation. No differences in skeletal anomalies were observed, but there were some differences in visceral anomalies, including increased incidence of levocardia at 2000 mg/kg bw. These anomalies were not considered treatment-related. The NOAEL for maternal toxicity was 2000 mg/kg bw/day, and for developmental and reproductive effects it was 800 mg/kg bw/day.

Groups of 25 mated female rats were dosed dermally with 0 and 2000 mg/kg bw ditridecyl adipate following the same study protocol as above. Again, there were no signs of maternal toxicity. No developmental toxicity was reported, and there were no visceral anomalies or levocardia.

Tridecyl adipate, 2000 mg/kg bw, was applied, unoccluded, to groups of 10 male Sprague-Dawley rats, 5 days/wk for 13 wks, and the effect on sperm morphology was evaluated.[87] (The 'Subchronic Dermal Toxicity' study was described earlier.) No differences in sperm morphology were observed between control and test animals.

Diisobutyl Adipate

The fetotoxic and teratogenic effects of diisobutyl adipate were evaluated following the procedure described in the earlier i.p. study.[114] These rats were dosed i.p. with 0.1983-1.9833 ml/kg diisobutyl adipate. The numbers of live and dead fetuses were not statistically significantly different between treated and blunt-needle control groups, but there was a statistically significant decrease in the mean fetal weight of the 1.1900 and 0.9833 ml/kg dose groups. The number of resorptions was similar between treated animals and both the blunt needle and pooled controls. Gross abnormalities, but not skeletal or visceral, were statistically significantly increased in fetuses of the 0.5950 and 1.9833 ml/kg groups.

Diethylhexyl Adipate

The reproductive effects of diethylhexyl adipate were studied in Swiss mice. Groups of 10 male mice were dosed i.p. with ≤9.3 g/kg and then mated with undosed females. A reduction in the number of gravid females was considered an anti-fertility effect, and the dominant lethal mutation was determined directly from the dose-dependent increase in the number of early fetal deaths and indirectly from the dose- and time-dependent decrease in implantations. There were no test article-related changes in the incidence of late fetal deaths. It was noted that the experimental design and interpretation have been questioned by some. Diethylhexyl adipate, ≤9.3 g/kg, was administered by i.p. injection to groups of 5 gravid Sprague Dawley rats on day 5, 10, and 15 of gestation. Resorption rates were similar to controls. A decrease in the mean fetal body

weight and a significant increase in gross fetal abnormalities at the high dose were observed when compared to pooled control values. However teratogenic effects were not observed when compared to concurrent controls. It was stated that the lack of historical and positive controls affected the validity of the results.[2]

Groups of 15 male and 30 female Wistar rats were fed a diet containing 0, 0.03, 0.18, or 1.2% diethylhexyl adipate (calculated as 28, 170, or 1080 mg/kg bw/day) for 10 weeks prior to mating.[87] Dosing was terminated, and the animals were mated. (A different source indicated that dosing continued throughout the study).[128] A reduction in body weight gain was reported during gestation for the dams of the 1.2% group. No test article-related effects on fertility were observed. Fetal weight, total litter weight, and litter size were reduced in the 1.2% group, but the number of pups born live, or their survival, was not affected. The NOAEL was 170 mg/kg bw/day and the LOAEL was 1080 mg/kg bw/day.

In another study in which gravid females were fed the same doses as above on days 1-22 of gestation, maternal body weight and feed consumption were statistically decreased in the 1.2% group. No significant effects on fetal weight or litter size were reported. Animals of the 0.18 and 1.2% groups had slightly increased incidences of minor skeletal abnormalities; this increase was attributed to fetotoxicity. The NOEL for maternal toxicity was 170 mg/kg bw/day. The NOAELs for developmental toxicity and fetotoxicity were 170 and 28 mg/kg bw/day, respectively. The LOAEL was 1080 mg/kg bw day.

A dose-range finding study was performed using groups of 8 gravid Wistar rats that were dosed by gavage with 2 ml/kg of 0, 800, or 1200 mg/day diethylhexyl adipate, in peanut oil, from day 7 of gestation until day 17 after parturition.[129] No signs of toxicity were reported in any of the groups. In the 800 mg/kg bw group, the only statistically significant observation made was decreased body weights of male and female pups on day 3. In the 1200 mg/kg bw group, statistically significant effects were observed for a number of parameters, including decreased maternal weight gain during days 7-21 of gestation, increased length of gestation (by 1 day), decreased pup body weights at birth and day 3, and an increase in perinatal loss per litter. (Perinatal loss was 42% in the 1200 mg/kg bw groups, as compared to 4.6% in controls.)

Based on the results of the dose-range finding study, groups of 20 gravid Wistar rats were dosed with 2 ml/kg of 0, 200, 400, or 800 mg/kg bw diethylhexyl adipate, in peanut oil, from day 7 of gestation until post-natal day 17. At postnatal day 21, all dams and pups were killed, with the exception that one male and one female pup per litter was kept for further evaluation. No signs of toxicity were reported in any of the groups. No significant effects were observed in the 200 mg/kg bw group. In the 400 mg/kg bw dose groups, the number of postnatal deaths per number of pups was statistically significant increased. In the 800 mg/kg bw group, statistically significant effects were observed for a number of parameters, including increased length of gestation (by 1 day), decreased pup body weights at birth and days 3 and 13, increased mean number of postnatal deaths, and an increase in postnatal death per number of pups. The percentage of perinatal loss per litter was twice as high in the 400 and 800 mg/kg bw groups (23%) as compared to controls (11%), but the change was not statistically significant. Testicular testosterone levels were unaffected in any of the pups that were killed on postnatal day 21 or the adult male offspring, and serum luteinizing hormone and prolactin levels were similar to controls. None of the sperm parameters that were evaluated were affected by dosing. The only statistically significant effects, noted in the 800 mg/kg bw group, were increased relative liver to body weights in male pups on day 21 and increased body weights and decreased adrenal weights in adult male offspring. Diethylhexyl adipate did not produce any antiandrogenic effects in the study. Fetal steroidogenesis was not evaluated. NOAEL was 200 mg/kg bw.

Groups of 10 female Crl:CD(SD) rats were dosed with 5 ml/kg, by gavage, of 0, 200, 1000, or 2000 mg/kg bw diethylhexyl adipate in corn oil for 2 wks prior to mating with undosed males, throughout mating, and until day 7 of gestation.[120] The dams were killed on day 14 of gestation. All animals survived until study termination. Body weights and

body weight gains were significantly decreased in the 2000 mg/kg bw dose group prior to mating. Staining around the perineum was observed in the 1000 and 2000 mg/kg bw dose groups. No statistically significant differences were observed for the 200 mg/kg bw group compared to controls. The mean estrous cycle length was statistically significantly increased in the 1000 and 2000 mg/kg bw groups, and the post-implantation loss rate was also statistically significantly increased in these groups. Additionally, in the 2000 mg/kg bw group, there was a significant decrease in implantation rate, and the number of live embryos was statistically significantly decreased and the pre-implantation loss rate statistically significantly increased. The researchers stated that the effects observed in this fertility study, in conjunction with the ovarian effects described earlier in the repeated dose study, suggest that diethylhexyl adipate disturbed ovulation. This correlated with the effect on estrous cycle length.

The testicular toxicity of diethylhexyl adipate was examined using male F344 rats.[130] Groups of six rats were fed a diet containing 6000 or 25,000 ppm diethylhexyl adipate for 4 wks, and the controls were given untreated feed. Some groups were dosed i.p. with 200 mg/kg bw thioacetamide, 3x/wk for 4 wks, and prior to dosing with diethylhexyl adipate to evaluate whether liver disease enhanced testicular effects. (There was a 1-wk rest period prior to dosing with diethylhexyl adipate.) The final body weights of animals given 25,000 ppm diethylhexyl adipate, with and without prior administration of thioacetamide, were statistically significantly decreased compared to their respective controls. The relative liver to body weights of these animals were statistically significantly increased. No significant effect on the relative weights of the testes or epididymis was seen for any of the test groups. Diethylhexyl adipate did not have any testicular toxic effects, with or without the induction of hepatic damage.

Diisononyl Adipate

In a subchronic dietary study described earlier, groups of male and female Beagle dogs were fed 0, 0.3, 1.0, or 3.0% (wks 1-8) and 6.0% (wks 9-13) diisononyl adipate for 13 wks.[87] Reproductive tissues were evaluated. No significant findings were reported for the 0.3 and 1.0% groups. In the high dose group, testes weight was decreased. At microscopic examination, it was found that the epididymal ducts were devoid of spermatozoa, the seminiferous tubules were composed of Sertoli cells and spermatogonia, spermatocytes and spermatids were not evident, and there was almost total aspermatogenesis. Ovaries were not weighed at necropsy. There were no gross or microscopic changes in the ovaries of the high dose group compared to controls.

Dibutyl Sebacate

A test group of 20 male and 20 female Sprague-Dawley rats was fed a diet containing 6.25% dibutyl sebacate for 10 wks, while a control group of 12 male and 12 female rats were fed the basal diet, and then animals of each group were then mated.[115] The dams were allowed to deliver their litters, and at weaning, 24 male and 24 female offspring were randomly chosen, fed the test diet for 21 days, and then killed for necropsy. The study results indicated that ingestion of a diet containing 6.25% dibutyl sebacate had no adverse effect on fertility, litter size, or survival of offspring. Growth was decreased during the pre-weaning and post-weaning periods. However, no gross pathological changes were found among young rats killed at the end of the 21-day post-weaning period.

Diethylhexyl Sebacate

Reproduction, suckling and growth were normal in a four-generation study of rats fed a diet containing 200 ppm diethylhexyl sebacate (~10 mg/kg bw/day).[1] No reproductive or developmental toxicity was observed.

Dimethyl Glutarate/Dimethyl Succinate/Dimethyl Adipate Mixture

The developmental toxicity produced by the inhalation of dibasic esters (mixture of 65.1% dimethyl glutarate, 17.8%

dimethyl succinate, and 16.8% dimethyl adipate) was evaluated in rats.[131] Groups of 24 gravid Crl:CD rats were exposed for 6 h/day to 0, 0.16, 0.4, or 1.0 mg/l dibasic esters, by whole body inhalation, on days 7-16 of gestation. The aerosol particle size in the 1.0 mg/l chamber was 5.3-5.4 µm, with 72-74% of the aerosol <10 µm. The animals were killed on day 21 of gestation. All animals survived until study termination. Body weight gains were statistically significantly decreased in the 0.4 and 1.0 mg/l groups. Feed consumption by these groups was reduced during the first 6 exposures; statistical significance was not given. Statistically significant differences in absolute and relative liver to body weights were not observed, but there was a significant trend of decreased absolute, but not relative, liver weights. The only significant clinical signs observed were perinasal staining and wet fur of rats in the 1.0 mg/l group. Reproductive and developmental effects were not observed, and the dibasic esters mixture was not a developmental toxicant in rats following inhalation of ≤1.0 mg/l.

Groups of 20 Crl:CD(SD)BR rats/gender were exposed for 6 h/day, 5 days/wk, to 0, 0.16, 0.40, or 1.0 mg/l dibasic esters by whole body inhalation for 14 wks prior to mating, and then 7 days/wk for 8 wks of mating, gestation, and lactation.[132] The mean aerosol particle size in the 1.0 mg/l chamber was 6.2 µm, with 69% of the aerosol <10 µm. Exposure was discontinued from day 19 of gestation through day 3 post-partum. All parental rats and 10 pups/gender were killed and necropsied on day 21 post-partum. The remaining pups were not necropsied. Maternal body weights in the 0.40 mg/l group were decreased during the last week of the study, while body weights of male and female rats of the 1.0 mg/l group were slightly decreased from wk 7 on. Relative liver to body weights were slightly, but not significantly, decreased in the 0.4 and 1.0 mg/l groups. Other differences in organ weights were not considered dose-related. With the exception of a statistically significant decrease in pup body weights at birth and day 21, no reproductive or developmental effects were observed. The only microscopic findings were squamous metaplasia in the olfactory epithelium of all treated parental rats. This effect was minimal in the 0.16 mg/l group and mild to moderate in the 0.4 and 1.0 mg/ml groups. The NOEL for reproductive parameters was 1.0 mg/l.

Endocrine Disruption

Diethylhexyl Adipate

A 28-day repeated-dose toxicity study was performed to determine whether diethylhexyl adipate has endocrine-mediated activities.[133] Groups of 10 male and 10 female Crj:CD (SD) rats were dosed orally by gavage with 0, 40, 200, or 1000 mg/kg bw diethylhexyl adipate in corn oil, at a volume of 10 ml/kg, for a minimum of 28 days. In addition to clinical observations, a functional observation battery was performed during wk 4, estrous cycling was assessed from day 22, hormone analysis was measured at the end of the test period, and sperm morphology and sperm count were examined. Male animals were killed and necropsied on day 29, while females were killed and necropsied on days 30-34 when in diestrous. Signs of toxicity were not observed, and no clinical chemistry or hematological findings were recorded. Hormonal and spermatological analyses were normal. Statistically significant increases were seen in relative kidney to body weights in males of the 200 and 1000 mg/kg bw groups, relative liver to body weights of males in the 1000 mg/kg bw group, and in relative liver, kidney, and adrenal to body weights in females of the 1000 mg/kg bw group. Microscopically, increased eosinophilic bodies and hyaline droplets were seen in the kidneys of male rats of the 1000 mg/kg bw group. Ovarian follicle atresia was observed in 4 females of the 1000 mg/kg bw group, accompanied by a prolonged estrous cycle in 2 of these rats. A change in the estrous cycle is an important endpoint for determination of endocrine-mediated effects in the enhanced TG 407 assay. The researchers stated that this effect, in conjunction with the microscopic findings, appears to be related to endocrine-mediated effects of diethylhexyl adipate. However, it was also stated that these findings may be attributable to the disturbance of ovarian function according to the hypothalamic-pituitary-gonad axis. The changes in relative organ to body

weights were considered toxic effects, and the NOEL was 40 mg/kg bw/day.

The effect diethylhexyl adipate. at concentrations of 1 x 10^{-10} to 5 x 10^{-5} M, on estrogen receptor and thyroid hormone (TH) functions was also examined.[134] The TH-like activity was assessed using the rat pituitary tumor cell line GH3 expressing intracellular TH and estrogen receptors and responding to physiological concentration of TH by proliferation. At "low potency", diethylhexyl adipate stimulated the TH- dependent rat pituitary GH3 cell proliferation in a concentration-dependent manner. Cotreatment of GH3 cells with diethylhexyl adipate potentiated the L-3,5,3'-triiodothyronine (T3)-EC_{50} potentiated the T3-induced GH3 cell proliferation.

GENOTOXICITY

Details of the genotoxicity studies on esters of dicarboxylic acids are described in Table 12. Details of the available genotoxicity data on esterase metabolites are also summarized in this table.

Diethyl Malonate

Diethyl malonate was not mutagenic in an Ames test or a cytogenetic assay using human peripheral lymphocytes at concentrations ≤5000 µg/plate.[14]

Dimethyl Malonate

Dimethyl malonate was not mutagenic in an Ames test at concentrations ≤5000 µg/plate.[14]

Dimethyl Succinate

Dimethyl succinate was not mutagenic in an Ames tests with concentrations of ≤ 20,000 µg/plate[135] or in a preincubation assay with concentrations of ≤10,000 µg/plate.[136]

Dimethyl Glutarate

Dimethyl glutarate was not mutagenic in a preincubation assay with concentrations of ≤10,000 µg/plate.[137]

Dimethyl Adipate

Dimethyl adipate was not mutagenic in a preincubation assay with concentrations of ≤10,000 µg/plate.[138]

Dibutyl Adipate

Dibutyl adipate was mutagenic in an Ames test at concentrations of ≤5000 µg/plate. It was not genotoxic in an in vivo mouse micronucleus assay in which the animals were dosed with ≤2000 mg/kg bw.[5]

Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid

Di-C7-9 branched and linear alkyl esters of adipic acid were not mutagenic in an Ames test at concentrations of ≤10.0 µl/plate.[87]

Ditridecyl Adipate

Ditridecyl adipate was not mutagenic in an Ames test at concentrations of 0-10 µl/plate, and it was not clastogenic in an *in vivo* micronucleus assay using rats dosed dermally with 0, 800, or 2000 mg/kg bw ditridecyl adipate.[87]

Diethylhexyl Adipate

Diethylhexyl adipate was not mutagenic in an Ames (concentrations tested were not provided) test.[2]

Diethylhexyl adipate was not mutagenic in a number of genotoxicity studies. In vitro, negative results were reported in Ames tests at concentrations ranging from ≤150 -10,000 µg/plate,[87,139-141] in an NTP preincubation assay,[142] in a liquid suspension assay,[143] and in a forward mutation assay using L5178Y cells at concentrations ≤1000 µg/ml .[144] In an assay for sister chromatid exchanges and chromosomal aberrations using concentrations of ≤200 µg/plate, results were negative,[145] while in another assay with ≤400 µl/plate, results were negative without, but equivocal with, metabolic activation in the sister chromatid exchange assay and there was some evidence of genotoxicity without, but none with, metabolic activation in the

chromosomal aberration assay.[146] In a ^{3}H-thymidine assay, there was a dose-dependent inhibition of ^{3}H-thymidine incorporation into replicating DNA, with a dose-dependent increase in the ratio of acid-soluble DNA-incorporated ^{3}H-thymidine.[140] *In vivo*, results were negative in micronucleus tests[87,147] and chromosomal aberration assays.[148,149]

An Ames test was performed on urine of rats dosed with diethylhexyl adipate to assess whether mutagenic substances occur in the urine following diethylhexyl adipate adminstration.[150] Groups of ≥6 male Sprague-Dawley rats were dosed orally by gavage with 0 or 2000 mg/kg bw diethylhexyl adipate in corn oil for 15 days. Urine was collected daily. The urine was not mutagenic in the Ames test, indicating that diethylhexyl adipate is not converted to mutagenic urinary metabolites. The urine of rats dosed with 1000 mg/kg bw 2-ethylhexanol by gavage for 15 days was also tested in an Ames assay. The urine of these rats also was not mutagenic. Urine from rats that were dosed with a known mutagen gave a positive response in an Ames test.

Diisononyl Adipate

Diisononyl adipate was not mutagenic in an Ames assay at ≤1000 µg/plate, and it was not genotoxic in a mouse lymphoma assay, a transformation assay, or a BALB/3t3 assay at concentrations of ≤100, 1000, or 1.3 µg/ml, respectively.[151]

Diethyl Sebacate

Diethyl sebacate was non-mutagenic in an *Escherichia coli* Sd-4-73 reversion (streptomycin dependence to independence) assay.[152]

Dibutyl Sebacate

Dibutyl sebacate, ≤10,000 µg/plate, was not mutagenic in the Ames assay.[153,154]

Diethylhexyl Sebacate

Diethylhexyl sebacate was not mutagenic in an Ames assay at concentrations of ≤10,000 µg/plate.[139,155] In the rat liver foci test, diethylhexyl sebacate demonstrated no evidence of promotion activity when administered orally at 500 mg/kg bw 3x/wk for 11 wks, following a single oral treatment with a known carcinogen.[156]

CARCINOGENICITY

Diethylhexyl Adipate

In an NTP carcinogenicity study, administration of ≤25,000 ppm diethylhexyl adipate to rats in the diet for 103 wks did not produce carcinogenic effects. However, mice fed the same amount for 103 wks had dose-related body weight reductions and a higher incidence of hepatocellular adenoma and carcinoma than the controls. In another study in which rats were fed ≤2.5% diethylhexyl adipate for 2 yrs, tumor incidence for the test animals was similar to that of controls. The same researchers found no tumors in dogs fed up to 0.2% diethylhexyl adipate for 1 yr. A single 10 mg dose of diethylhexyl adipate given by s.c. injection was not carcinogenic in mice. In a lifetime study, diethylhexyl adipate caused no skin tumors when 10 mg was applied weekly to the back skin of mice.[2]

Research has shown that other compounds with a 2-ethylhexyl group that have been evaluated for carcinogenicity had some evidence of hepatocarcinogenicity, ranging from very strong to equivocal, in rodents. [157]

In an evaluation of the carcinogenic risk of diethylhexyl adipate, the IARC stated that there was limited evidence in experimental animals for the carcinogenicity of diethylhexyl adipate.[17] Therefore, the overall evaluation of diethylhexyl adipate was *not classifiable as to its carcinogenicity to humans (Group 3).*

Diethylhexyl Sebacate

No evidence of carcinogenicity was observed in an unspecified number of rats fed a diet providing about 10 mg diethylhexyl sebacate/kg/day for up to 19 months.[1] No further study details were provided.

Tumor Promotion

Diethylhexyl Adipate

A group of 14 male F344 rats were used to assess the carcinogenic potential of diethylhexyl adipate in a medium-term liver bioassay.[158] The rats were given a single i.p. dose of diethylnitrosamine, and 2 wks later they were given 20,000 ppm diethylhexyl adipate in the diet. At wk 3, a partial hepatectomy was performed. Positive results for carcinogenic potential were indicated by a significant increase in GST-P positive foci. Diethylhexyl adipate did not have an enhancing effect on the development of GST-P-positive foci.

CLINICAL ASSESSMENT OF SAFETY

Human Exposure

Diethylhexyl Adipate

Diethylhexyl adipate can migrate into food, and it is most marked when plasticized PVC film comes in direct contact with fatty foods.[90] Using the analyses of a range of typical food, a maximum intake of 16 mg/person/day for diethylhexyl adipate was estimated. The amount of diethylhexyl adipate used in PVC films was reduced, and the maximum intake estimate was revised to 8.2 mg/day.

Dibutyl Sebacate

Dibutyl sebacate, a component of PVC, can pass from the packing films to the enclosed food.[43]

Dermal Irritation and Sensitization

Clinical dermal irritation and sensitization data on esters of dicarboxylic acids are presented in Table 13. The available dermal irritation and sensitization data on esterase metabolites are also summarized in this table.

Dimethyl Malonate

The sensitization potential of 8% dimethyl malonate in petrolatum was evaluated in a maximization test using 25 subjects.[14] Dimethyl malonate was not a sensitizer.

Dibutyl Adipate

Undiluted dibutyl adipate was not irritating in a 24-hr clinical patch test with 10 subjects. Slight reactions (not defined) were reported for 4 of 18 subjects in a 24-h patch test with dibutyl adipate, 20% in alcohol.[5]

Diisopropyl Adipate

The dermal irritation and sensitization of diisopropyl adipate was evaluated in a number of studies. Undiluted diisopropyl adipate produced no irritation in 4 h patch tests, but was moderately irritating in a 21-day cumulative irritancy test. Formulations containing 0.26-20.75% diisopropyl adipate caused minimal to mild irritation, but no sensitization.[2]

Diethylhexyl Adipate

The dermal irritation and sensitization of diethylhexyl adipate was evaluated in a number of studies with formulations containing 0.01-9% diethylhexyl adipate. Mild reactions were seen with a formulations containing 0.01%. Using a formulation containing 0.7%, on subject reacted strongly following the second challenge, with erythema and papules observed. Strong reactions were seen for 3 subjects in a patch test of a formulation containing 9.0% diethylhexyl adipate.[2]

Diisostearyl Adipate

A human repeat insult patch test (HRIPT) using 50 subjects was used to evaluate the irritation and sensitization potential of diisostearyl adipate.[159] Two-tenths ml was applied neat to the back of each subject under an occlusive patch for 24 h, after which time the subject removed the patch. This procedure was performed 3 times per wk for 3 wks, for a total of 9 induction patches. Following a 10-14 day non-treatment period, a 24 h challenge patch was applied to a previously untreated

site, and reactions were scored at 24 and 48 h. No adverse reactions were observed, and diisostearyl adipate was not a primary irritant or a sensitizer.

Diethyl Sebacate

A single insult occlusive patch test (SIOPT) was performed using 20 subjects to determine the irritation potential of a body cream containing 1.5% diethyl sebacate.[160] The test patch was applied for 24 h. The PII was 0.00, and the body cream containing 1.5% diethyl sebacate was non-irritating.

The sensitization potential of a body cream containing 1.5% diethyl sebacate was evaluated in a maximization study.[161] During induction, 0.05 ml of 0.25% aq. sodium lauryl sulfate (SLS) was applied under an occlusive patch for 24 h. At that time, the patch was removed and 0.05 ml of the test material was applied to the same site under an occlusive patch for 48-72 h. If no irritation was present at the test site upon patch removal, an occlusive patch with 0.25% aq. SLS was applied for 24 h, followed by a patch of the test material. This sequence was used for 5 induction patches. If irritation developed during induction, the SLS patch was eliminated. After a 10-day non-treatment period, a challenge was performed at a previously untreated site. The challenge site was pretreated with 0.05 ml of 5.0% aq. SLS under an occlusive patch for 1 h, followed by an occlusive patch of the test material for 48 h. Twenty-five subjects completed the study. No reactions were seen at challenge, and a body cream containing 1.5% diethyl sebacate did not have contact-sensitizing potential.

Diisopropyl Sebacate

An SIOPT was performed using 20 subjects to determine the irritation potential of a foundation containing 1.8% diisopropyl sebacate.[162] The patch was applied for 24 h. The foundation containing 1.8% diisopropyl sebacate was not irritating.

The irritation and sensitization potential of diisopropyl sebacate was evaluated in a patch test that consisted of four 24-h applications of diisopropyl sebacate as supplied (approximately 100%) during weeks 1, 2, 3, and 6 on a 2 cm x2 cm area of skin on the right upper arm of each subject.[163] Examinations were performed immediately after patch removal. The induction phase was performed during wks 1-4 using 107 subjects. No clinically significant effects were detected on any of the subjects during this phase. During wk 6, the challenge phase was conducted on 105 subjects. No clinically significant effects were noted in any of the subjects during this phase. Diisopropyl sebacate was not observed to have any significant skin-irritating or sensitizing activity under the conditions of this study.

A maximization assay was performed, using a modified protocol of the maximization assay procedure described earlier, to determine the contact-sensitization potential of a foundation containing 2.2% diisopropyl sebacate.[164] In this study, the test material was allowed to volatilize for 30 min before the occlusive patch was applied. Twenty-five subjects completed the study. No reactions were seen at challenge, and a foundation containing 2.2% diisopropyl sebacate did not have contact-sensitizing potential.

Two heat protection hair spray products containing 1% diisopropyl sebacate were tested using a modified Draize HRIPT procedure to determine the potential of those products to induce irritation and contact sensitization.[165] The products were tested neat and allowed to volatilize prior to patch application. Samples were patched under semi-occlusive conditions. Approximately 0.2ml was used in each patch. One hundred ten subjects completed the study. Generally transient, barely perceptible (0.5-level) to mild (1-level) patch test responses on 22 test subjects for one formulation and only barely perceptible (0.5-level) patch test response on 15 test subjects with the other formulation during the induction and/or challenge phases of the study were reported. Both products were considered to be non-irritating and non-sensitizing.

A heat protection hair spray product containing 7.2% diisopropyl sebacate was tested using an HRIPT to determine

the potential of this product to induce irritation and contact sensitization.[166] The product was tested neat under semi-occlusive conditions. Approximately 0.2 ml sample was used in each patch. Fifty-one subjects completed the study. No skin reactivity was observed in any of the test subjects during the course of the study.

Diethylhexyl Sebacate

Diethylhexyl sebacate was applied neat using occlusive patches to the skin of 15-30 subjects (sex not specified) for 48-h.[1] No local reactions were observed in the challenge phase (48-h covered contact with neat liquid) that was carried out 2 weeks later, presumably due to limited induction.

Dioctyldodecyl Dodecanedioate

An HRIPT with 50 subjects was performed to evaluate the irritation and sensitization potential of dioctyldodecyl dodecanedioate.[159] Two-tenths ml of the test material, neat, was applied to the back of each subject under an occlusive patch for 24 h, after which time the subject removed the patch. This procedure was performed 3 times per wk for 3 wks, for a total of 9 induction patches. Following a 10-14 day non-treatment period, a 24 h challenge patch was applied to a previously untreated site, and reactions were scored at 24 and 48 h. No adverse reactions were observed, and dioctyldodecyl dodecanedioate was not a primary irritant or a sensitizer.

Diisocetyl Dodecanedioate

An HRIPT with 50 subjects was performed as described above to evaluate the irritation and sensitization potential of diisocetyl dodecanedioate.[159] No adverse reactions were observed, and diisocetyl dodecanedioate was not a primary irritant or a sensitizer.

Phototoxicity and Photosensitization

Dibutyl Adipate

Dibutyl adipate, as a 10% dilution in liquid paraffin, was not phototoxic in a clinical phototoxicity study using 30 subjects.[5]

Diisopropyl Adipate

In photopatch test studies using 49-98 subjects, formulations containing 0.7-17.0% diisopropyl adipate were not phototoxic, primary irritants, or sensitizers.[2]

Diethylhexyl Adipate

In a photopatch test on 9.0% diethylhexyl adipate using 25 subjects, no phototoxic or photoallergic reactions were observed.[2]

Ocular Irritation

Dibutyl Adipate

Dibutyl adipate, 0.1% in paraffin oil, was not an ocular irritant in two subjects.[5]

Comedogenicity

Dibutyl Adipate

Dibutyl adipate, 10-100% (vehicle not stated), was not comedogenic in clinical testing.[5]

Case Reports

A number of investigators have reported cases of allergic contact dermatitis in response to diethyl sebacate-containing products, and have demonstrated diethyl sebacate to be the substance, or one of several substances in the products, eliciting the dermatitis.[39,167-171] Two case studies were reported of allergic reactions to lotion containing diisopropyl sebacate.[172,173] In a case study where one patient was sensitized to other sebacate esters, diethylhexyl sebacate was not irritating.[172] For

stearyl alcohol, a metabolite of distearyl succinate, contact sensitization was reported in 3 individuals.[174] These case reports are described in Table 14.

Risk Assessment

Diethylhexyl Adipate

According to the Integrated Risk Information System of the EPA, the weight-of-evidence classification for diethylhexyl adipate was "possible human carcinogen".[128] The classification was based on an absence of human data and increased liver tumors in female mice. The only genotoxic effect was a positive dominant lethal assay. It was noted that diethylhexyl adipate exhibits structural relationships to other non-genotoxic compounds that are classified as probable and possible carcinogens.

SUMMARY

This safety assessment includes sebacic acid and other alkyl α,ω-dicarboxylic acids, salts, monoesters and diesters, for a total of 56 ingredients. The dicarboxylic acids are terminally functionalized straight alkyl chains characterized by a separation between the acid functional groups of one to 10 carbons. The simple alkyl di-esters are the result of the condensation of alkyl dicarboxylic acids and two equivalents of alkyl alcohols. These ingredients can be metabolized via hydrolysis back to the parent alcohol, the mono-ester, and the parent dicarboxylic acid. The simple alkyl esters (mono- and di-) of these dicarboxylic acids have straight or branched side chains ranging in length from one to 18 carbons. This safety assessment is divided into two parts – (1) 12 dicarboxylic acids and their salts and (2) 44 esters of dicarboxylic acids.

A safety assessment of diethylhexyl adipate (called dioctyl adipate at the time of that assessment) and diisopropyl adipate was published in 1984 with the conclusion that these ingredients are safe as used in cosmetics. This conclusion was reaffirmed in 2006. Additionally, dibutyl adipate was previously reviewed in 1996 and the available data were found insufficient to support the safety of dibutyl adipate in cosmetic formulations. When re-reviewed in 2006, additional data were made available to address the needs identified by the CIR Expert Panel, and an amended conclusion was issued stating that dibutyl adipate is safe for use in cosmetic formulations.

While many of the alkyl dicarboxylic acids occur in natural products, commercial production of these acids has historically occurred via alkali pyrolysis of lipids.

A relationship exists between the molecular weight and the log octanol – water partioning coefficient. Physical properties change as chain length increases, and the water solubility of these acids is inversely proportional to their chain length. Odd versus even chain length also plays a role. The alternating effects are believed to be the result of the inability of odd carbon number compounds to assume an in-plane orientation of both carboxyl groups with respect to the hydrocarbon chain. The diesters, in contrast, are much more lipid soluble and more difficult to dissolve in water. The short-chain alkyl mono- and diesters are more soluble in water, less lipophilic, and relatively more volatile than the corresponding longer-chain alkyl esters.

The ingredients included in this review would not be expected to have any meaningful ultraviolet absorption.

The ingredients in this report function in cosmetics as pH-adjusters, fragrance ingredients, plasticizers, skin-conditioning agents and/or solvents and corrosion inhibitors. The majority of the dicarboxylic acids function in cosmetics as pH adjusters or fragrance ingredients. Six of the 12 dicarboxylic acids and their salts and 24 of the 44 esters included in this safety assessment are reported to be used in cosmetic formulations. For the dicarboxylic acids and their salts, disodium succinate has the greatest number of reported uses, with a total of 45. The acid with the greatest concentration of use is succinic acid, 26%; use at this concentration is in a bath product that will be diluted during use. The greatest leave-on concentration is

0.4%, disodium succinate, with dermal contact exposure. For the esters, diisopropyl adipate has the greatest number of uses, with 70 reported. The concentration of use is greatest for dimethyl glutarate, 15% in a dermal rinse-off product. The ingredients with the greatest leave-on use concentrations, which are all dermal contact exposures, are diethylhexyl adipate, 14%, diisostearyl adipate, 10%, and diisopropyl sebacate, 10%.

Dicarboxylic Acids and Their Salts

Dicarboxylic acids are natural metabolic products of the ω-oxidation of monocarboxylic acids when the β-oxidation of free fatty acids is impaired. Under normal physiological conditions, dicarboxylic acids are rapidly β-oxidized, resulting in very low cellular concentrations and practically non-detectable concentrations in the plasma. Oxidation of odd- and even-numbered chains proceeds to different end points; even chains are completely, while odd-number chains are not completely, oxidized.

Unchanged dicarboxylic acid was found in the urine of rats. With oral dosing, approximately 53-67% adipic acid, 40% azelaic acid, and 50% dodecanedioate was recovered with the respective acid. With i.v. dosing, 59-71% adipic acid and 35% sebacate was recovered. In humans, 6.76-61 adipic acid, and 61% azelaic acid were found in the urine after dosing with the respective acid. With azelaic acid and dodecanedioic acid, radioactivity was found in all tissues, and it decreased after 24 h in all tissues except adipose tissue. Radioactivity was found in expired carbon dioxide at 24 h after dosing adipic acid (70%), azelaic acid (14.5%), and disodium sebacate (25%). For rats dosed orally with azelaic, sebacic, undecanedioic, and dodecanedioic acid, 2.5, 2.1, 1.8, and 1.6% of the respective acid was found in the urine unchanged. The amount recovered decreased with increasing chain length. After oral dosing, 60, 17, 5, and 0.1% of azelaic, sebacic, decanedioic, and undecane-dioic acids, respectively, were recovered unchanged in the urine. In the plasma of both animals and humans, dicarboxylic acid catabolites that were 2-, 4-, or 6- carbons shorter than the corresponding dicarboxylic acid were found.

Adipic acid did not induce peroxisome proliferation. Dicarboxylic acids did have some cellular effects and inhibited mitochondrial oxidoreductases, reversibly inhibited microsomal NADPH and cytochrome P450 reductase, and competitively inhibited tyrosinase *in vitro*.

The oral LD_{50} values of the dicarboxylic acids had a wide range; for example, adipic acid had values in rats ranging from 0.94 g/kg to greater than the highest dose tested (11 g/kg). Most reported values for the acids were >2 g/kg. The reported dermal LD_{50} values ranged from >6 g/kg dodecanedioic acid to >10 g/kg glutaric acid.

In short-term oral toxicity studies, ≤3000 mg/kg bw/day adipic acid did not produce significant toxicological effects in rats. Signs of toxicity were seen at >3600 mg/kg bw/day. No toxicity was observed with guinea pigs fed 400-600 mg/day adipic acid. Short-term inhalation exposure to 126 mg/m^3 adipic acid to rats did not produce signs of toxicity, but exposure of mice to 460 mg/m^3 resulted in decreased weight gain and produced effects in the upper respiratory tract, liver, kidneys, and central nervous system.

In a subchronic oral study, 10 male and 10 female rats exposed to 10% sodium succinate in the drinking water died, but no compound-related lesions were found. Body weights were decreased in rats given ≥2.5% sodium succinate for 13 wks, but toxicological treatment-related changes were not observed. Glutaric acid had a low degree of toxicity to rats (at 2%) and dogs (concentration not specified) when given in the feed. Dietary administration of ≤3400 mg/kg bw/day adipic acid for 19 wks produced slight effects in the liver of male rats; the NOAEL was 3333 mg/kg bw. A mixture of adipic, glutaric, and succinic acids had a low degree of toxicity in rats when tested at 3% for 90-days. Signs of toxicity were reported in a sub-chronic inhalation study in which mice were exposed to 13 or 120 mg/m^3 adipic acid.

Slight effects were seen in the livers of rats fed ≤3200 mg/kg bw/day adipic acid for 33 wks, and the NOAEL for rats

fed a diet containing adipic acid for 2 yrs was 1%; no significant toxicological effects were seen at concentrations of ≤5%. No significant toxicological effects were observed for mice fed ≤280 mg/kg bw or rabbits fed ≤400 mg/kg bw azelaic acid for 180 days. Disodium sebacate was not toxic to rats or rabbits fed up to 1000 mg/kg bw for 6 mos.

For the dicarboxylic acids, the severity of ocular irritation seems to decrease with increasing carbon number. Succinic acid was a severe ocular irritant, glutaric acid was moderately irritating, and dodecanedioic acid was a slight irritant. Ocular irritation produced by adipic acid was dose-dependent. Slight to mild dermal irritation was observed in rabbits for succinic, glutaric, and adipic acid, while dodecanedioic acid was not an irritant in rabbits. Using guinea pigs, adipic acid, dodecanedioic acid, and a mixture of succinic, glutaric, and adipic acids are not sensitizers.

Reproductive and developmental effects were not seen upon oral dosing with the dicarboxylic acids or disodium sebacate. Malonic acid, at 0.1% *in vitro*, has a spermicidal effect on human spermatozoa. Glutaric acid was tested at doses of ≤1300 mg/kg bw in rats and 500 mg/kg bw in rabbits, adipic acid at doses of ≤263 mg/kg bw in mice, 288 mg/kg bw in rats, 205 mg/kg bw in hamsters, or 250 mg/kg bw in rabbits, azelaic acid at doses of ≤140 mg/kg bw in rats and 200 mg/kg bw in rabbits, disodium sebacate at 500 mg/kg bw in rats and 1000 mg/kg bw in rabbits, and dodecanedioic acid was tested at ≤1000 mg/kg bw using rats. Embryotoxic effects were reported in a reproductive study of 2500 mg/kg bw/day azelaic acid using rats and in reproductive studies with ≤500 mg/kg bw/day azelaic acid using rabbits and monkey. *In vitro*, sodium salts of some dicarboxylic acid had a specific inhibitory effect on muscle activity of the uterine horn, and this effect progressively increased with chain length.

The dicarboxylic acids are not genotoxic, and consistently were not mutagenic in Ames tests. Positive results were seen in a transformation assay on glutaric acid using Balb/c-3T3 cells, both with and without metabolic activation. The results of a mouse lymphoma assay, with and without metabolic activation, on glutaric acid were negative in a neutral pH range. Equivocal results were obtained in an *in vitro* chromosomal aberration assay of ≤15 mg/ml disodium succinate using Chinese hamster fibroblast cells. The dicarboxylic acids were not genotoxic in *in vivo* assays.

Carcinogenicity was not seen in rats given up to 2% sodium succinate in the drinking water or 5% adipic acid in feed for 2 yrs. An increase in the incidence of C-cell adenoma/carcinoma of the thyroid in females given 2% sodium succinate, and a positive trend in the occurrence of this tumor, was considered a function of experimental variability and not related to dosing. Adipic acid was not carcinogenic when given orally to rats at up to 5% in the diet.

In a cumulative irritancy test, the cumulative irritation of a 15% azelaic acid gel increased with successive patching. It is not known if the vehicle played a role in the irritation scores. Daily application of a 20% azelaic cream causes erythema and irritation.

Esters of Dicarboxylic Acids

The metabolism of diesters in animals is expected to occur, initially, via enzymatic hydrolysis, leading to the corresponding dicarboxylic acids and the corresponding linear or branched alcohol. These dicarboxylic acids and alcohols can be further metabolized or conjugated to polar products that are excreted in urine, or, the enzymatic hydrolysis may be incomplete and result, at least for some diesters, in the production of monoesters.

In *in vitro* absorption studies using pig skin, 8.8 and 3% of undiluted diethyl malonate was found in the skin and receptor fluid, respectively, after 50 h. Absorption was enhanced when diethyl malonate was diluted with ethanol and reduced when diluted in acetone. Using human skin, 16% of the applied diethyl malonate penetrated in 24 h. In vivo, absorption of diethyl malonate, estimated from urinary and fecal recovery, was 15% in nude mice, 4% in human skin grafted to nude mice, 6% in pig skin grafted to nude mice, 2.5% in pigs, and 4% in dogs.

Approximately 11% of ditridecyl adipate was absorbed through the skin of rats; 5.5-7.4% of the applied dose was found in the tissues, 3.5-4.7%was found in the urine, and 0.4-0.7% was found in the feces after 4 days. Prior dosing with ditridecyl adipate did not significantly affect absorption.

In vitro, diethylhexyl adipate was readily hydrolyzed to mono-(2-ethylhexyl) adipate (MEHA) or adipic acid in rat liver, pancreas, and small intestine tissue preparations. In rats, diethylhexyl adipate is hydrolyzed to adipic acid and 2-ethylhexanol or MEHA. 2-Ethylhexanol is converted to 2-ethylhexanoic acid, which may form a glucuronide conjugate or may be subjected to ω- and (ω-1)-oxidation and further metabolism. More than 98% of diethylhexyl adipate administered orally to rats was excreted in 48 h; 21-45% of the radioactivity was expired in carbon dioxide and 34-52% was excreted in the urine. Diethylhexyl adipate and MEHA are not found in the blood or urine; diethylhexyl adipate or the metabolites are recovered in the tissues. Metabolism studies have shown that excretion in the urine is not as unchanged diethylhexyl adipate; mostly adipic acid is found. In humans, peak urinary elimination of all metabolites occurred within 8 h of dosing.

Diethylhexyl sebacate is not readily absorbed through the skin of guinea pigs. Metabolism in rodents and humans may follow partially common pathways, producing 2-ethylhexanol as an intermediary metabolite.

Diethylhexyl adipate is a peroxisome proliferator requiring extensive phase I metabolism to produce the proximate peroxisome proliferator, which in both mice and rats appears to be 2-ethylhexanoic acid. Diethylhexyl adipate is not as potent a proliferator as diethylhexyl phthalate. Peroxisome proliferation causes an increase in liver weights and can induce hepatocarcinogenicity in rats and mice. Peroxisome proliferation is not believed to pose the risk of inducing hepatocarcinogenesis in humans, as a species difference in response to peroxisome proliferators exists.

Diethylhexyl adipate did not bind covalently to hepatic DNA in mice. It did stimulate DNA synthesis in livers of rats. In another study, a statistically significant increase in 8-OH-dG occurred in the liver DNA, but not the kidney DNA, at wk 1 and 2. The IARC remarked that the weight of evidence for diethylhexyl adipate demonstrated that rodent peroxisome proliferators do not act as direct DNA-damaging agents.

The oral and dermal LD_{50} values are greater than 2 g/kg. No mortality occurred in rats exposed to concentrated vapors of diethyl malonate diethyl succinate, dibutyl adipate, or diethylhexyl adipate for 8 h. Some deaths, possibly due to thermal decomposition were seen in rats and rabbits exposed to 940 mg/m3 for 7 h. In a 4-hr inhalation toxicity study, a mixture of dimethyl glutarate, dimethyl succinate, and methyl adipate, the anterior and posterior nasal passageways were affected.

Oral administration of ≤1000 mg/kg bw dibutyl adipate for 28 days did not produce toxic effects in rats. In short-term oral dosing with diethylhexyl adipate, decreased weight gain was reported for rats and mice. The NOELs for rats and mice were 2 and 0.63%, respectively, in feed; 5/5 female mice fed 10% dibutyl adipate in feed died. In 2- and 4-wk studies of diethylhexyl adipate, the oral NOAEL for ovarian toxicity was 200 mg/kg bw in rats; an increase in atresia of the large follicle and a decrease in currently formed corpora lutea were seen in females dosed with 1000 and 2000 mg/kg bw diethylhexyl adipate.

In a short-term dermal study in which 10 rabbits were dosed dermally with 0.5 or 1.0 ml/kg of a 20% dispersion of dibutyl adipate for 6 wks, there was a significant decrease in body weights in the high dose group, and renal lesions in one animal of each group. There were no signs of toxicity in guinea pigs in an immersion study with 20.75% diisopropyl adipate, diluted to an actual concentration of 0.10% adipate. Dermal administration of diethylhexyl adipate to rabbits for 2 wks resulted in slight to moderate erythema at the test site, but toxic effects were not reported for most of the animals.

In a 90-day oral toxicity study in which rats were fed 36-41 mg/kg bw diethyl malonate, no treatment-related effects were observed. Dietary administration of ≤2.5% di-C7-9 branched and linear alkyl esters of adipic (approx. 1500 and 1900

mg/kg bw/day for males and females, respectively) for 90 days did not result in systemic toxicity. The NOAELS for male and female rats were 1500 and 1950 mg/kg bw/day, respectively. Subchronic oral administration of diethylhexyl adipate to rats caused significant decreases in body weight gains and increases in liver and kidney weights. The dietary NOEL for rats in a 90-day study was 610 mg/kg bw. A decrease in body weights was seen in mice fed a diet with 1.2 and 2.5% diethylhexyl adipate. For diisononyl adipate, dietary administration of up to 500 mg/kg bw to rats for 13 wks resulted in a statistically significant increase in relative kidney weights, but there were no toxicological findings. With dogs, 3.0% dietary diisononyl adipate resulted in a decrease in body weights, testes weight, and feed consumption, increased liver weight, elevated enzyme levels, liver and kidney discoloration, and microscopic changes in the liver, testes, spleen, and kidneys.

No adverse effects were reported with whole-body application of a 6.25% emulsion of dibutyl adipate to dogs 2x/wk for 3 mos. Unoccluded dermal application of up to 2000 mg/kg bw ditridecyl adipate for 13 wks to rats produced slight erythema, but no systemic toxicity.

In a 6-month study in which rats were dosed intragastrically with diethylhexyl adipate, hepatic detoxification appeared depressed at the beginning of the study, while in a 10-mos study, a decrease in central nervous system excitability was noted. Dietary administration of ≤1.25% dibutyl sebacate for 1 yr or ≤6.25% for 2 yrs did not have an effect on growth

Ocular irritation appeared to lessen in severity as chain length of the dicarboxylic acid esters increased. Undiluted diethyl malonate was slightly to moderately irritating to rabbit eyes. Dibutyl, diisopropyl, and diethylhexyl adipate, at concentrations ranging from 0.1 – 100%, were non- or minimal ocular irritants. Diisopropyl sebacate was minimally irritating. Diethylhexyl sebacate was non-irritating in an MTT viability assay. Undiluted dioctyldodecyl and diisocetyl dodecanedioate were not irritating to rabbit eyes.

The esters of dicarboxylic acids were mostly non- or mildly irritating to rabbit skin. Some minimal irritation was seen with diisopropyl adipate, undiluted or at 5-20.75% in formulation, and moderate erythema was reported with undiluted dibutyl adipate. Dimethyl malonate, dibutyl and diethylhexyl adipate, diethylhexyl sebacate, and dioctyldodecyl dodecanedioate were not sensitizers in guinea pigs or rabbits. Perfume formulations containing 1.1% diisopropyl adipate were not phototoxic in rabbits.

Oral administration of up to 1000 mg/kg bw dimethyl malonate to Wistar rats did not have an effect on fertility, and no developmental toxicity was reported. The NOAEL was 300 mg/kg bw for repeated dose and maternal toxicity and 1000 mg/kg bw for fertility and developmental toxicity. Oral administration of up to 100 mg/kg bw dibutyl adipate to Sprague-Dawley rats did not cause any reproductive effects, and the NOEL for parental and offspring toxicity was 300 mg/kg bw/day and for reproductive toxicity was 100 mg/kg bw/day. Oral administration of ≤7000 mg/kg bw di-C7-9 branched and linear alkyl esters of adipic acid to Sprague-Dawley rats did not result in developmental toxicity. Dietary administration of up to 1.2% diethylhexyl adipate did not affect fertility when fed to rats prior to mating. Fetal weight, total litter weight, and litter size were reduced with 1.2% diethylhexyl adipate. In a study in which gravid rats were fed the same doses during gestation, no significant effects on fetal weight or litter size were reported. An increased incidence of minor skeletal abnormalities was attributed to fetotoxicity. In a study in which diethylhexyl adipate was given orally to rats from day 7 of gestation until post-natal day 17, antiandrogenic effects were not observed, although some increase in post-natal death was observed. Administration of up to 2000 mg/kg bw diethylhexyl adipate prior to dosing and through day 7 of gestation did have an effect on the mean estrous cycle length at a dose of 1000 and 2000 mg/kg bw, and did appear to disturb ovulation. Significant decreases were also seen in implantation rate and number of live embryos, as well as an increase in pre-implantation loss. Diethylhexyl adipate did not produce testicular toxic effects in male F344 rats when fed at up to 25,000 ppm in the diet for 4 wks. Dietary

administration of 6.25% dibutyl sebacate to male and female Sprague-Dawley rats for 10 wks prior to mating had no adverse effects on fertility, litter size, or survival of offspring. Diethylhexyl sebacate, 200 ppm in the diet, did not produce reproductive or developmental effects in rats.

Dermal applications of 2000 mg/kg bw ditridecyl adipate did not have an effect on sperm morphology. Some visceral anomalies were reported. The NOAELs for maternal toxicity and developmental and reproductive effects were 2000 and 800 mg/kg bw/day, respectively.

Dimethyl, diethyl, dipropyl, dibutyl, diisobutyl, and diethylhexyl adipate were evaluated for fetotoxic and teratogenic effects in rats when administered i.p. at 1/3 – 1/30 of the i.p. LD_{50} values. Some effect on resorptions and abnormalities were seen with all but diethyl adipate.

Inhalation by rats of ≤1.0 mg/l of a mixture of dimethyl glutarate, dimethyl succinate, and dimethyl adipate on days 7-16 of gestation or for 14 days prior to mating, during mating and gestation, and lactation, no adverse developmental or reproductive effects were observed. The only exception was a statistically significant decrease in pup weight at birth and day 21.

Diethylhexyl adipate appeared to have endocrine-mediated effects in Crj:CD (SD) rats in a 28-day oral study; however, it was stated that the findings may be attributable to the disturbance in ovarian function according to the hypothalamic-pituitary-gonad axis. Diethylhexyl adipate simulated thyroid hormone-dependent rat pituitary GH3 cell proliferation in a concentration-dependent manner.

The esters of dicarboxylic acids were not mutagenic or genotoxic in a battery of *in vitro* and *in vivo* tests. The only non-negative results reported were equivocal results in a sister-chromatid exchange assay with ≤400 µg/ml diethylhexyl adipate in the presence of metabolic activation and a dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, with a dose-dependent increase in the ratio of acid-incorporated ^{3}H-thymidine with ≤0.01 M diethylhexyl adipate.. (The same effect was seen in the ^{3}H-thymidine assay with 2-ethylhexanol.)

In an NTP 2-yr dietary study, ≤25,000 ppm diethylhexyl adipate did not produce tumors in male or female rats, but it did increase the incidence of hepatocellular adenoma and carcinoma in male and female mice. Diethylhexyl adipate did not cause skin tumors with weekly application of 10 mg to the back of mice in a lifetime study. Other compounds with a 2-ethylhexyl group that have been evaluated for carcinogenicity had some evidence of hepatocarcinogenicity, ranging from very strong to equivocal, in rodents. Feeding of diethylhexyl sebacate to rats for 19 mos did not result in carcinogenic effects.

In a number of clinical irritation and sensitization studies, the diesters of dicarboxylic acids are not irritants or sensitizers. The only exception noted was that undiluted diisopropyl adipate was moderately irritating in one cumulative irritancy test, and some slight irritation was seen with formulations containing diethylhexyl adipate. A 10% dilution of dibutyl adipate tested on 30 subjects and formulations containing 0.7-17% diisopropyl adipate, tested on 49-98 subjects, and 9% diethylhexyl adipate, tested on 25 subjects, were not phototoxic.

Cases of allergic contact dermatitis in response to diethyl sebacate-containing products have been reported, and it has been demonstrated that diethyl sebacate was the substance, or one of several substances in the products, eliciting the dermatitis. Two case studies were reported of allergic reactions to lotion containing diisopropyl sebacate.

According to the Integrated Risk Information System of the EPA, the weight-of-evidence classification for diethylhexyl adipate was “possible human carcinogen”. The classification was based on an absence of human data and increased liver tumors in female mice. The IARC has stated that diethylhexyl adipate is not classifiable as to its carcinogenicity in humans.

DISCUSSION

The Expert Panel reviewed the available data on dicarboxylic acids and their salts, and the data on the esters of dicarboxylic acids, and determined that these ingredients are safe as used in cosmetics. In reaching this conclusion, the Expert Panel considered a number of issues.

The Expert Panel noted gaps in the available safety data for some of the dicarboxylic acid and salts and esters of dicarboxylic acids in this safety assessment. The available data on many of the ingredients are sufficient, however, and similar structural activity relationships, biologic functions, and cosmetic product usage, suggest that the available data may be extrapolated to support the safety of the entire group. For example, a concern regarding the extent of dermal absorption for certain long-chain, branched diesters is addressed, because dermal penetration of long chain alcohols is likely to be low and the dermal penetration for the diesters is likely to be even lower, inferring toxicity characteristics from ingredients where toxicity data were available was appropriate

The CIR Expert Panel considered the dangers inherent in using animal-derived ingredients, namely the transmission of infectious agents. While tallow may be used in the manufacture of some ingredients in this safety assessment and is clearly animal-derived, the Expert Panel notes that tallow is highly processed and tallow derivatives even more so. The Panel agrees with determinations by the U.S. FDA that tallow derivatives are not risk materials for transmission of infectious agents.

The Panel noted that the only significant toxic effect of the dicarboxylic acids was irritation to the skin and eyes, which would be expected for acids. Dicarboxylic acids reviewed in this safety assessment are not expected to be appreciably absorbed from cosmetic formulations, exhibit low single-dose or repeated-dose toxicity in animal studies, and are not genotoxic or carcinogenic in animal studies. Since a use of these acids in cosmetics is as a pH adjuster, the irritating property of these acids would be lost. The highest use of an acid in leave-on formulations is 0.3% azelaic acid, of a salt is 0.4% disodium succinate, and of an ester is 14% diethylhexyl adipate. Although bath products can contain higher concentrations of these acids, salts, or esters, contact time is short and the product will be diluted as it is being rinsed.

Case studies have reported reactions to products containing diethyl sebacate. Follow-up patch tests performed with ≥5% diethyl sebacate, which is greater than the reported use concentration, had positive results. Diethyl sebacate is reported to be used in cosmetics at 1.5%, and no irritation or sensitization was reported in clinical studies of a formulation containing 1.5% diethyl sebacate.

The Expert Panel also noted that esters of dicarboxylic acids, in particular diethylhexyl adipate, have the potential to induce peroxisome proliferation. This effect has been examined because ethylhexyl adipate is structurally related to a notable peroxisome proliferator, diethylhexyl phthalate. Diethylhexyl adipate is not as potent a peroxisome proliferator as diethyl-hexyl phthalate, and, while peroxisome proliferation is toxicologically well-studied, this is an effect observed only in rodents and is not relevant to humans. Accordingly, the hepatocarcinogenic effects observed in rodents are related to this effect and not believed to pose the risk of inducing hepatocarcinogenesis in humans.

The reproductive and developmental toxicity of the dicarboxylic acids and their esters were generally well studied. The results of these studies did not cause any concern for the Panel.

The potential adverse effects of inhaled aerosols depend on the specific chemical species, the concentration and the duration of the exposure and their site of deposition within the respiratory system. In practice, aerosols should have at least 99% of their particle diameters in the 10 – 110 µm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 µm. Particles with an aerodynamic diameter of ≤ 10µm are respirable. In the absences of inhalation toxicity data, the panel determined that dicarboxylic acids and their salts and the esters of dicarboxylic acids can be used safely in hair

sprays, because the product size is not respirable.

CONCLUSION

The CIR Expert Panel concluded that dicarboxylic acids and their salts, and the esters of dicarboxylic acids, as listed below, are safe in the present practices of use and concentration. Were ingredients in these groups not in current use (as indicated by *) to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in these groups.

Acids and salts:

malonic acid*
succinic acid
sodium succinate
disodium succinate
glutaric acid*
adipic acid
azelaic acid
dipotassium azelate*
disodium azelate*
sebacic acid
disodium sebacate*
dodecanedioic acid*

Esters:

diethyl malonate
decyl succinate*
dimethyl succinate
diethyl succinate*
dicapryl succinate
dicetearyl succinate*
diisobutyl succinate*
diethylhexyl succinate
dimethyl glutarate
diisobutyl glutarate*
diisostearyl glutarate
dimethyl adipate
diethyl adipate*
dipropyl adipate*
dibutyl adipate
dihexyl adipate
dicapryl adipate
di-C12-15 alkyl adipate*
ditridecyl adipate*
dicetyl adipate*
diisopropyl adipate
diisobutyl adipate
diethylhexyl adipate
diisooctyl adipate*
diisononyl adipate*
diisodecyl adipate
dihexyldecyl adipate*
diheptylundecyl adipate
dioctyldodecyl adipate
diisocetyl adipate*
diisostearyl adipate
isostearyl sebacate
diethyl sebacate
dibutyl sebacate*
dicaprylyl/capryl sebacate*
diisopropyl sebacate
diethylhexyl sebacate
dibutyloctyl sebacate*
diisooctyl sebacate
dihexyldecyl sebacate*
dioctyldodecyl sebacate
diisostearyl sebacate*
dioctyldodecyl dodecanedioate
diisocetyl dodecanedioate

Figure 1a. Map of the malonic and succinic ester ingredients in this assessment, and associated esterase metabolites

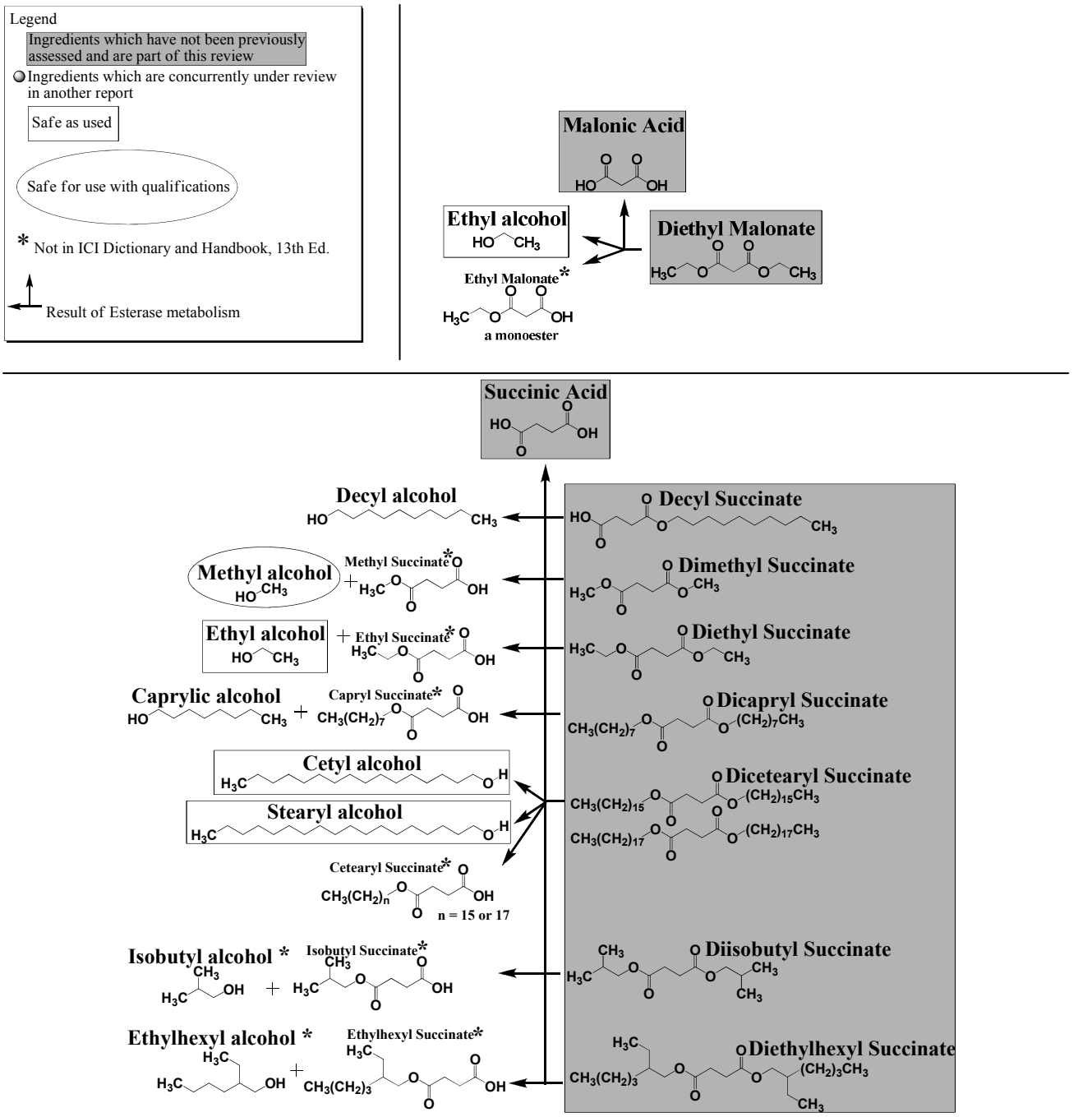
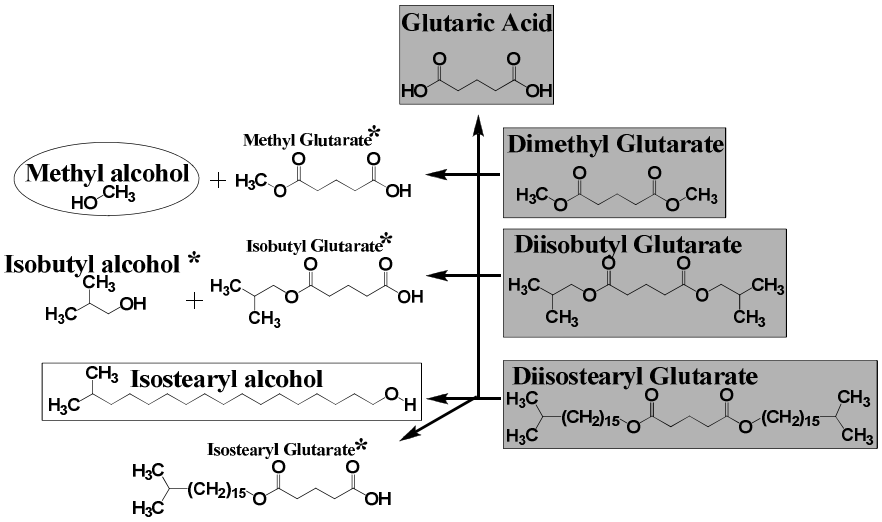


Figure 1b. Map of the glutaric and straight-chain adipic ester ingredients in this assessment, and associated esterase metabolites



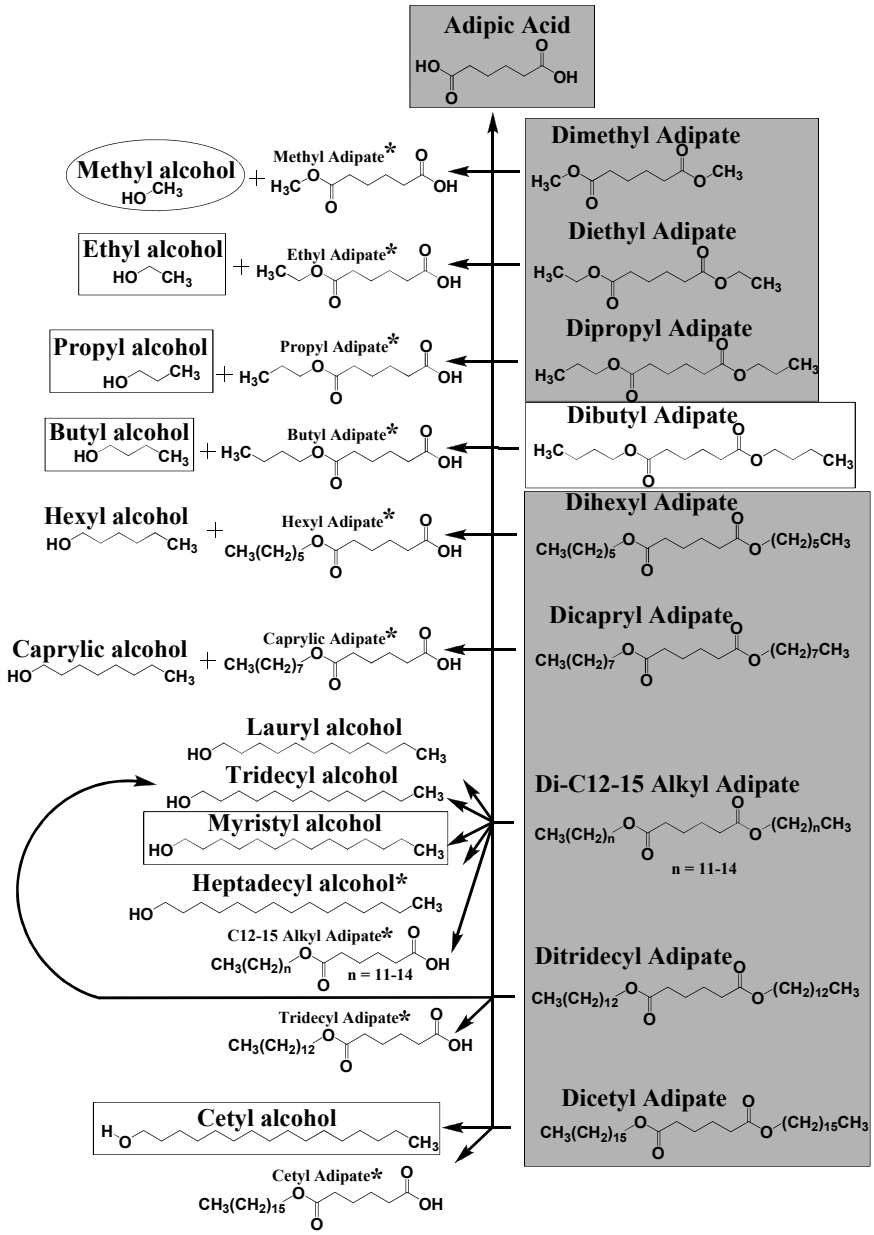


Figure 1c. Map of the branched chain adipic ester ingredients in this assessment, and associated esterase metabolites

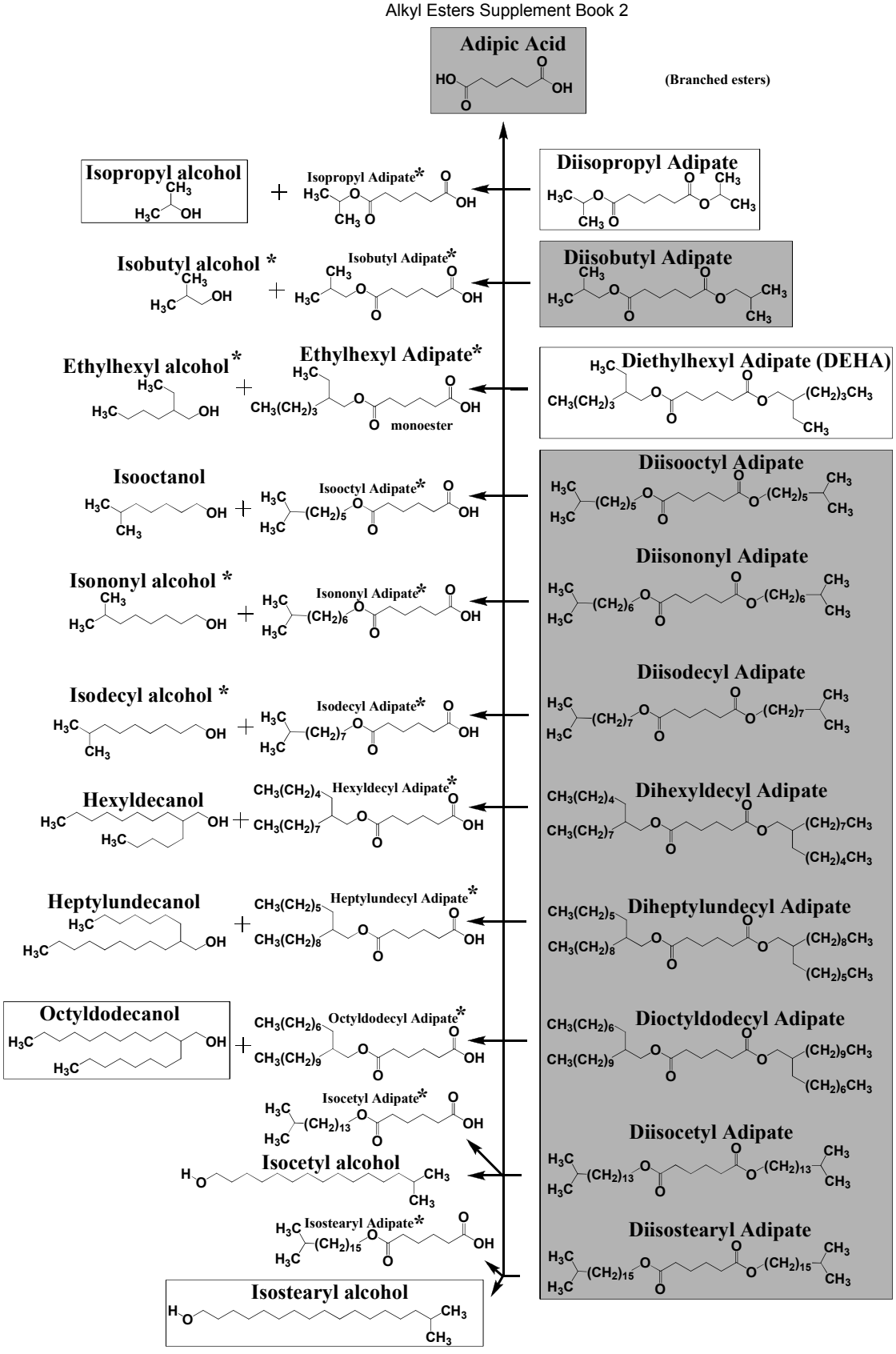


Figure 1d. Map of the sebacic and dodecanedioic ester ingredients in this assessment, and associated esterase metabolites

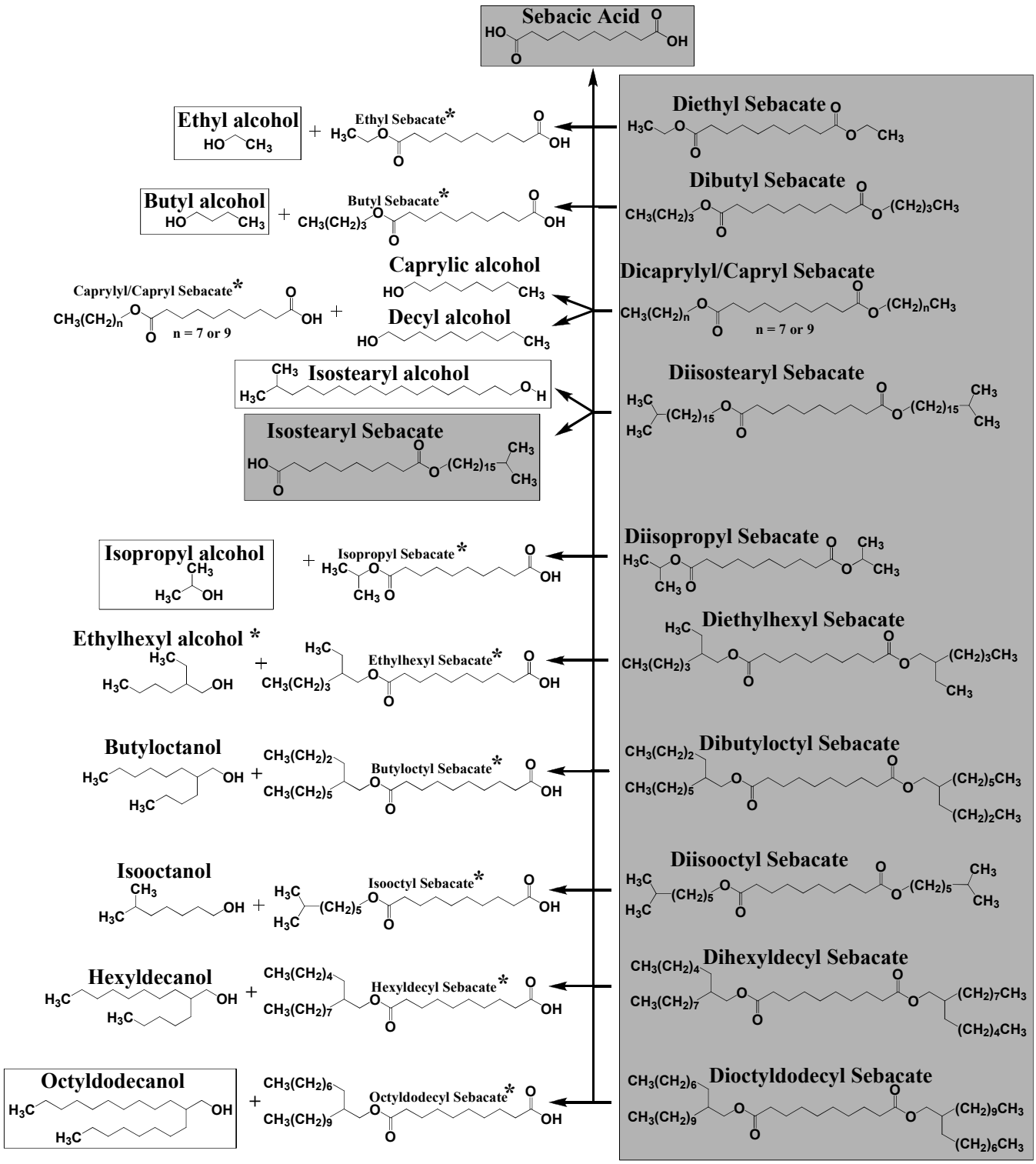


Figure 2. Sebacic acid synthesis from castor oil.

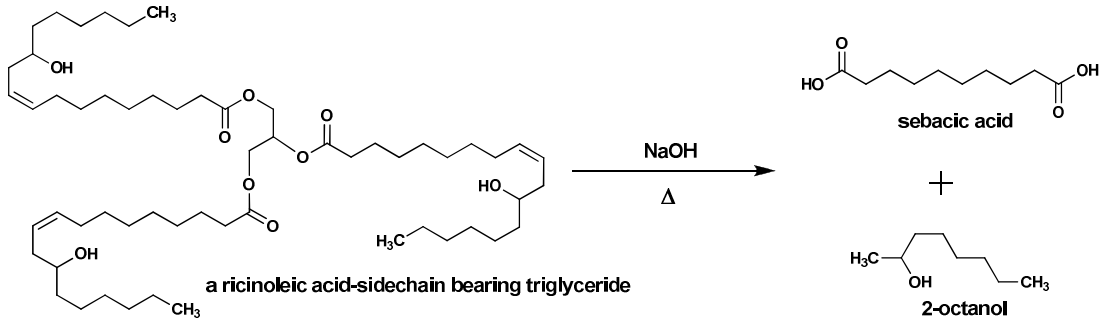
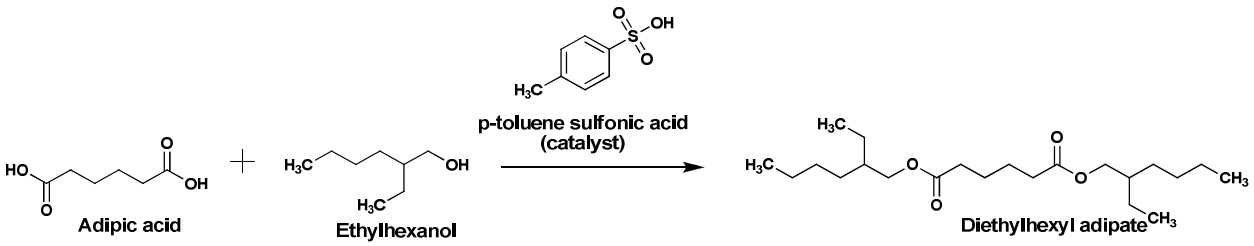


Figure 3. Diethylhexyl adipate synthesis from adipic acid.



CHARTS

Chart 1. Dicarboxylic Acids; Log K_{ow} vs Molecular Weight

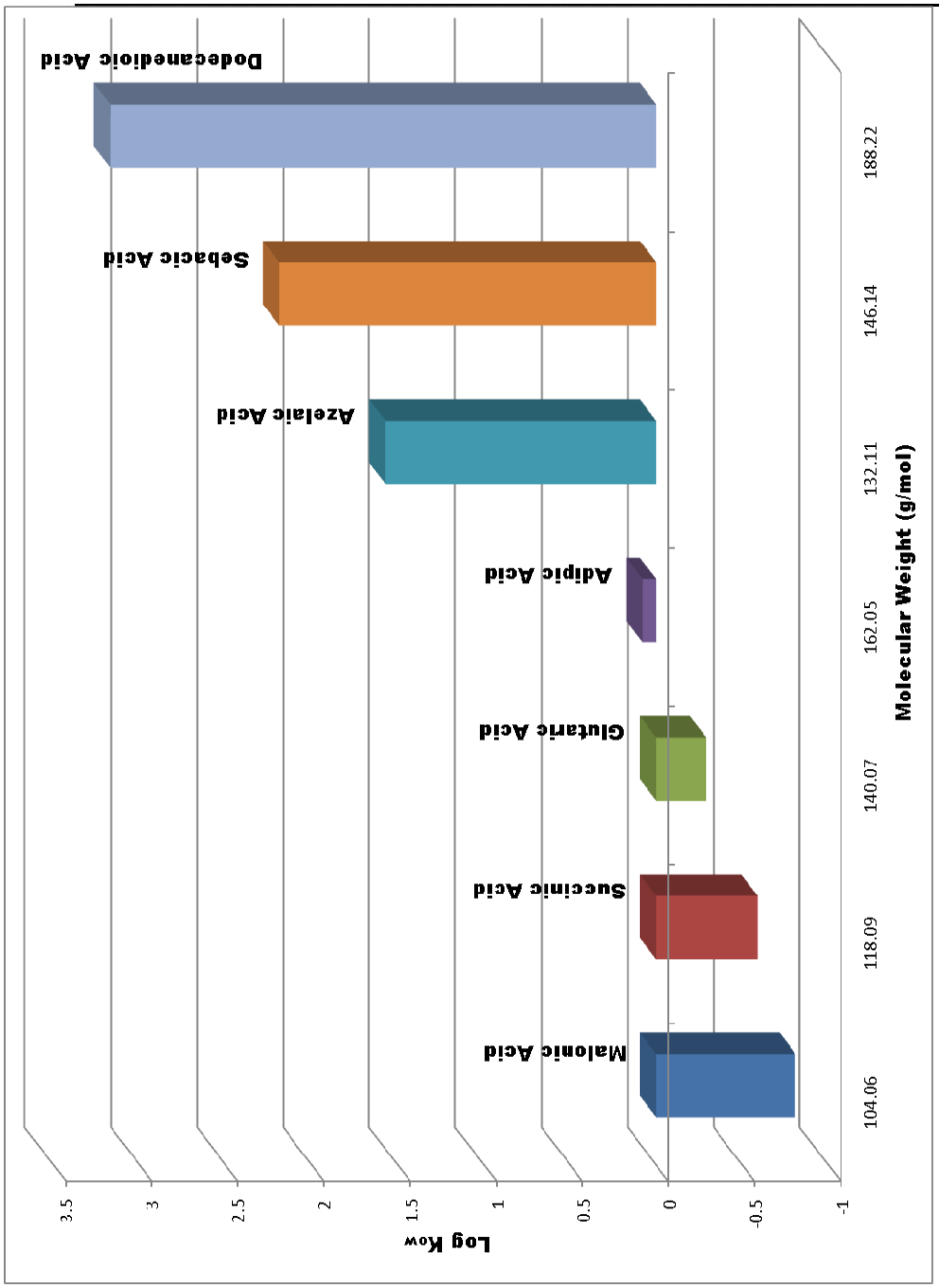


Chart 2. Dicarboxylic Acids and their Salts; Log K_{ow} vs Molecular Weight

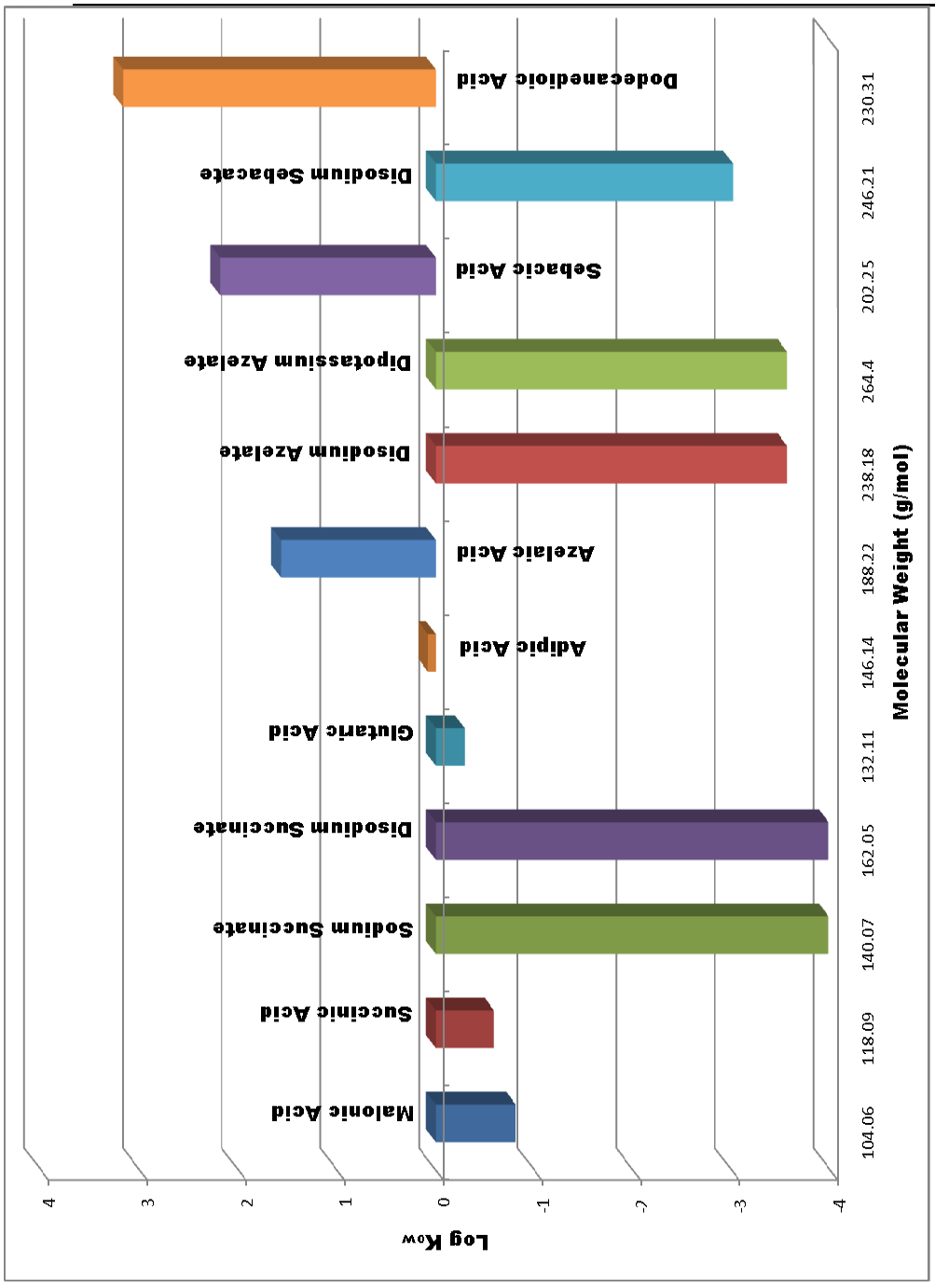
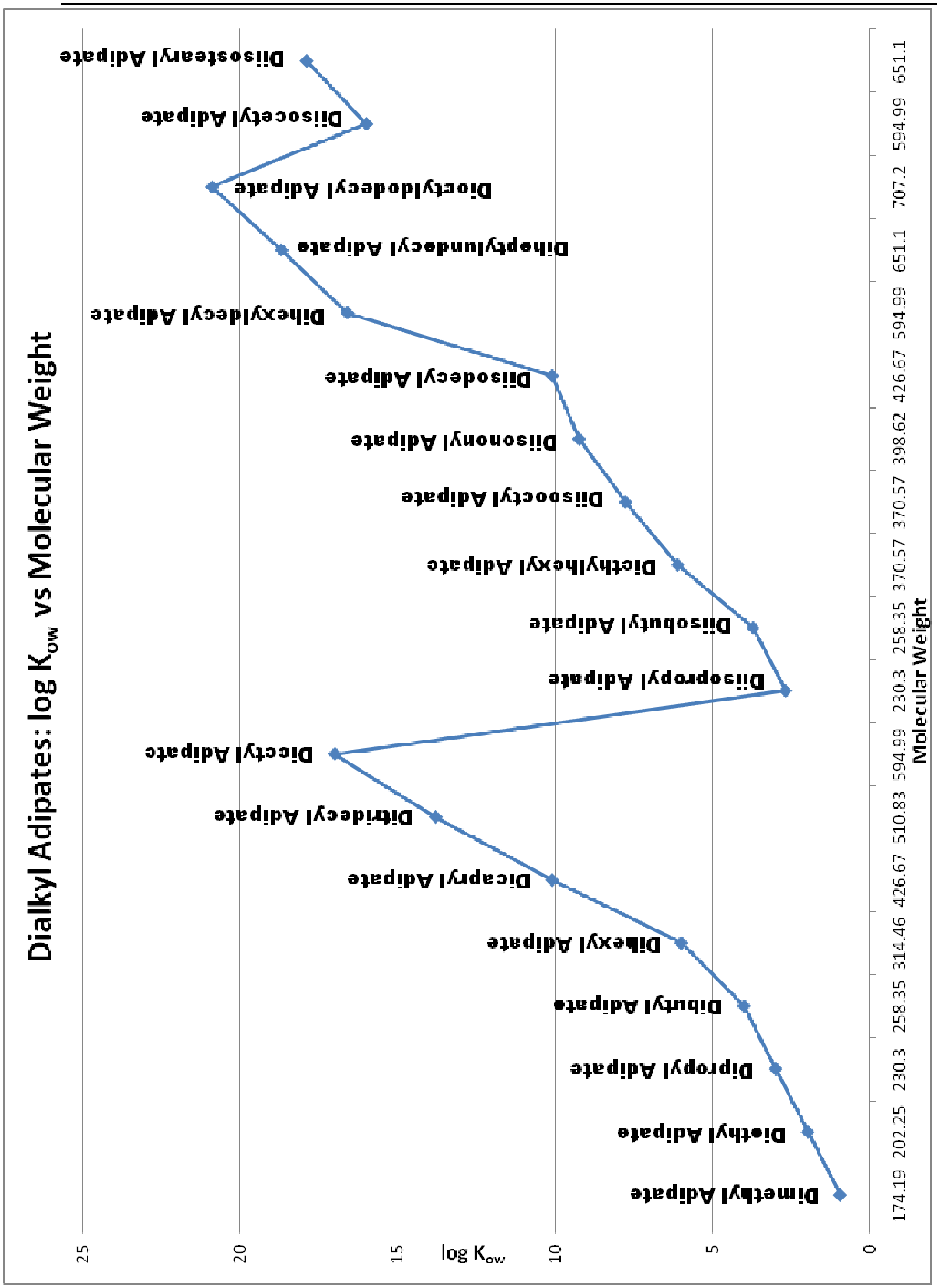


Chart 3. Example of the effects of chain length and branching on solubility. Log K_{ow} vs Molecular Weight



TABLES

Table 1. Definitions, functions and structures of dicarboxylic acid, salt and ester ingredients in this safety assessment

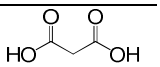
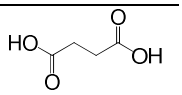
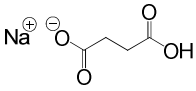
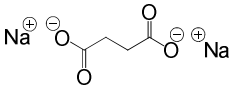
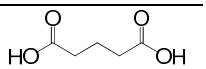
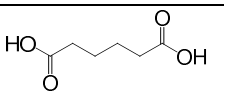
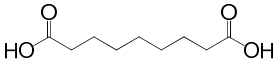
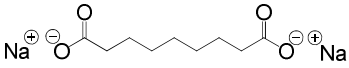
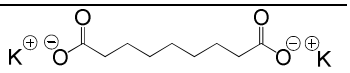
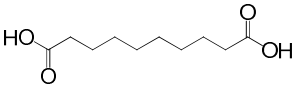
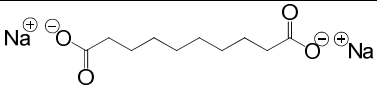
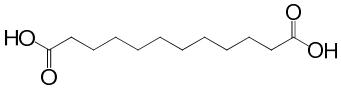
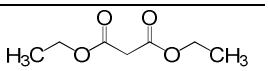
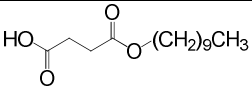
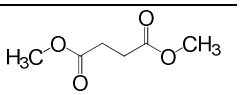
Ingredient CAS No.	Definition	Function(s)	Formula/structure
Dicarboxylic Acids and Metal Salts			
Malonic Acid 141-82-2	Malonic Acid is the organic compound that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Succinic Acid 110-15-6	Succinic Acid is the dicarboxylic acid that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Sodium Succinate 2922-54-5	Sodium Succinate is the sodium salt of succinic acid.	Buffering Agents; pH Adjusters	
Disodium Succinate 150-90-3	Disodium Succinate is the disodium salt of Succinic Acid .	Fragrance Ingredients; Not Reported	
Glutaric Acid 110-94-1	Glutaric Acid is the organic compound that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Adipic Acid 124-04-9	Adipic Acid is the organic dicarboxylic acid that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Azelaic Acid 123-99-9	Azelaic Acid is the dicarboxylic acid that conforms to noted structure.	Fragrance Ingredients; pH Adjusters	
Disodium Azelate 17265-13-3	Disodium Azelate is the disodium salt of azelaic acid.	Not Reported	
Dipotassium Azelate 19619-43-3	Dipotassium Azelate is the organic salt that conforms to noted structure.	Not Reported	
Sebacic Acid 111-20-6	Sebacic Acid is the organic dicarboxylic acid that conforms to noted structure.	pH Adjusters	
Disodium Sebacate 17265-14-4	Disodium Sebacate is the disodium salt of Sebacic Acid . It conforms to the noted structure.	Not Reported	
Dodecanedioic Acid 693-23-2	Dodecanedioic Acid is the organic compound that conforms to noted structure.	Skin-Conditioning Agents - Miscellaneous	
Malonic Diester Ingredient			
Diethyl Malonate 105-53-3	Diethyl Malonate is the organic compound that conforms to noted structure.	Fragrance Ingredients	
Succinic Ester Ingredients			
Monoester			
Decyl Succinate 54482-22-3 (wrong CAS No. 2530-33-8)	Decyl Succinate is the monoester of decyl alcohol and succinic acid.	Skin-Conditioning Agents - Emollient	
Diesters			
Dimethyl Succinate 106-65-0	Dimethyl Succinate is the diester of methyl alcohol and Succinic Acid.	Nail Polish and Enamel Removers	

Table 1. Definitions, functions and structures of dicarboxylic acid, salt and ester ingredients in this safety assessment

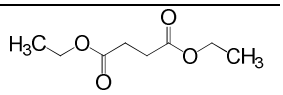
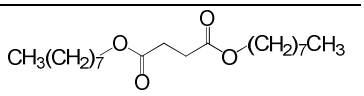
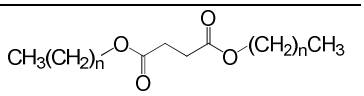
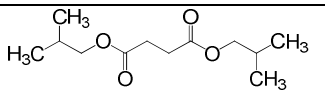
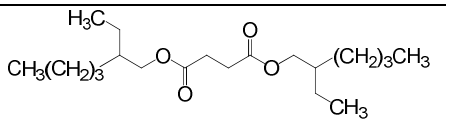
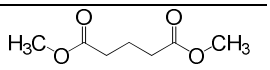
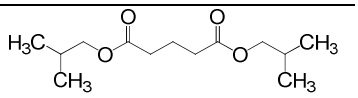
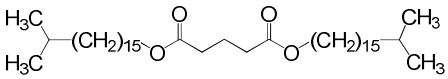
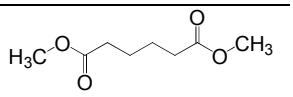
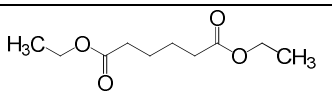
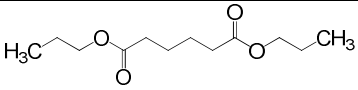
Ingredient CAS No.	Definition	Function(s)	Formula/structure
Diethyl Succinate 123-25-1	Diethyl Succinate is the diester of ethyl alcohol and Succinic Acid .	Fragrance Ingredients; Plasticizers; Solvents	
Dicapryl Succinate 14491-66-8	Dicapryl Succinate is the organic compound that conforms to noted structure.	Film Formers; Hair Conditioning Agents; Nail Conditioning Agents; Plasticizers; Skin-Conditioning Agents - Emollient	
Dicetearyl Succinate 93280-98-9	Dicetearyl Succinate is the diester of Cetearyl Alcohol and Succinic Acid .	Skin-Conditioning Agents - Miscellaneous	 wherein n=15 or 17
Branched			
Diisobutyl Succinate 925-06-4	Diisobutyl Succinate is the organic compound that conforms to the noted structure.	Plasticizers	
Diethylhexyl Succinate 2915-57-3	Diethylhexyl Succinate is the diester of 2-ethylhexyl alcohol and Succinic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Glutaric Ester Ingredients			
Dimethyl Glutarate 1119-40-0	Dimethyl Glutarate is the diester of methyl alcohol and glutaric acid.	Nail Polish and Enamel Removers	
Branched			
Diisobutyl Glutarate 71195-64-7	Diisobutyl Glutarate is the organic compound that conforms to noted structure.	Plasticizers	
Diisostearyl Glutarate No CAS No.	Diisostearyl Glutarate is the diester of isostearyl alcohol and glutaric acid.	Skin-Conditioning Agents - Emollient	One example of an “iso” 
Adipic Ester Ingredients			
Dimethyl Adipate 627-93-0	Dimethyl Adipate is the diester of methyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diethyl Adipate 141-28-6	Diethyl Adipate is the diester of ethyl alcohol and adipic acid.	Fragrance Ingredients; Skin-Conditioning Agents - Emollient	
Dipropyl Adipate 106-19-4	Dipropyl Adipate is the diester of propyl alcohol and adipic acid.	Skin-Conditioning Agents - Emollient; Solvents	

Table 1. Definitions, functions and structures of dicarboxylic acid, salt and ester ingredients in this safety assessment

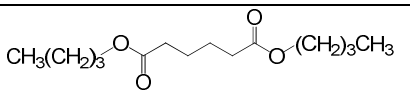
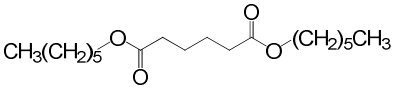
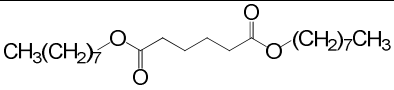
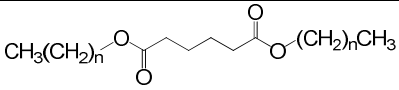
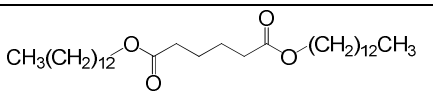
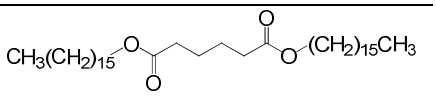
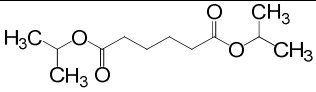
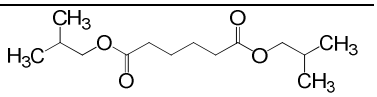
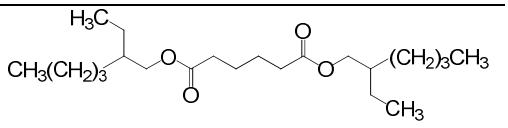
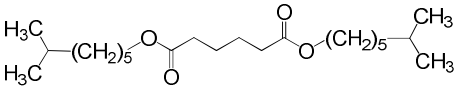
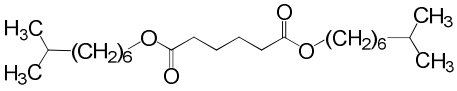
Ingredient CAS No.	Definition	Function(s)	Formula/structure
Dibutyl Adipate 105-99-7	Dibutyl Adipate is the diester of butyl alcohol and adipic acid.	Nail Polish and Enamels; Suntan Gels, Creams, and Liquids	
Dihexyl Adipate 110-33-8	Dihexyl Adipate is the diester of hexyl alcohol and adipic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dicapryl Adipate 105-97-5	Dicapryl Adipate is the diester of capryl alcohol and adipic acid.	Plasticizers	
Di-C12-15 Alkyl Adipate No CAS No.	Di-C12-15 Alkyl Adipate is the diester of C12-15 Alcohols and adipic acid.	Skin-Conditioning Agents - Emollient	 wherein n=11, 12, 13 or 14
Ditridecyl Adipate 16958-92-2	Ditridecyl Adipate is the diester of Tridecyl Alcohol and Adipic Acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dicetyl Adipate 26720-21-8	Dicetyl Adipate is the diester of cetyl alcohol and adipic acid.	Skin-Conditioning Agents - Emollient	
Branched			
Diisopropyl Adipate 6938-94-9	Diisopropyl Adipate is the diester of isopropyl alcohol and Adipic Acid.	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diisobutyl Adipate 141-04-8	Diisobutyl Adipate is the diester of isobutyl alcohol and Adipic Acid.	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diethylhexyl Adipate 103-23-1	Diethylhexyl Adipate is the diester of a 2-ethylhexyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diisooctyl Adipate 108-63-4	Diisooctyl Adipate is the organic compound that conforms to noted structure.	Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Diisononyl Adipate 33703-08-1	Diisononyl Adipate is the diester of isononyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 

Table 1. Definitions, functions and structures of dicarboxylic acid, salt and ester ingredients in this safety assessment

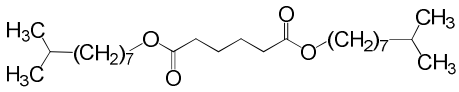
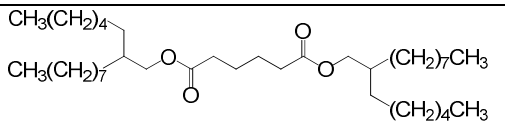
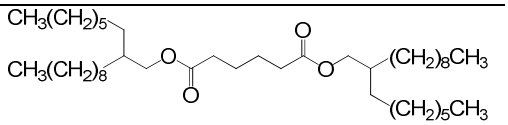
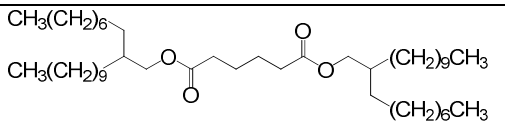
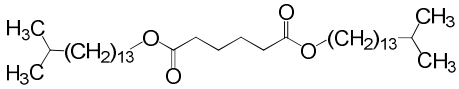
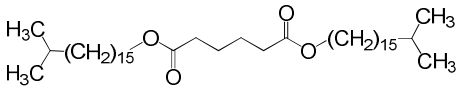
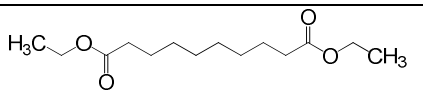
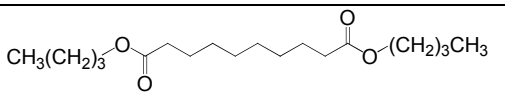
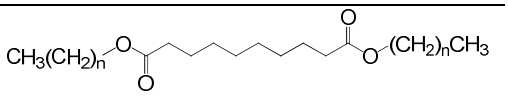
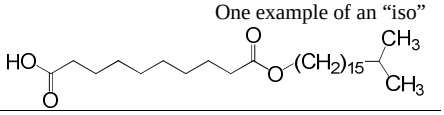
Ingredient CAS No.	Definition	Function(s)	Formula/structure
Diisodecyl Adipate 27178-16-1	Diisodecyl Adipate is the diester of isodecyl alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Dihexyldecyl Adipate 57533-90-1	Dihexyldecyl Adipate is the diester of hexyldecanol and adipic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Diheptylundecyl Adipate 155613-91-5	Diheptylundecyl Adipate is the diester of adipic acid and heptylundecanol.	Skin-Conditioning Agents - Emollient; Solvents	
Dioctyldodecyl Adipate 85117-94-8	Dioctyldodecyl Adipate is the diester of octyldodecanol and adipic acid .	Plasticizers; Skin-Conditioning Agents - Emollient	
Diisocetyl Adipate 59686-69-0 *sec:* 58262-41-2	Diisocetyl Adipate is the diester of hexadecyl alcohol and adipic acid .	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	One example of an "iso" 
Diisostearyl Adipate 62479-36-1	Diisostearyl Adipate is the diester of Isostearyl Alcohol and Adipic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient	One example of an "iso" 
Sebacic Ester Ingredients			
Diethyl Sebacate 110-40-7	Diethyl Sebacate is the diester of ethyl alcohol and Sebacic Acid	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Dibutyl Sebacate 109-43-3	Dibutyl Sebacate is the diester of butyl alcohol and sebacic acid.	Fragrance Ingredients; Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Dicaprylyl/ Capryl Sebacate No CAS. No.	Dicaprylyl/Capryl Sebacate is the organic compound that conforms generally to the noted structure.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	 wherein n=7 or 9
Branched Monoester			
Isostearyl Sebacate 478273-24-4	Isostearyl Sebacate is the half-ester of isostearyl alcohol and sebacic acid.	Skin-Conditioning Agents - Miscellaneous	One example of an "iso" 

Table 1. Definitions, functions and structures of dicarboxylic acid, salt and ester ingredients in this safety assessment

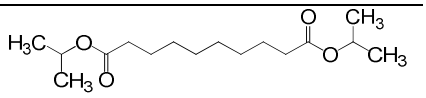
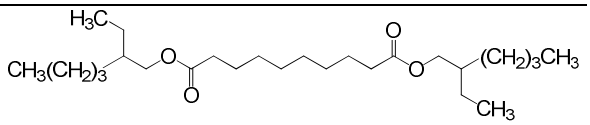
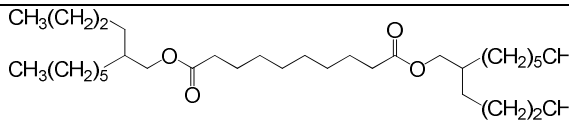
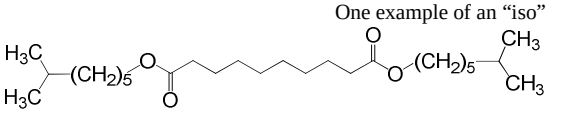
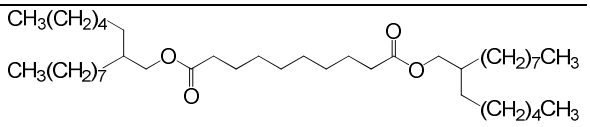
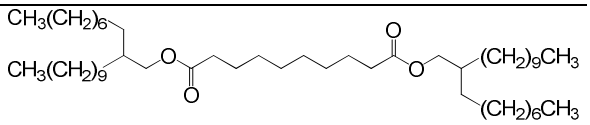
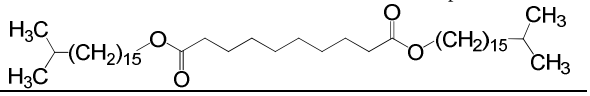
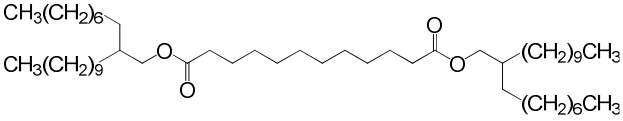
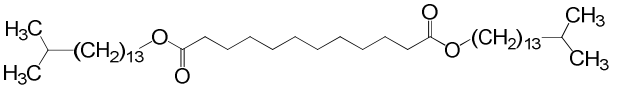
Ingredient CAS No.	Definition	Function(s)	Formula/structure
Branched Diesters			
Diisopropyl Sebacate 7491-02-3	Diisopropyl Sebacate is the diester of isopropyl alcohol and Sebacic Acid.	Plasticizers; Skin-Conditioning Agents - Emollient; Solvents	
Diethylhexyl Sebacate 122-62-3	Diethylhexyl Sebacate is the diester of 2-ethylhexyl alcohol and Sebacic Acid.	Fragrance Ingredients; Plasticizers; Solvents	
Dibutyloctyl Sebacate 184706-97-6	Dibutyloctyl Sebacate is the diester of butyloctyl alcohol and sebacic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Diisooctyl Sebacate 10340-41-7	Diisooctyl Sebacate is the organic compound that conforms to noted structure.	Antioxidants; Plasticizers; Skin-Conditioning Agents - Emollient	One example of an "iso" 
Dihexyldecyl Sebacate 359073-59-9	Dihexyldecyl Sebacate is the diester of hexyldecyl alcohol and sebacic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Dioctyldodecyl Sebacate 69275-01-0	Dioctyldodecyl Sebacate is the diester of octyldodecanol and sebacic acid.	Skin-Conditioning Agents - Emollient; Solvents	
Diisostearyl Sebacate No CAS No.	Diisostearyl Sebacate is the diester of isostearyl alcohol and sebacic acid.	Skin-Conditioning Agents - Emollient	One example of an "iso" 
Dodecanoic Ester Ingredients			
Dioctyldodecyl Dodecanedioate 129423-55-8	Dioctyldodecyl Dodecanedioate is the diester of octyldodecanol and dodecanedioic acid.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous	
Diisocetyl Dodecanedioate 131252-83-0	Diisocetyl Dodecanedioate is the organic compound that conforms to noted structure.	Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents	One example of an "iso" 

Table 2a. Physical and Chemical properties of the alkyl dicarboxylic acid and salt ingredients.[33,175,176]

INCI Name	**Malonic Acid**	**Succinic Acid**	**Sodium Succinate**	**Disodium Succinate**	**Glutaric Acid**	**Adipic Acid**
Appearance	small crystals	colorless prisms	crystalline	crystalline	large monoclinic prisms	white, monoclinic prisms
Molecular Weight (g/mol)	104.06	118.09	140.07	162.05	132.11	146.14
Melting/Boiling Point (°C)	135 (dec.)/ 264 (est.)	185-187/ 235	206 (est.)/ 486 (est.)	156 (est.)/ 426 (est.)	97.5-98/ 302-304	152/265
Density (g/cm^3)	1.63	1.56	--	--	1.429	1.360
Vapor pressure (mm Hg @ 25°C)	0.001 (est.)	0.0000002	$7.3\ E^{-10}$ (est.)	$8.7\ E^{-8}$ (est.)	0.000003	0.07
Solubility (g/L water @ 25°C)	1520	83	1000 (est.)	31 (est.)	639	30
Log K_{ow}	-0.81	-0.59	-3.98 (est.)	-3.98 (est.)	-0.29	0.08

INCI Name	**Azelaic Acid**	**Disodium Azelate**	**Dipotassium Azelate**	**Sebacic Acid**	**Disodium Sebacate**	**Dodecanedioic Acid**
Appearance	monoclinic prismatic needles	crystalline	crystalline	Monoclinic prismatic tablets	crystalline	--
Molecular Weight (g/mol)	188.22	238.18	264.40	202.25	246.21	230.31
Melting/Boiling Point (°C)	106.5/ 286.5	186 (est.)/ 484 (est.)	186 (est.)/ 484 (est.)	134.5/ 294.5	194/496 (est.)	128/383 (est.)
Density (g/cm^3)	1.0291	--	--	1.207	--	1.16
Vapor pressure (mm Hg @ 25°C)	0.00002 (est.)	$1.4\ E^{-9}$ (est.)	$1.4\ E^{-9}$ (est.)	0.000007 (est.)	$5.9\ E^{-10}$ (est.)	0.000002 (est.)
Solubility (g/L water @ 20°C)	2.4	1000 (est.)	1000 (est.)	1.0	1000 (est.)	0.040
Log K_{ow}	1.57	-3.56 (est.)	-3.56 (est.)	2.19 (est.)	-3.01 (est.)	3.17 (est.)

Table 2b. Physical and Chemical properties of the mono- and di-carboxylic acid esters. [33,175,176]

INCI Name	**Diethyl Malonate**	**Decyl Succinate**	**Dimethyl Succinate**	**Diethyl Succinate**	**Dicapryl Succinate**	**Dicetearyl Succinate**	**Diisobutyl Succinate**
Appearance	colorless liquid	--	--	liquid	--	--	liquid
Molecular Weight (g/mol)	160.17	258.35	146.14	174.19	342.51	566-623	230.30
Melting/Boiling Point (°C)	-50/ 198-199	96/377 (est.)	19.5/196.1	-21.3/217.7	14 (est.)/ 375 (est.)	--/--	-48 (est.)/ 216
Density (g/cm^3)	1.055	1.002 (est.)	1.1	1.04	0.94 (est.)	--	0.967
Vapor pressure (mm Hg @ 25°C)	0.269	0.000001 (est.)	0.4 (est.)	0.126	0.000008 (est.)	--	0.019 (est.)
Solubility (g/L water @ 25°C)	20	20 (est.)	50 (est.)	10 (est.)	0.0015 (est.)	--	0.60 (est.)
Log K_{ow}	0.96	4.57 (est.)	0.26 (est.)	1.28 (est.)	7.39 (est.)	--	3.00 (est.)

INCI Name	**Diethylhexyl Succinate**	**Dimethyl Glutarate**	**Diisobutyl Glutarate**	**Diisostearyl Glutarate**	**Dimethyl Adipate**	**Diethyl Adipate**	**Dipropyl Adipate**
Appearance	--	liquid	--	--	--	--	--
Molecular Weight (g/mol)	342.51	160.17	244.33	637.07	174.19	202.25	230.30
Melting/Boiling Point (°C)	-12 (est.)/ 359 (est.)	-42.5/ 214.2	-38 (est.)/ 237	212 (est.)/ 600 (est.)	210/229 (est.)	24-26/ 248-249	-15.7/ 274 (est.)
Density (g/cm^3)	0.933	1.0876	0.97 (est.)	--	1.062	1.08	0.98
Vapor pressure (mm Hg @ 25°C)	0.00002	0.185 (est.)	0.008 (est.)	$7.8\ E^{-13}$ (est.)	0.073 (est.)	0.027 (est.)	0.0055 (est.)
Solubility (g/L water @ 25°C)	0.002 (est.)	27 (est.)	0.29 (est.)	$1.16\ E^{-16}$ (est.)	14 (est.)	2.8 (est.)	0.62 (est.)
Log K_{ow}	7.08 (est.)	0.57 (est.)	3.44 (est.)	17.5 (est.)	0.95 (est.)	1.97 (est.)	2.99 (est.)

INCI Name	**Dibutyl Adipate**	**Dihexyl Adipate**	**Dicapryl Adipate**	**Di-C12-15 Alkyl Adipate**	**Ditridecyl Adipate**	**Dicetyl Adipate**	**Diisopropyl Adipate**
Appearance	--	liquid	--	--	--	--	liquid
Molecular Weight (g/mol)	258.35	314.46	426.67	482-567	510.83	594.99	230.30
Melting/Boiling Point (°C)	37.5/300 (est.)	-8/351 (est.)	26.5-27.1/ 442 (est.)	--/--	45.9/503 (est.)	56.5-57/ 559 (est.)	-1.1/253 (est.)
Density (g/cm^3)	0.96	0.95 (est.)	0.92 (est.)	--	0.91 (est.)	0.897 (est.)	0.982 (est.)
Vapor pressure (mm Hg @ 25°C)	0.0011 (est.)	0.00004 (est.)	0.00000005 (est.)	--	$3.0\ E^{-10}$ (est.)	$1.5\ E^{-12}$ (est.)	0.0192 (est.)
Solubility (g/L water @ 25°C)	0.14 (est.)	0.0082 (est.)	0.000041 (est.)	--	0.0000011 (est.)	0.00000005 (est.)	0.78 (est.)
Log K_{ow}	4.0 (est.)	6.0 (est.)	10.1 (est.)	--	13.8 (est.)	17 (est.)	2.68 (est.)

Table 2b. Physical and Chemical properties of the mono- and di-carboxylic acid esters

INCI Name	**Diisobutyl Adipate**	**Diethylhexyl Adipate**	**Diisooctyl Adipate**	**Diisononyl Adipate**	**Diisodecyl Adipate**	**Dihexyldecyl Adipate**
Appearance	liquid	liquid	--	--	--	--
Molecular Weight (g/mol)	258.35	370.57	370.57	398.62	426.67	594.99
Melting/Boiling Point (°C)	-20/278-280	-67.8/390	9 (est.)/ 382 (est.)	56 (est.)/ 230	51 (est.)/ 426 (est.)	181 (est.)/ 548 (est.)
Density (g/cm^3)	0.95	0.925	0.93 (est.)	--	--	0.896 (est.)
Vapor pressure (mm Hg @ 25°C)	0.0036 (est.)	0.0000009	0.000004 (est.)	$3.3\ E^{-6}$ (est.)	$1.9\ E^{-6}$ (est.)	$4.6\ E^{-12}$ (est.)
Solubility (g/L water @ 25°C)	0.18	0.00078	0.00067 (est.)	$4.0\ E^{-5}$ (est.)	$5.2\ E^{-6}$ (est.)	0.00000006 (est.)
Log K_{ow}	3.70 (est.)	6.11	7.77 (est.)	9.24 (est.)	10.1 (est.)	16.6 (est.)

INCI Name	**Diheptylundecyl Adipate**	**Dioctyldodecyl Adipate**	**Diisocetyl Adipate**	**Diisostearyl Adipate**	**Diethyl Sebacate**	**Dibutyl Sebacate**
Appearance	--	--	--	--	liquid	liquid
Molecular Weight (g/mol)	651.10	707.20	594.99	651.10	258.35	314.46
Melting/Boiling Point (°C)	229 (est.)/ 584 (est.)	267 (est.)/ 619 (est.)	181 (est.)/ 565 (est.)	229 (est.)/ 611 (est.)	5/298	-10/ 344-345
Density (g/cm^3)	0.892 (est.)	0.888 (est.)	0.896 (est.)	--	0.969 (est.)	0.94
Vapor pressure (mm Hg @ 25°C)	$1.26\ E^{-13}$ (est.)	$3.17\ E^{-15}$ (est.)	$1.4\ E^{-11}$ (est.)	$2.4\ E^{-13}$ (est.)	0.00054 (est.)	0.00004 (est.)
Solubility (g/L water @ 25°C)	$9.8\ E^{-9}$ (est.)	$2.1\ E^{-9}$ (est.)	$4.0\ E^{-12}$ (est.)	$3.6\ E^{-14}$ (est.)	0.15 (est.)	0.0085 (est.)
Log K_{ow}	18.7 (est.)	20.9 (est.)	16.0 (est.)	17.9 (est.)	3.92 (est.)	5.96 (est.)

INCI Name	**Dicaprylyl/Capryl Sebacate**	**Isostearyl Sebacate**	**Diisopropyl Sebacate**	**Diethylhexyl Sebacate**	**Dibutyloctyl Sebacate**	**Diisooctyl Sebacate**
Appearance	--	--	--	--	--	--
Molecular Weight (g/mol)	426-482	454.73	286.41	426.67	538.89	426.67
Melting/Boiling Point (°C)	--/--	215 (est.)/ 545 (est.)	-7 (est.)/ 308 (est.)	-48/436 (est.)	135 (est.)/ 510 (est.)	51 (est.)/ 428 (est.)
Density (g/cm^3)	--	0.929 (est.)	0.953 (est.)	0.91	0.901 (est.)	0.916 (est.)
Vapor pressure (mm Hg @ 25°C)	--	$2.5\ E^{-13}$ (est.)	0.0007 (est.)	$8.7\ E^{-8}$ (est.)	$1.6\ E^{-10}$ (est.)	$1.6\ E^{-7}$ (est.)
Solubility (g/L water @ 25°C)	--	0.0013 (est.)	0.046	0.00006 (est.)	0.0000006 (est.)	0.00006 (est.)
Log K_{ow}	--	11.2 (est.)	4.63 (est.)	9.72 (est.)	14.1 (est.)	9.72 (est.)

Table 2b. Physical and Chemical properties of the mono- and di-carboxylic acid esters

INCI Name	**Dihexyldecyl Sebacate**	**Dioctyldodecyl Sebacate**	**Diisostearyl Sebacate**	**Dioctyldodecyl Dodecanedioate**	**Diisocetyl Dodecanedioate**
Appearance	--	--	--	--	--
Molecular Weight (g/mol)	651.10	763.31	707.20	791.36	679.15
Melting/Boiling Point (°C)	229 (est.)/ 584 (est.)	299 (est.)/ 652 (est.)	268 (est.)/ 568 (est.)	314 (est.)/ 668 (est.)	247 (est.)/ 635 (est.)
Density (g/cm^3)	0.892 (est.)	0.885 (est.)	--	0.884 (est.)	--
Vapor pressure (mm Hg @ 25°C)	1.3 E^{-13} (est.)	7.4 E^{-17} (est.)	4.8 E^{-15} (est.)	1.1 E^{-17} (est.)	3.6 E^{-14} (est.)
Solubility (g/L water @ 25°C)	0.0000001 (est.)	6.8 E^{-10} (est.)	3.2 E^{-16} (est.)	3.6 E^{-10} (est.)	3.4 E^{-15} (est.)
Log K_{ow}	18.4 (est.)	22.6 (est.)	19.9 (est.)	23.7 (est.)	18.9 (est.)

"(est.)" = estimated value by EPI Suite
"(dec.)" = some decomposition occurred
"--" = Value not found
"E^{-13}" = divided by 10^{13}

Table 3a. Frequency[22] and concentration[23,25] of use by duration and exposure - Dicarboxylic Acids and Their Salts

	No. of Uses	*Conc. of Use (%)*	*No. of Uses*	*Conc. of Use (%)*	*No. of Uses*	*Conc. of Use (%)*
	Succinic Acid		**Sodium Succinate**		**Disodium Succinate**	
Totals	**4**	**0.001-26**	**7**	**NR**	**45**	**0.0005-0.4**
Duration of Use						
Leave-On	*2*	*0.001-0.2*	*3*	*NR*	*38*	*0.005-0.4*
Rinse Off	*2*	*0.001-26*	*4*	*NR*	*7*	*0.0005*
Exposure Type						
Eye Area	NR	NR	NR	NR	4	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR
Dermal Contact	2	0.01-26	5	NR	40	0.0005-0.4
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR
Hair, Non-Coloring	2	0.001-0.2	2	NR	5	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	0.2	1	NR	NR	NR
Bath Products	NR	26	1	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Adipic Acid		**Azelaic Acid**		**Sebacic Acid**	
Totals	**25**	**0.000001-18**	**9**	**0.007-10**	**12**	**0.0009-1**
Duration of Use						
Leave-On	*2*	*0.000001*	*7*	*0.007-0.3*	*9*	*0.0009-0.03*
Rinse Off	*23*	*0.5-18*	*2*	*10*	*3*	*0.001-1*
Exposure Type						
Eye Area	NR	0.000001	NR	NR	NR	NR
Possible Ingestion	NR	0.000001	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR
Dermal Contact	1	0.000001-18	25	0.007-10	12	0.0009-1
Deodorant (Underarm)	NR	NR	NR	NR	NR	0.0009
Hair, Non-Coloring	24	0.5	NR	NR	NR	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	-	NR	NR	NR	1	0.04
Bath Products	1	15-18	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

NR – not reported to be used

Table 3b. Frequency[22] and concentration[23,25] of use by duration and exposure - Esters of Dicarboxylic Acids

	No. Uses	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*
	Diethyl Malonate		**Dimethyl Succinate**		**Dicapryl Succinate**		**Diethylhexyl Succinate**		**Dimethyl Glutarate**		**Dimethyl Adipate**	
Totals	**NR**	**0.004-0.02**	**12**	**0.002-5**	**12**	**NR**	**38**	**0.02-6**	**13**	**0.5-15**	**12**	**0.2**
Duration of Use												
Leave-On	*NR*	*0.02*	*NR*	*0.002*	*9*	*NR*	*34*	*0.02-6*	*NR*	*NR*	*NR*	*NR*
Rinse Off	*NR*	*0.004-0.01*	*12*	*0.2-5*	*NR*	*NR*	*4*	*3-5*	*13*	*0.5-15*	*12*	*0.2*
Exposure Type												
Eye Area	NR	NR	NR	0.002	NR	NR	1	NR	NR	NR	NR	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	3	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	1	NR	NR	1	NR	NR	NR	NR
Dermal Contact	NR	0.004-0.02	NR	0.002-5	8	NR	34	1-6	NR	15	NR	NR
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair, Non-Coloring	NR	NR	NR	NR	1	NR	4	0.02-5	NR	NR	NR	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	12	0.2	NR	NR	NR	NR	13	0.5	12	0.2
Mucous Membrane	NR	NR	NR	NR	2	NR	1	NR	NR	NR	NR	NR
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Dihexyl Adipate		**Dicapryl Adipate**		**Diisobutyl Adipate**		**Diisodecyl Adipate**		**Diheptylundecyl Adipate**		**Dioctyldodecyl Adipate**	
Totals	**1**	**3**	**43**	**NR**	**22**	**0.001-3**	**1**	**NR**	**NR**	**6**	**3**	**NR**
Duration of Use												
Leave-On	*1*	*NR*	*38*	*NR*	*22*	*0.001-3*	*1*	*NR*	*NR*	*6*	*3*	*NR*
Rinse Off	*NR*	*3*	*5*	*NR*	*NR*	*0.002-0.5*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type												
Eye Area	NR	3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3	NR
Inhalation	NR	NR	1	NR	5	0.05-3	NR	NR	NR	NR	NR	NR
Dermal Contact	1	3	43	NR	8	0.002-3	1	NR	NR	6	3	NR
Deodorant (Underarm)	NR	NR	30	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair, Non-Coloring	NR	NR	NR	NR	5	0.05-0.2	NR	NR	NR	NR	NR	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	9	0.001-0.7	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	0.009	NR	NR	NR	NR	NR	NR
Bath Products	NR	NR	5	NR	NR	0.5	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 3b. Frequency[22] and concentration[23,25] of use by duration and exposure - Esters of Dicarboxylic Acids (continued)

	No. Uses	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*	*No. Uses*	*Conc. of Use (%)*
	Diisostearyl Adipate		**Isostearyl Sebacate**		**Diethyl Sebacate**		**Diisostearyl Sebacate**		**Diisopropyl Sebacate**		**Diethylhexyl Sebacate**	
Totals	**6**	**3-10**		**0.005-0.7**	**NR**	**1.5**	**NR**	**0.005-0.7**	**30**	**0.06-10**	**13**	**0.5-5**
Duration of Use												
Leave-On	*4*	*10*		*0.005-0.7*	*NR*	*1.5*	*NR*	*0.005-0.7*	*29*	*0.06-10*	*13*	*0.5-5*
Rinse Off	*2*	*3*		*NR*	*NR*	*NR*	*NR*	*NR*	*1*	*2*	*NR*	*1*
Exposure Type												
Eye Area	NR	NR		NR	NR	NR	NR	NR	1	NR	4	NR
Possible Ingestion	4	10		NR	NR	NR	NR	NR	NR	NR	NR	NR
Inhalation	NR	NR		NR	NR	NR	NR	NR	1	NR	NR	1
Dermal Contact	6	3-10		0.005-0.7	NR	1.5	NR	0.005-0.7	23	0.06-10	11	0.5-5
Deodorant (Underarm)	NR	NR		NR	NR	NR	NR	NR	4	1	NR	0.5
Hair, Non-Coloring	NR	NR		NR	NR	NR	NR	NR	6	8	2	NR
Hair, Coloring	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR		NR	NR	NR	NR	NR	1	0.08	NR	NR
Mucous Membrane	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
Bath Products	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR	NR

	Diisooctyl Sebacate		**Dioctyldodecyl Sebacate**		**Dioctyldodecyl Dodecanedioate**		**Diisocetyl Dodecanedioate**	
Totals	**NR**	**1-3**	**NR**	**3-8**	**5**	**6**	**2**	**0.9-7**
Duration of Use								
Leave-On	*NR*	*1-3*	*NR*	*3-8*	*5*	*6*	*2*	*0.9-7*
Rinse Off	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Possible Ingestion	NR	NR	NR	8	1	6	NR	NR
Inhalation	NR	NR	NR	3-5	2	NR	NR	0.9-3
Dermal Contact	NR	1-3	NR	3-8	5	6	2	0.9-7
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair, Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair, Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

NR - not reported to be used

Table 3c. Current and historical frequency and concentration of use according to duration and type of exposure - previously reviewed esters

	Dibutyl Adipate						**Diisopropyl Adipate**					
	# of Uses			*Conc. of Use (%)*			*# of Uses*			*Conc. of Use (%)*		
data year	*1994*[177]	*2002*[5]	*2010*[22]	*1996*[177]	*2002*[5]	*2010*[25]	*1981*[2]	*2002*[4]	*2010*[22]	*1981*[2]	*2003*[4]	*2010*[25]
Totals	**1**	**NR**	**6**	**NR**	**5-8**	**NR**	**112**	**66**	**70**	**≤0.1-25**	**0.01-15**	**0.005-8**
Duration of Use												
Leave-On	*1*	*NR*	*6*	*NR*	*5-8*	*NR*	*92*	*60*	*64*	*≤0.1-25*	*0.01-15*	*0.005-8*
Rinse Off	*NR*	*NR*	*0*	*NR*	*NR*	*NR*	*20*	*6*	*6*	*≤0.1-26*	*001-8*	*2-7*
Exposure Type												
Eye Area	NR	NR	2	NR	NR	NR	2	NR	2	1-25	NR	1
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	1	NR	NR	NR
Inhalation	1	NR	2	NR	NR	NR	47	33	21	0.1-25	1-15	0.005-8
Dermal Contact	1	NR	3	NR	8	NR	102	62	50	≤0.1-25	0.01-15	0.005-8
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	6	NR	0.01	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	10	3	17	≤0.1-5	0.1-3	0.5-3
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	1	NR	5	NR	NR	1	NR	NR	3	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	1	NR	NR	0.1-1	NR	NR
Bath Products	NR	NR	NR	NR	NR	NR	8	6	1	1-25	5-8	2
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Diethylhexyl Adipate					
	# of Uses			*Conc. of Use (%)*		
data year	*1981*[2]	*2002*[4]	*2010*[22]	*1981*[2]	*2003*[4]	*2010*[25]
Totals	**27**	**49**	**48**	**≤0.1-25**	**0.4-38**	**0.6-14**
Duration of Use						
Leave-On	*21*	*44*	*39*	*≤0.1-10*	*0.4-38*	*0.9-14*
Rinse Off	*6*	*5*	*9*	*1-25*	*NR*	*0.6*
Exposure Type						
Eye Area	NR	2	3	NR	0.4-2	NR
Possible Ingestion	5	1	1	1-5	NR	NR
Inhalation	6	5	5	1-5	NR	NR
Dermal Contact	25	47	43	≤0.1-25	0.4-38	0.6-14
Deodorant (underarm)	1	NR	NR	0.1-1	8	0.9
Hair - Non-Coloring	NR	NR	1	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	2	2	4	1-5	NR	2-3
Mucous Membrane	NR	4	1	NR	NR	NR
Bath Products	4	NR	NR	10-25	NR	NR
Baby Products	NR	NR	1	NR	NR	NR

NR - not reported to be used

Table 3d. Ingredients not reported to be used

Dicarboxylic Acids and Their Salts
Malonic Acid
Glutaric Acid
Disodium Azelate
Dipotassium Azelate
Disodium Sebacate
Dodecanedioic Acid

Esters of Dicarboxylic Acids
Decyl Succinate
Diethyl Succinate
Dicetearyl Succinate
Diisobutyl Succinate
Diisobutyl Glutarate
Diisostearyl Glutarate
Diethyl Adipate
Dipropyl Adipate
Di-C 12-15 Alkyl Adipate
Ditridecyl Adipate
Dicetyl Adipate
Diisooctyl Adipate
Diisononyl Adipate
Dihexyldecyl Adipate
Diisocetyl Adipate
Dibutyl Sebacate
Dicaprylyl/Capryl Sebacate
Dibutyloctyl Sebacate
Dihexyldecyl Sebacate
Diisostearyl Sebacate

Table 4. Acute toxicity - Dicarboxylic Acids and Their Salts

Animals	No./Gender/Group	Dose	median lethal dose/conc.	Reference
		ORAL		
Succinic Acid				
rats	not specified	not specified	2260 mg/kg	178
Sodium Succinate				
rats	4 males, 4 females	0.5-8 g/kg	8 g/kg	71
Glutaric Acid				
rats	5/dose, male and female	50% aq. solution	2750 mg/kg	68
Adipic Acid				
mice	13 males	1500-2500 mg/kg of a 6% suspension in 0.5% methyl cellulose	1900 mg/kg	47
mice	not specified	not specified	4175 mg/kg	47
mice	not specified	not specified	4200 mg/kg	47
rats	M/F, no. not specified	20% in corn oil	5050 mg/kg	68
rats	5 or 10 males	100-3000 mg/kg (n=5) or 5000 mg/kg (n=10) adipic acid in 0.85% saline	940 mg/kg	47
Wistar rats	not specified	not specified	approx. 3600 mg/kg	47
rats	10 males	5000 mg/kg of a 33.3% suspension in 0.85% saline	grater than highest dose tested	47
rats	5 males, 5 females	14.7-10,000 mg/kg as a 14.7-50% suspension in carboxymethyl cellulose (CMC)	5560 mg/kg	47
rats	not specified	10,000 mg/kg	greater than highest dose tested	47
rat and rabbit	not specified	not specified	greater than highest dose tested	47
rabbits	not specified	2430 or 4860 mg/kg of a 20% partially neutralized soln (75% sodium adipate)	>2430 and <4860 mg/kg	47
Adipic/Glutaric/Succinic Mixture (percentages not given)				
rats	10 males	5000-7500 mg/kg aq.	6829 mg/kg	68
Azelaic Acid				
Wistar rats	6 males 6 females	500-4000 mg/kg	greater than highest dose tested	72
New Zealand rabbits	6 males 6 females	500-4000 mg/kg	greater than highest dose tested	72
Disodium Sebacate				
Wistar rats	4 males, 4 females	0-5000 mg/kg	greater than highest dose tested	67
New Zealand rabbits	4 males, 4 females	0-6000 mg/kg	greater than highest dose tested	67
Dodecanedioic Acid				
rats	m/f; no. not specified	not specified	>3000 mg/kg	69
		DERMAL		
Glutaric Acid				
rabbits	1 rabbit/group; M/F	50% aq. solution	>10,000 mg/kg	68
Adipic Acid				
rabbits	1- 2/group; male and female	5010 (n=1) or 7940 mg/kg (n=2) 40% adipic acid in corn oil, with occlusion	greater than highest dose tested	47
Adipic/Glutaric/Succinic Mixture (percentages not given)				
rats	not specified	not specified	>200 mg/kg	68
New Zealand white rabbits	not specified	40% aq. solution; 24 h occlusive exposure	>7940 mg/kg	68
Dodecanedioic Acid				
albino rabbits	males; no. not specified	not specified	>6000 mg/kg	**69**
		INHALATION		
Adipic Acid				
rats	20/group; males and females	5.4 or 7.7 mg/l; head/nose-only exposure; $MMAD_{50}$ <3.5 µm	greater than highest dose tested	70

Table 4. Acute toxicity - Dicarboxylic Acids and Their Salts

Animals	No./Gender/Group	Dose	median lethal dose/conc.	Reference
Adipic/Glutaric/Succinic Mixture				
rats	20, gender not specified	4 h exposure' percentages not given	>0.03 mg/l	68
rats	Crl:Cd/BR; 42 males	5.9 mg/l ; 4 h nose-only exposure; 66.0% dimethyl glutarate; 16.5% dimethyl succinate; 17.0% dimethyl adipate	anterior and posterior nasal passages were affected; nasal lesions distributed along inspiratory airflow routes; lesions in posterior nasal cavity were less severe	117
		PARENTERAL		
Disodium Succinate				
mice	not specified	i.v.	4500 mg/kg	178
Adipic Acid				
mouse	not specified	i.p., 0.681-50% solution in 0.5% CMC	approx. 170 mg/kg	47
mouse	not specified	i.p., 600 and 900 mg/kg aq.	600 mg/kg	47
mouse	not specified	i.p. admin	4000 mg/kg	47
rats	7 males	i.p., 200-350 mg/kg	275 mg/kg	47
mouse	not specified	i.v., 650-700 mg/kg 2% solution	680 mg/kg	47
rabbit	not specified	i.v., 2430 mg/kg 20% soln, partially neutralized	2430 mg/kg	47
Disodium Azelate				
rats	6 males, 6 females	i.p., 0-1198 mg/kg	greater than highest dose tested	72
rabbits	6 males, 6 females	i.p., 0-1198 mg/kg	greater than highest dose tested	72
Disodium Sebacate				
Wistar rats	4 males, 4 females	i.p., 0-7000 mg/kg	5500 mg/kg; dehydration and ascites formation was noted	67
New Zealand rabbits	4 males, 4 females	i.p., 0-8000 mg/kg	6000 mg/kg; dehydration and ascites formation was noted	67
Wistar rats	10	i.v. , 0-1000 mg/kg	560 mg/kg; dehydration and ascites formation was noted	67
New Zealand rabbits	10	i.v., 0-1800 mg/kg	1400 mg/kg; dehydration and ascites formation was noted	67

Table 5. Ocular Irritation - Dicarboxylic Acid and Their Esters

Concentration	Animals	Procedure	Results	Reference
Succinic Acid				
not specified	not specified	ocular irritation study (details not specified)	severe ocular irritant	68
Glutaric Acid				
not specified	not specified	ocular irritation study (details not specified)	moderate ocular irritant	68
Adipic Acid				
undiluted	2 albino rabbits	10 or 57.1 mg placed in eye; eye of 1 animal rinsed	10 mg: mild conjunctival irritation in the rinsed and unrinsed eyes; the rinsed eye was normal at 3 days and the unrinsed eye was normal at 14 days; 57.1 mg: mild conjunctival irritation with transient corneal opacity in the rinsed eye; the eye was normal by day 3; moderate to mild conjunctival irritation with mild corneal opacity and iritic effects in the unrinsed eye; the eye was normal at day 7	68
undiluted	6 rabbits; gender not specified	0.1 ml instilled into the eye; eyes were not rinsed	severely irritating - primary irritation index of 41.5/110; irritated conjunctiva and scar formation, increased corneal opacity and iridal inflammation; not cleared by day 8	152
undiluted	3 rabbits; gender not specified	100m g instilled following GLP; acute eye irritation/corrosion test	severe irritation; corneal opacity and iridal irritation; cleared within 16 days	152
undiluted	2 rabbits; gender not specified	50 mg placed in eye; eyes were not rinsed	severely irritating; corneal opacity still present at day 8	152
Dodecanedioic Acid				
not specified	male rabbits, no. not specified	ocular irritation study (GLP; details not provided)	slight irritant; irritation index 11.96/110	69
not specified	rabbits; no./gender not specified	ocular irritation study (details not provided)	small area of corneal opacity and mild conjunctival irritation; cleared within 7 days	69

Table 6. Dermal irritation and sensitization - Dicarboxylic Acids and Their Salts

Dose/Conc..	Animals	Procedure	Results	Reference
		IRRITATION		
Succinic Acid				
not specified	rabbits, no./gender not specified	irritation studies (details not provided)	slight to mild irritation	68
Glutaric Acid				
not specified	rabbits, no./gender not specified	irritation studies (details not provided)	slight irritation	68
Adipic Acid				
500 mg of 50% aq.	6 rabbits	occlusive application to a 5 cm x 5 cm area of abraded or intact skin for 24 h	intact skin: erythema (score 2-3/4), cleared by day 3; abraded skin: mild to severe erythema and edema (2/4 at 24 h; 0-2 at 72 h), cleared by day 7	47
undiluted or 80% aq. paste	2 rabbits/group	occlusive application to intact skin on the back and the ear for 20 h	no irritation on the back; erythema on the ear at 24 h (score of 2/4), with clearing by 72 h	47
not specified	rabbits, no./gender not specified	occlusive application for 24 h	not irritating	47
undiluted or 50% paste in propylene glycol (PG)	6 rabbits	semi-occlusive application of 500 mg for 24 h	slight to mild irritation in 3/6 rabbits with 50%; no corrosion with undiluted test material	47
50% in PG	10 guinea pigs, gender not specified	applied to intact skin	no irritation	47
Succinic/Glutaric/Adipic Acids Mixture (percentages not specified)				
not given	guinea pigs, no./gender not specified	irritation study (details not provided)	no to mild irritation	70
Dodecanedioic Acid				
not specified	male rabbits, no. not specified	irritation study; 4 h exposure (GLP; details not provided)	not an irritant; irritation index 0/8	69
0.5 g	male rabbits, no. not specified	FHSA procedures	not an irritant	69
		SENSITIZATION		
Adipic Acid				
induction: 0.1 ml of 1.0% aq. soln; challenge: 0.05 ml of 50 and 25% in PG	10 guinea pigs/group	induction: 4 sacral intradermal injections, 1/wk; challenge: dermal application after a 2 wk rest period	very mild to no irritation; no sensitization	47
Succinic/Glutaric/Adipic Acids Mixture (percentages not specified)				
not given	guinea pigs, no./gender not specified	sensitization study (details not provided)	not a sensitizer	70
Dodecanedioic Acid				
induction: 0.5%; challenge: 25 and 50%	female guinea pigs, no. not specified	Magnusson-Kligman maximization test (intracutaneous admin at induction; dermal admin at challenge)	not a sensitizer	69

Table 7. Genotoxicity studies - Dicarboxylic acids and Their Salts

Concentration	Vehicle	Procedure	Test System	Results	Reference
		IN VITRO			
Malonic Acid					
≤3333 µg/plate	water	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	76
Succinic Acid					
≤5 mg/plate	phosphate buffer	Ames test	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98	negative	77
≤1.0 mg/ml	saline	chromosomal aberration assay	Chinese hamster fibroblasts cells	negative	77
Sodium Succinate					
≤10 µg/plate	distilled water	Ames test, +/- metabolic activation	*S. typhimurium TA97, TA102*	negative	179
Disodium Succinate					
≤5 mg/plate	phosphate buffer	Ames test	*S. typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98*	negative	77
≤10,000 µg/plate	distilled water	Ames test, +/- metabolic activation	*S. typhimurium* TA97, TA102	negative	178
≤15.0 mg/ml	saline	chromosomal aberration assay	Chinese hamster fibroblasts cells	equivocal	77
Glutaric Acid					
0-5000 µg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA 1537, TA1538	negative	68
0-8295 µg/ml	not specified	mouse lymphoma assay,+/-metabolic activation	L5178Y/ TK cells	negative with neutral pH	68
0-12.5 mg/ml w/out; 0-26.3 mg/ml w/met. act.	DMSO	transformation assay, +/- metabolic activation	Balb/c-3T3 cells	positive, +/- activation	68
≤10,000 µg/plate	water	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	180
Adipic Acid					
≤10,000 µg/plate	DMSO	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	80
≤10 mg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *E. coli* WP2	negative	47
≤5 mg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *E. coli* WP2uvrA	negative	47
≤200 mg/l	not specified	Ames test, without metabolic activation	*S. typhimurium* TA1530, G-46	negative	47
≤200 mg/l	not specified	yeast gene mutation assay, without metabolic activation	*S. cerevisiae* D-3	negative	47
≤2000 µg/plate	DMSO	mouse lymphoma assay, +/- metabolic activation	*L5178Y/TK ± cells*	negative	81
≤200 mg/l	not specified	cytogenetic assay, without metabolic activation	human embryonic lung fibroblasts	negative	47
≤1000 µg/ml	not specified	viral enhanced cell transformation assay	Syrian hamster ovary cells	negative	47
Adipic/Glutaric/Succinic Acid Mixture					
0-3000 µg/plate	50% aq. solution	Ames test, +/- metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA 1537, TA1538	negative	68
≤5000 µg/ml	not specified	unscheduled DNA synthesis	F344 rat hepatocytes	negative	68
≤2500 µg/ml	not specified	HGPRT assay, without metabolic activation	not specified	negative	68
≤3500 µg/ml	not specified	HGPRT assay, with metabolic activation	not specified	negative	68
≤1500 µg/plate	distilled water	transformation assay, without metabolic activation	CHO cells	negative	68

Table 7. Genotoxicity studies - Dicarboxylic acids and Their Salts

Concentration	Vehicle	Procedure	Test System	Results	Reference
≤2500 µg/plate	distilled water	transformation assay, with metabolic activation	CHO cells	positive at 2000 µg/ml	68
Azelaic Acid					
20%	cream	Ames test; no details	not specified	negative	51
20%	cream	HGRPT test; no details	Chinese hamster ovary cells	negative	68
20%	cream	human lymphocyte test, no details	human lymphocytes	negative	68
Sebacic Acid					
≤5000 µg/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *E. coli* WP2	negative	181
Dodecanedioic Acid					
10-5000 µg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	69
		IN VIVO			
Glutaric Acid					
0, 800 mg/kg	distilled water	micronucleus assay	4 male and 4 female CD-1 mice/group	negative	68
Adipic Acid					
≤375 mg/kg; 1 or 5 doses	not specified	cytogenetic assay; animals dosed orally by gavage	male rats	negative	47
5000 mg/kg (1 dose); 2500 mg/kg (5 doses)	not specified	cytogenetic assay; animals dosed orally by gavage	male rats	negative	47
≤375 mg/kg; 1 or 5 doses	not specified	dominant lethal assay; animals dosed orally be gavage	male rats	negative	47
5000 mg/kg (1 dose); 2500 mg/kg (5 doses)	not specified	dominant lethal assay; animals dosed orally be gavage	male rats	negative	47
Adipic/Glutaric/Succinic Acid Mixture					
2750 mg/kg (males), 1375 mg/kg (females)	not specified	cytogenetic assay; animals dosed orally by gavage	male and female Sprague Dawley rats; 1 dose	negative	68
Azelaic Acid					
20%	cream	dominant lethal assay	mice	negative	68
Dodecanedioic Acid					
≤5000 mg/kg	not specified	micronucleus assay	Crl:CD-1(CR)BR mice	negative	69

Table 8. Induction of peroxisome proliferation – Esters of Dicarboxylic Acids

Test System/Procedure	Test Compound/Dose	Results/Observations	Reference
Diethylhexyl Adipate			
hepatocytes from male Swiss mice and rats	diethylhexyl adipate (DEHA)	no peroxisome proliferation	Cornu et al. 1992
	1° metabolites: MEHA; 2-ethyl-hexanol, 0.5 mM	5-fold induction of peroxisomal β-oxidation in mouse hepatocytes, as measured by cyanide-insensitive palmitoyl CoA oxidase (PCO) activity; 4-5 fold increase in rat hepatocytes	
	2° metabolite: 2-ethylhexanoic acid, 1mM 2° metabolite: 2-ethyl-5-hydroxy-1-oic acid, 2mM	25-fold induction of PCO activity in mouse hepatocytes;9-fold increase in rat hepatocytes; 2-ethyl-hexanoic acid was the proximate peroxisome proliferator 5-fold stimulation of PCO	
cultured guinea pig hepatocytes	DEHA and metabolites, ≤2 mM	did not stimulate PCO	Cornu et al. 1992
cultured marmoset hepatocytes	DEHA and metabolites, ≤2 mM	did not stimulate PCO	
male and female Wistar rats and Swiss mice, 5/gender/group; dosed orally by gavage for 14 days in corn oil	DEHA, 0-2.5 g/kg 2-ethylhexanol, 0-1.75 g/kg 2-ethylhexanoic acid, 0-1.0 g/kg	- relative liver to body weights increased dose-dependently -on a molar basis, DEHA was twice as potent as 2-ethylhexanol or 2-ethylhexanoic acid -peroxisomal β-oxidation was induced in a linear dose-response manner; -PCO was stimulated to the greatest effect in male mice -2-ethylhexanoic acid was the primary proliferator	Keith et al. 1992
male and female F344 rats or female $B6C3F_1$ mice, 5/gender/group; dosed orally by gavage for 14 days in corn oil	≤2.5 g/kg/day DEHA	-PCO activity was increased to the greatest extent, 15-fold, in male rats -dose-related peroxisome proliferation was statistically significantly increased in both rat and mice -relative liver weights were increased in a dose-dependent manner	Keith et al. 1992
female F344 and $B6C3F_1$ mice, 5-8/group;dosed for 1, 4,or 13 wks	0-4.0% DEHA in the diet (rats) 0-2.5% DEHA in the diet (mice)	-PCO induction was markedly increased in rats and mice at all 3 time frames -microsomal cytochrome activity and stimulation of replicative DNA was significantly increased in mice, but not in rats	Lake et al. 1997
male F344 rats and female $B6C3F_1$ mice, 5/group; 5 ml/kg for 14 days; route of administration not specified	0-2 g/kg DEHA	-PCO and catalase activity, but not glutathione activity, were statistically significantly increased -steady-state hydrogen peroxide activity increased 2-fold compared to controls	Tomaszewski et al. 1986
F344 rats, 3-4/group; dietary administration, 30 days	0.25-2% DEHA 0.25-2% diethylhexyl phthalate 0.001-0.02% ciprofibrate (a very potent peroxisome proliferator)	-hepatomegalic potencies of diethylhexyl phthalate were 200 and of ciprofibrate were 1000—fold greater than DEHA -DEHA produced moderate peroxisome proliferation at 2%, but not at lower concentrations	Reddy et al., 1986
rats, 2 males and 2 females/group; dietary administration, 21 days	≤2.5% DEHA	at 2.5%, peroxisome proliferation was markedly increased in males and moderately increased in females; overall, however, activity was weak	Barber et al. 1987
Diethylhexyl Sebacate			
4 male F344 rats; dietary administration for 3 wks	2% diethylhexyl sebacate	hepatic peroxisome proliferation was observed, evidenced by increased liver size, hepatic activities of peroxisome-associated enzymes, and hypolipidemia	Moody et al. 1978

Table 9. Acute toxicity - Esters of Dicarboxylic Acids

Animals	No./Gender/Group	Dose	median lethal dose/concentration, or result	Reference
		ORAL		
Diethyl Malonate				
rats	not specified	not specified	15,000 mg/kg	14
Dimethyl Malonate				
rats	not specified	not specified	>2000 mg/kg	14
Diethyl Succinate				
rats	not specified	not specified	8530 mg/kg	119
Dibutyl Adipate				
rats	*not specified*	*20% dispersion*	*11,260-12,900 mg/kg*	*5*
rats	*not specified*	*undiluted*	*1520 mg/kg*	*5*
rats	not specified	not specified	1290 mg/kg	105
rats	not specified	undiluted	12,900 mg/kg	87
Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid				
Sprague-Dawley rats	5-10; males/females	2000-15,800 mg/kg, undiluted	greater than highest dose tested	87
Ditridecyl Adipate				
Sherman Wistar rats	5/gender	16,000 mg/kg	greater than highest dose tested	87
Wistar rats	5/gender	15,000 mg/kg	greater than highest dose tested	87
Diisopropyl Adipate				
Sprague-Dawley rats	*5 males/5 females*	*formulation containing 1.08%*	*1 female died*	*2*
Sprague-Dawley rats	*5 males/5 females*	*formulation containing 1.08%*	*no animals died*	*2*
Sprague-Dawley rats	*5 males/5 females*	*formulation containing 5%*	*no animals died*	*2*
[2]rats	*5 males/f5 females*	*formulation containing 0.7%*	*>76,800 mg/kg*	*2*
rats	*not specified*	*formulation containing 20.75%*	*>15,000 mg/kg*	*2*
Diisobutyl Adipate				
NMRI mice	5 males	2000 mg/kg	greater than highest dose tested	113
Diethylhexyl Adipate				
mice	*5 males/5 females*	*≤20,000 mg/kg in corn oil*	*males: 15,000 mg/kg; females: 24,600 mg/kg*	*2*
rats	*5 males/5 females*	*≤20,000 mg/kg, undiluted*	*2 males of the 10,000 mg/kg group died; 1 male and 1 female of the 20,000 mg/kg group died*	*2*
albino rats	*5 males/5 females*	*7400 mg/kg*	*1 animal died*	*2*
rats	*not specified*	*not specified*	*single oral toxic dose - 9.11 g/kg*	*2*
rats	*not specified*	*not specified*	*no-effect dose: 6000 mg/kg; central nervous system effects seen at higher concentrations*	*2*
Harlan-Wistar rats	*5 males/5 females*	*formulations containing 0.175%*	*>6500 mg/kg*	*2*
rats	not specified	not specified	9110 mg/kg	116
rats	5 males/females	7380 mg/kg, undiluted	>7300 mg/kg	87
rats	not specified	not specified	9.1 g/kg	87
Diisooctyl Adipate				
rats	5/group	2000-64,000 mg/kg, undiluted	greater than highest dose tested	87
guinea pigs	not specified	not specified	>5 ml/kg	87
Diisononyl Adipate				
rats	5/group	346-10,000 mg/kg, undiluted	greater than highest dose tested	87
Diisodecyl Adipate				
NMRI mice	5 male	2000 mg/kg	greater than highest dose tested	111
rats	not specified	undiluted	20,500 mg/kg	87
Dioctyldodecyl Adipate				
NMRI mice	5 female	2000 mg/kg	greater than highest dose tested	112
rats	not specified	not specified	NOAEL <4000 mg/kg	43
Diisocetyl Adipate				
NMRI mice	5 males	2000 mg/kg	greater than highest dose tested	110
Diisopropyl Sebacate				
NMRI mice	5 female	2000 mg/kg	greater than highest dose tested	126
Diethylhexyl Sebacate				
NMRI mice	5 female	2000 mg/kg	greater than highest dose tested	109
mice	not specified	undiluted	9.5 g/kg	87

Table 9. Acute toxicity - Esters of Dicarboxylic Acids

Animals	No./Gender/Group	Dose	median lethal dose/concentration, or result	Reference
rats	not specified	undiluted	5.0 cc/kg	87
rats	not specified	undiluted	12.8 g/kg	87
rats	not specified	undiluted	17 g/kg	87
Dioctyldodecyl Dodecanedioate				
Wistar rats	5 male/5 female	5000 mg/kg	greater than highest dose tested	123
Diisocetyl Dodecanedioate				
Wistar rats	5 male/5 female	5000 mg/kg	greater than highest dose tested	107
Esterase Metabolites *(summary information/results only provided)*				
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
rats			1516-7000 mg/kg	118
mice			2500-3768 mg/kg	118
Hexyl Alcohol (metabolite of dihexyl adipate)				
rats			3131-4900 mg/kg	118
mice			103-1950 mg/kg	118
Butyloctanol (metabolite of dibutyloctyl sebacate)				
rats			12,900 mg/kg	119
Decyl Alcohol (metabolite of decyl succinate)				
rats			9800 mg/kg	119
Isooctyl Alcohol (metabolite of diisooctyl adipate and diisooctyl sebacate)				
rats		mixture of C7-9 branched alkyl alcohols	>2000 mg/kg	118
Nonyl Alcohol (metabolite of diisononyl adipate)				
rats		mixture of C8-10 branched alkyl alcohols	3000 mg/kg	118
Isodecyl Alcohol (metabolite of diisodecyl adipate)				
rats		mixture of C9-11 branched alkyl alcohols	4600 mg/kg	118
		DERMAL		
Diethyl Malonate				
rabbits	not specified	not specified	16,700 mg/kg	14
Dibutyl Adipate				
rabbits	*not specified*	*96%*	*20 ml/kg*	*5*
rats	not specified	i.m.	NOAEL >8000 mg/kg	115
Ditridecyl Adipate				
rabbits	3	2000 mg/kg	greater than highest dose tested	87
rabbits	10	5000 m/kg to abraded skin; semi-occlusive	greater than highest dose tested	87
Diethylhexyl Adipate				
rabbits	*8*	*≤8700 m/kg to abraded skin; occlusive*	*mild irritation; no systemic toxic effects*	*2*
rabbits	1 male/1 female	≤8660 mg/kg for 24 h, occluded, 1 intact and 1 abraded site	>8670 mg/kg	116
Diisononyl Adipate				
rabbits	4/group	50-3160 mg/kg to abraded skin	greater than highest dose tested	87
Diethylhexyl Sebacate				
guinea pigs	not specified	not specified	<10,000 mg/kg	1
Dioctyldodecyl Dodecanedioate				
NZW rabbits	5 male/5 female	2000 mg/kg, intact skin, 24 h occlusive	>2000 mg/kg	108
Esterase Metabolites *(summary information/results only provided)*				
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
rats			>3000 mg/kg	118
rabbits			1980-2600 mg/kg	118
Hexyl Alcohol (metabolite of dihexyl adipate)				
rats			1500 mg/kg	118
rabbits			1500 - >500 mg/kg	118
Butyloctanol (metabolite of dibutyloctyl sebacate)				

Table 9. Acute toxicity - Esters of Dicarboxylic Acids

Animals	No./Gender/Group	Dose	median lethal dose/concentration, or result	Reference
rabbits			3.36 ml/kg	119
Decyl Alcohol (metabolite of decyl succinate)				
rabbits			3.5 ml/kg	119
Isooctyl Alcohol (metabolite of diisooctyl adipate and diisooctyl sebacate)				
rats		mixture of C7-9 branched alkyl alcohols	>2600 mg/kg	118
Nonyl Alcohol (metabolite of diisononyl adipate)				
rats		mixture of C8-10 branched alkyl alcohols	3160 mg/kg	118
Isodecyl Alcohol (metabolite of diisodecyl adipate)				
rats		mixture of C9-11 branched alkyl alcohols	>2600 mg/kg	118
INHALATION				
Diethyl Malonate				
rats	not specified	concentrated vapors for 8 h	no deaths	14
Diethyl Succinate				
rats	not specified	concentrated vapors for 8 h	no deaths	119
Dibutyl Adipate				
albino rats	*6 male*	*flowing stream of saturated air, 8 h*	*no mortality*	*2*
Diethylhexyl Adipate				
rats	not specified	concentrated vapors for 8 h	no deaths	119
Diethylhexyl Sebacate				
rats	not specified	250 mg/m^3 for 4 h	no effect on lung or liver	1
rats	3	saturated vapor, 6 h	no lung toxicity	1
rats	4	940 mg/m^3, 7 h	3 rats died, may be attributable to thermal decomp products	1
guinea pigs	2	940 mg/m^3, 7 h	no animals died	1
rabbits	4	940 mg/m^3, 7 h	2 rabbits died, may be attributable to thermal decomp products	1
Esterase Metabolites *(generally, summary information/results only provided)*				
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
rats	3 males/3 females	vapor conc. of 0.89 mg/l or aerosol/ vapor conc of 5.3 mg/l, 4 h	0.89 mg/l: all animals survived; 5.3 mg/l: all animals died	118
mice, rats, and guinea pigs	10	227 ppm, 6 h	all animals survived	118
Hexyl Alcohol (metabolite of dihexyl adipate)				
rats		21 mg/l, 1 h	greater than highest dose tested	118
Butyloctanol (metabolite of dibutyloctyl sebacate)				
rats		concentrated vapors for 8 h	no deaths	119
Decyl Alcohol (metabolite of decyl succinate)				
rats		concentrated vapors for 8 h	no deaths	119
PARENTERAL				
Dimethyl Adipate				
Sprague-Dawley rats	not specified	i.p.	1.8 ml/kg	114
Diethyl Adipate				
Sprague-Dawley rats	not specified	i.p.	2.5 ml/kg	114
Dipropyl Adipate				
Sprague-Dawley rats	not specified	i.p.	3.8 ml/kg	114
Dibutyl Adipate				
rats	*not specified*	*i.p.*	*5.2 ml/kg*	*5*
Diisopropyl Adipate				
rats	*not specified*	*i.v.*	*640 mg/kg*	*2*
Diethylhexyl Adipate				
rats	*not specified*	*i.v.*	*900 mg/kg*	*2*
rabbits	*not specified*	*i.v.*	*540 mg/kg*	*2*
Sprague-Dawley rats	not specified	i.p.	>50 ml/kg	114

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Table 10. Ocular Irritation - Esters of Dicarboxylic Acids*

Concentration	Animals/System	Procedure	Results	Reference
Diethyl Malonate				
undiluted	rabbits, no./gender not specified	0.1 ml	slight to moderate irritation	14
Dimethyl Malonate				
undiluted	rabbits, no./gender not specified	0.1 ml ,unrinsed	slight to moderate irritation; cleared by day 8	14
Dibutyl Adipate				
undiluted	*rabbits, no. not specified*	*unrinsed*	*minimally irritating*	*5*
undiluted	*2 New Zealand rabbits*	*unrinsed*	*slight irritation*	*5*
0.1% in olive oil	*rabbits*	*unrinsed*	*non-irritating*	*5*
Diisopropyl Adipate				
undiluted	*6 albino rabbits*	*0.1 ml, unrinsed*	*negligible irritation*	*2*
undiluted	*6 albino rabbits*	*0.1 ml, unrinsed*	*non-irritating*	*2*
0.7% in formulation	*9 albino rabbits*	*0.1 mi, undiluted, rinsed*	*some corneal stippling*	*2*
5% in formulation	*6 albino rabbits*	*not specified*	*non-irritating*	*2*
20.75% in formulation	*6 albino rabbits*	*not specified*	*non-irritating*	*2*
undiluted	3 albino rabbits	0.1 ml, unrinsed	non-irritating	113
Diethylhexyl Adipate				
undiluted	*6 albino rabbits*	*0.1 ml, unrinsed*	*non-irritating*	*2*
0.01% in formulation	*6 albino rabbits*	*0.1 ml, unrinsed*	*non-irritating*	*2*
0.175% in formulation	*6 albino rabbits*	*0.1 ml, unrinsed*	*mild transient irritant*	*2*
Diisopropyl Sebacate				
	6 rabbits	0.1 ml, unrinsed	minimally irritating	121
Diethylhexyl Sebacate				
1.2% in formulation	EpiOcular MTT viability assay	undiluted	non-irritating	122
Dioctyldodecyl Dodecanedioate				
undiluted	6 rabbits	0.1 ml, unrinsed	MMTS = 0.0; non-irritating	106
Diisocetyl Dodecanedioate				
undiluted	6 rabbits	0.1 ml, unrinsed	MMTS = 0.0; non-irritating	124
Esterase Metabolites *(generally, summary information/results only provided)*				
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)				
	rabbits	20 µg	moderately severe corneal irritation	118
Isopropyl Alcohol (metabolite of diisopropyl adipate and diisopropyl sebacate)				
	rabbits		severely irritating	182
Hexyl Alcohol (metabolite of dihexyl adipate)				
	rabbits		highly irritating	118

*Data from original safety assessments are in italics.

Table 11. Dermal irritation and sensitization - Esters of Dicarboxylic Acids*

Dose/Conc.	Animals	Procedure	Results	Reference
		DERMAL IRRITATION		
Diethyl Malonate				
not specified	rabbits	occlusive application; 4 h	slightly irritating	14
Dimethyl Malonate				
not specified	rabbits	semi-occlusive application; 4 h	not irritating; slight erythema at 30-60 min after patch removal	14
Dibutyl Adipate				
undiluted	*rabbits*	*applied to belly*	*PII of 2/8*	5
undiluted	*5 albino rabbits*	*0.1 ml, applied 8x in 4 h*	*moderate erythema at 24 h*	
undiluted	*3 rabbits*	*impregnated bands, 3 d application, 3 wks*	*moderate erythema*	5
undiluted	*5 rabbits*	*impregnated bands, applied 2w/wk for 6 applications*	*no progressive skin damage*	5
undiluted	*3 rabbits*	*0.025 ml to intact and abraded skin, 3 applications at 3 h intervals for 3 days*	*erythema and capillary injection during the study; desquamation was observed*	5
10% in acetone	*5 hairless mice*	*applied to ear, 1x/day, 10 days*	*no adverse effect*	5
10% in acetone	*mice*	*application to backs, 2x/day, 14 days*	*no adverse effect*	5
Diisopropyl Adipate				
undiluted	*9 albino rabbits*	*24 h, 0.1 ml, occlusive*	*PII of 1.6/4; mild irritant*	2
undiluted	*9 albino rabbits*	*24 h, 0.1 ml, occlusive*	*PII of 1.3/4; mild irritant*	2
undiluted	*9 albino rabbits*	*24 h, 0.1 ml, occlusive*	*PII of 0.06/4; minimally irritating*	2
5% in formulation	*9 albino rabbits*	*24 h, 0.1 ml, occlusive*	*PII of 0.33; minimally irritating*	2
20.75% in formulation	*9 albino rabbits*	*24 h, 0.1 ml, occlusive*	*PII of 0.11; minimally irritating*	2
undiluted	3 albino rabbits	semi-occlusive application; 4 h, undiluted	non-irritating	113
Diethylhexyl Adipate				
undiluted	*6 albino rabbits*	*intact and abraded skin, 0.5 ml, 24 h, occlusive*	*very mild irritant*	2
0.175% in formulation	*3 albino rabbits*	*4, 0.5 ml applications*	*irritation index of 1.6/4*	2
Diisodecyl Adipate				
undiluted	3 albino rabbits	semi-occlusive application, 4 h, undiluted	non-irritating; scores of 0-1 for erythema and 0 for edema at 1-72 h; reversible	111
Dioctyldodecyl Adipate				
undiluted	3 albino rabbits	semi-occlusive application, 4 h, undiluted	non-irritating; scores of 0-1 for erythema and 0 or 1 for edema at 24-72 h; reversible	112
Diisocetyl Adipate				
undiluted	3 albino rabbits	semi-occlusive application, 4 h, undiluted	non-irritating; scores of 0-2 for erythema and 0 or 1 for edema at 1-72 h; reversible	110
Diethyl Sebacate				
undiluted	8 rabbits	intact and abraded skin, occlusive, 0.3 ml	PII of 0.0	125
30% in ethanol	8 rabbits	intact and abraded skin, occlusive, 0.3 ml	PII of 0.3	125

Table 11. Dermal irritation and sensitization - Esters of Dicarboxylic Acids*

Dose/Conc.	Animals	Procedure	Results	Reference
Diisopropyl Sebacate				
undiluted	6 rabbits	intact and abraded skin, occlusive, 0.5 ml	PII of 2.88; not a primary irritant	121
undiluted	3 albino rabbits	semi-occlusive application, 4 h, undiluted	non-irritating; scores of 1 for erythema, with a 2 at 24 h, and 0 or 1 for edema at 1-72 h; reversible	126
Diethylhexyl Sebacate				
undiluted	3 albino rabbits	semi-occlusive application, 4 h, undiluted	non-irritating; scores of 1 for erythema and 0 for edema at 1-72 h; reversible	109
undiluted	2-4 rabbits	occlusive application; 48 h	not irritating	
Dioctyldodecyl Dodecanedioate				
undiluted	6 NZW rabbits	occlusive application, 24 h, 0.5 ml	PII = 0; not a primary irritant	183
Diisocetyl Dodecanedioate				
undiluted	6 NZW rabbits	occlusive application, 24 h, 0.5 ml	PII = 0; not a primary irritant	184
Esterase Metabolites				
Ethylhexyl Alcohol (metabolite of diethylhexyl adipate and diethylhexyl sebacate)				
	3 male rabbits	occlusion, 4 h	irritating	118
	rabbits	occlusive, 0.5 ml	highly irritating; not reversible	118
Caprylic Alcohol (metabolite of dicapryl succinate, dicapryl adipate, and dicaprylyl/capryl sebacate)				
undiluted	rabbits		mild irritation	185
SENSITIZATION				
Dimethyl Malonate				
not specified	guinea pigs	Buehler method	not sensitizing	14
Dibutyl Adipate				
25%	*5 guinea pigs*	*maximization test*	*not sensitizing*	*5*
Diethylhexyl Adipate				
0.1% in olive oil	*10 male guinea pigs*	*induction: 10 injections; 2 wk non-treatment pd; challenge: 0.05 ml injection*	*not sensitizing*	*2*
Diethylhexyl Sebacate				
undiluted	rabbits	occlusive patches, details not provided	no reactions	1
Dioctyldodecyl Dodecanedioate				
0.1 ml for intraderm induction; 0.5 ml top. induction /challenge	10 female guinea pigs	maximization test	not sensitizing; slight erythema at induction	127
Esterase Metabolites				
Hexyl Alcohol (metabolite of dihexyl adipate)				
1% in petrolatum	guinea pigs	maximization test	not sensitizing	118

*Data from original safety assessments are in italics.

Table 12. Genotoxicity studies - Esters of Dicarboxylic Acids*

Concentration	Vehicle	Procedure	Test System	Results	Reference
			IN VITRO		
Diethyl Malonate					
≤5000 µg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA98, TA100	negative	14
≤5000 µg/plate	not specified	cytogenetic assay, +/- metabolic activation	human peripheral lymphocytes	negative; cytotoxic at 5000 µg/plate	14
Dimethyl Malonate					
≤5000 µg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA98, TA100	negative; cytotoxic at ≥1000 µg/plate	14
Dimethyl Succinate					
20,000 µg/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA98, TA100	negative	135
≤10,000 µg/plate	water	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	136
Dimethyl Glutarate					
≤10,000 µg/plate	DMSO	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	137
Dimethyl Adipate					
≤10,000 µg/plate	DMSO	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	138
Dibutyl Adipate					
≤5000 µg/plate		*Ames test, +/- metabolic activation*	S. typhimurium *TA98, TA100, TA1535, TA1537, TA1538*	*negative*	*5*
Di-C7-9 Branched and Linear Alkyl Esters of Adipic Acid					
≤10.0 µl/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA98, TA100	negative	87
Ditridecyl Adipate					
≤10 µl/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	87
Diisobutyl Adipate					
≤10,000 µg/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium* TA98, TA100, TA102, TA97, TA98, *E. coli* wp2	negative	186
Diethylhexyl Adipate					
≤5 mg/plate	*not specified*	*Ames test, +/- metabolic activation*	*S. typhimurium TA1535, TA1537, TA1538, TA98, TA100*	*negative*	*2*
5000 µg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	141
≤0.01 M	not specified	Ames test, +/- metabolic activation	*S. typhimurium*TA98, TA100	negative	140
10,000 µg/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537,. TA98, TA100	negative	139
10,000 µg/plate	95% ethanol	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537,. TA98, TA100	negative	139
10,000 µg/plate	acetone	NTP preincubation assay, +/- metabolic activation	*S. typhimurium TA100, TA1535, TA97, TA98*	negative	142
≤150 µg/plate	not specified	Ames test, +/- metabolic activation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	negative	87

Table 12. Genotoxicity studies - Esters of Dicarboxylic Acids*

Concentration	Vehicle	Procedure	Test System	Results	Reference
not specified	DMSO	liquid suspension assay	S. typhimurium *TA100*	negative	143
≤400 µg/ml	not specified	sister chromatid exchange assay, +/- metabolic activation	Chinese hamster ovary cells	negative w/out activation; equivocal w/activation	146
≤200µg/plate, 3 or 51 h	DMSO	sister chromatid exchange assay	female F344 rat hepatocytes	negative	145
≤400 µg/ml	not specified	chromosomal aberration assay, +/- metabolic activation	Chinese hamster ovary cells	some evidence w/out activation; negative w/activation	146
≤200µg/plate, 3 or 51 h	DMSO	chromosomal aberration assay	female F344 rat hepatocytes	negative	145
≤0.01 M	not specified	^{3}H-thymidine assay, +/- metabolic activation	splenic lymphoid cells	dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, w/a dose-dependent increase in the ratio of acid-soluble to DNA-incorporated ^{3}H-thymidine	140
≤1000 µg/plate		forward mutation assay, +/- metabolic act.	L5178Y cells	negative	144
urine of rats dosed with 2000 mg/kg diethylhexyl adipate					
	corn oil	Ames test		negative	150
Diisononyl Adipate					
≤1000 µg/plate		Ames test, +/- metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	negative	151
≤100 µg/ml		lymphoma assay, +/- metabolic activation	mouse lymphoma L5178Y cells	negative	
≤1000 µg/ml		transformation assay	Syrian hamster embryo cells	negative	151
≤1.3 µ/ml		BALB/3T3 assay		negative	151
Diethyl Sebacate					
		reversion assay	*E. coli* Sd-4-73	negative	152
Dibutyl Sebacate					
not specified	not specified	Ames test	*S, typhimurium* TA98, TA100, TA1535, TA1537, TA1538	negative	153
≤10,000 µg/plate	DMSO & Tween 80	Ames test, +/- metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537*; E. coli wp2 uvrA*	negative	154
Diethylhexyl Sebacate					
≤10,000 µg/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium TA98, TA100, TA1535, TA1537*	negative	139
≤5000 µg/plate	DMSO	Ames test, +/- metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537*; E. coli wp2 uvrA*	negative	155
Esterase Metabolites					
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)					
10,000 µg/plate		Ames test, +/- metabolic activation		negative	139
		Ames test		negative	151
≤0.01 M	not specified	Ames test, +/- metabolic activation	*S. typhimurium*TA98, TA100	negative	140
≤5000 µg/plate	not specified	Ames test, +/- metabolic activation		negative	118
0-1.5 mM	DMSO	liquid suspension assay	*S,. typhimurium* TA100	negative	143
not specified	not specified	mouse lymphoma assay		negative	151

Table 12. Genotoxicity studies - Esters of Dicarboxylic Acids*

Concentration	Vehicle	Procedure	Test System	Results	Reference
not specified	not specified	unscheduled DNA synthesis		negative	151
≤0.01 M	not specified	^{3}H-thymidine assay, +/- metabolic activation	splenic lymphoid cells	dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, w/a dose-dependent increase in the ratio of acid-soluble to DNA-incorporated ^{3}H-thymidine	140
1000 mg/kg	corn oil	Ames test performed on urine from rats dosed orally for 15 days		negative	150
MEHA (metabolite of diethylhexyl adipate)					
10,000 µg/plate		Ames test, +/- metabolic activation		negative	139
≤1000 µg/plate		Ames test		negative	86
Mono-(2-Ethyl-5-Hydroxyhexyl)Adipate (metabolite of diethylhexyl adipate)					
≤1000 µg/plate		Ames test		negative	86
Mono-(2-Ethyl-5-Oxohexyl)Adipate (metabolite of diethylhexyl adipate)					
≤1000 µg/plate		Ames test		negative	86
Propyl and Isopropyl Alcohol (metabolite of dipropyl adipate, diisopropyl adipate, and diisopropyl sebacate)					
		bacterial and mammalian cell assays		negative	182
Isooctyl Alcohol (metabolite of diisooctyl adipate and diisooctyl sebacate)					
C7-9 branched alkyl alcohols		bacterial and mammalian cell assays		negative	118
			IN VIVO		
Dimethyl Succinate					
≥1250 mg/kg	corn oil	micronucleus test, i.p.	male F344 rats	negative	187
Dimethyl Glutarate					
≥1250 mg/kg	corn oil	micronucleus test, i.p.	male F344 rats	negative	188
Dibutyl Adipate					
≤2000 mg/kg	*olive oil*	*mouse micronucleus test*	*mice*	*negative*	*5*
≥724 mg/kg	corn oil	micronucleus test, i.p.	male F344 rats	negative	189
Ditridecyl Adipate					
≤2000 mg/kg	none	micronucleus test; dosed dermally for 13 wks	groups of 10 male and 10 female Sprague Dawley rats	negative	87
Diethylhexyl Adipate					
2000 mg/kg	corn oil	micronucleus test; dosed i.p. for 3 days	5 male B3C3F$_1$ mice	negative	147
≤5000 mg/kg	corn oil	chromosomal aberration assay	8 male B3C3F$_1$ mice	negative	149
not specified	corn oil	chromosomal aberration assay	8 B6C3F$_1$ mice	negative	148
5000 mg/kg	corn oil	micronucleus test single i.p. dose	6 male/6 female B3C3F1 mice	negative	87
Dibutyl Sebacate					
943-2829 mg/kg	olive oil	micronucleus test, i.p.	micronucleus test	negative	153
Diethylhexyl Sebacate					
500 mg/kg	not specified	rat liver foci test	single dose of known carcinogen, the dosing 3x/wk for 11 wks	no activity	156

Table 12. Genotoxicity studies - Esters of Dicarboxylic Acids*

Concentration	Vehicle	Procedure	Test System	Results	Reference
Esterase Metabolites					
Ethylhexyl Alcohol (metabolite of diethylhexyl succinate, diethylhexyl adipate, and diethylhexyl sebacate)					
not specified	not specified	micronucleus test	mice	negative	151
not specified	not specified	transformation assay	BALB/3T3	negative	151
Propyl and Isopropyl Alcohol (metabolite of dipropyl adipate, diisopropyl adipate, and diisopropyl sebacate)					
C7-9 branched alkyl alcohols		micronucleus test		negative	182

*Data from original safety assessments are in italics.

Table 13. Clinical dermal irritation and sensitization - Esters of Dicarboxylic Acids*

Test Material	No. of Subjects	Procedure	Results	Reference
Dimethyl Malonate				
8% in petrolatum	25	maximization test	not a sensitizer	14
Dibutyl Adipate				
undiluted	*10*	*24 h patch test*	*no irritation at 24 or 48 h*	*5*
20% in alcohol	*10*	*24 h occlusive patch test*	*slight reactions in 4 subjects*	*5*
Diisopropyl Adipate				
undiluted	*19*	*24 h occlusive patch, 0.1 ml*	*no irritation*	*2*
undiluted	*19*	*24 h occlusive patch, 0.1 ml*	*no irritation*	*2*
undiluted	*15*	*24 h occlusive patch, 0.1 ml*	*no irritation*	*2*
undiluted	*15*	*24 h occlusive patch, 0.1 ml*	*no irritation*	*2*
undiluted	*16*	*cumulative irritancy test*	*moderately irritating; score of 395/945; irritation in 14/16 subjects on day 6*	*2*
0.7% in formulation	*13*	*cumulative irritancy test*	*non-irritating; score of 2/630*	*2*
1.1% in formulation	*17*	*cumulative irritancy test*	*low potential for hazard to consumer; score of 0.29/84*	*2*
1.1% in formulation	*17*	*cumulative irritancy test*	*low potential for hazard to consumer; score of 0.24/84*	*2*
20.75% in a bath oil	*7*	*cumulative irritancy test*	*score of 8/84*	*2*
20.75% in formulation diluted to 1.25%	*19*	*24 h occlusive patch, 0.1 ml*	*minimal irritation*	*2*
5.0% in formulation	*19*	*24 h occlusive patch, 0.1 ml*	*no irritation*	*2*
1.08% in formulation	*235*	*HRIPT*	*no sensitization; slight hyperpigmentation*	*2*
3.0% in formulation	*50*	*HRIPT*	*no irritation or sensitization*	*2*
5.0% in formulation	*108*	*HRIPT*	*no irritation or sensitization*	*2*
5.0% aq. dispersion of a product containing 20.75%	*116*	*HRIPT*	*minimal, faint erythema produced throughout the study*	*2*
0.7% in formulation	*25*	*maximization test*	*no contact sensitization potential*	*2*
Diethylhexyl Adipate				
0.175% in formulation	*11*	*cumulative irritancy test*	*slightly irritating; score of 72/630*	*2*
0.01% in formulation	*100*	*Schwartz-Peck prophetic patch test*	*not an irritant or a sensitizer*	*2*
0.01% in formulation	*49*	*Shelanski and Shelanski HRIPT*	*weak reactions in up to 4 subjects and strong reactions in 1 subject*	*2*
9.0% in formulation	*209*	*modified Draize-Shelanski patch test*	*3 strong reactions and 1 faint reaction at 2nd challenge*	*2*
9.0% in formulation	*151*	*modified Draize-Shelanski patch test*	*irritant reactions in 2 subjects; no sensitization*	*2*
product containing 0.7% of a 25% solution	*not given*	*Shelanski-Jordan RIPT*	*1-2 subjects had reactions during the study*	*2*
Diisostearyl Adipate				
undiluted	50	HRIPT	not a primary irritant or sensitizer	159
1.5% in formulation	20	SIOPT	not irritating	160
1.5% in formulation	25	maximization test	no contact sensitization potential	161

Table 13. Clinical dermal irritation and sensitization - Esters of Dicarboxylic Acids*

Test Material	No. of Subjects	Procedure	Results	Reference
Diisopropyl Sebacate				
1.8% in formulation	20	SIOPT	not irritating	162
undiluted	105	patch test	no irritation or sensitization	163
2.2% in formulation	27	maximization test	no irritation or sensitization	164
1% in formulation	110	modified HRIPT, semi-occlusive	not an irritant or a sensitizer	165
1% in formulation	110	modified HRIPT, semi-occlusive	not an irritant or a sensitizer	165
7.2% in formulation	51	HRIPT, semi-occlusive	no skin reactivity observed	166
Diethyl Sebacate				
1.5% in formulation	20	SIOPT	non-irritating; PII of 0.00	160
1.5% in formulation	25	maximization test	not sensitizing	162
Diethylhexyl Sebacate				
undiluted	15-30	occlusive patches	no reactions	1
Dioctyldodecyl Dodecanedioate				
undiluted	50	HRIPT	not a primary irritant or sensitizer	159
Diisocetyl Dodecanedioate				
undiluted	50	HRIPT	not a primary irritant or sensitizer	159
Esterase Metabolites				
Methanol (metabolite of dimethyl succinate, dimethyl glutarate, and dimethyl adipate				
			primary irritation of the skin	190
3.2%	274	provocative occupational study	positive results	190
5%		closed patch test	slight positive reaction (+)	190
7 and 70%		closed patch test	+++ reactions	190
Propyl Alcohol				
undiluted	20	24 h patch test	no reactions	191
undiluted	116	48 h patch test	no reactions	192
undiluted	16	24 h patch test	no reactions	193
undiluted	42	48 h patch test	no reactions	194
undiluted	16	24 h patch test	no reactions	195
undiluted	7	24 h patch test	no reactions	196
Isopropyl Alcohol (metabolite of diisopropyl alcohol and diisopropyl sebacate)				
80.74% spray concentration	9		no sensitization potential	197
2.85% in formulation	109	HRIPT	no sensitization	198
undiluted	12	24 h patch test	no reactions	199
Cetyl Alcohol (metabolite of dicetyl succinate and dicetyl adipate)				
11.5% in formulation	80	topical tolerance study	reaction in 1 subject	200
6.0% in formulation	12	cumulative irritancy test	mild cumulative irritation	200
8.4% in formulation	110	HRIPT	not a primary irritant or sensitizer	200
3.0% in formulation	25	HRIPT	not a sensitizer	200
Myristyl Alcohol (metabolite of dimyristyl adipate)				
0.80% in formulation	53	4 wk application	no irritation	200

Table 13. Clinical dermal irritation and sensitization - Esters of Dicarboxylic Acids*

Test Material	No. of Subjects	Procedure	Results	Reference
0.25% in formulation	51	4 wk application	1 reaction by 1 subject	200
0.25% in formulation	229	10 - 24 h occlusive patch	not an irritant or an allergen	200
Stearyl Alcohol ((metabolite of distearyl succinate)				
undiluted		SIOPT	mild irritation	174
Isostearyl Alcohol (metabolite of diisostearyl glutarate, diisostearyl adipate, or diisostearyl sebacate)				
25% in petrolatum	19		no irritation	200
25.0% in formulation			no irritation	200
27.0% in formulation			no irritation	200
28.0% in formulation			no irritation	200
25% in 95% isopropyl alcohol	12	HRIPT	3 subject slight erythema at induction; no sensitization	200
5% in formulation	148	HRIPT, with rechallenge for reactors; add'l challenge with 5% in ethanol	12 subjects had possible sensitization reactions at 1st challenge; 6 reacted at rechallenge; all 6 had positive reactions to 5% in alcohol	200
5% in formulation	60	HRIPT, rechallenge of 5% in ethanol for reactors	5 subjects reacted at 1 challenge1/5 rechallenged reacted	200
Caprylic Alcohol (metabolite of dicapryl succinate, dicapryl adipate, and dicaprylyl/capryl sebacate)				
2% in petrolatum	25	48 h closed patch	no irritation	185
Decyl Alcohol (metabolite of decyl succinate and didecyl sebacate)				
3% in petrolatum	25	48 h closed patch	no irritation	185

*Data from original safety assessments are in italics.

Table 14. Case reports - Esters of Dicarboxylic Acids

Subject	Presentation	Follow-Up Testing/Discussion	Reference
Diethyl Sebacate			
1 subject	severe contact dermatitis after 7 days of treatment with a drug ointment	patch test with 20% diethyl sebacate in ethanol gave +++ reaction	39
1 subject	quick onset of contact dermatitis after use of drug ointment	patch test with 20% diethyl sebacate in ethanol gave +++ reaction	39
24 yr old female	swelling and erythema with 3 mos of use of a cream; diethyl sebacate was in the vehicle	patch test results were positive to the cream containing diethyl sebacate and negative to the lotion that did not; patch testing with 30 add'l subjects was negative	169
28 yr old female	erythema developed with use of an ointment; did not subside when a new ointment was used	positive patch test to 1st, but not 2nd ointment; upon patch testing with a panel of items, including diethyl sebacate, only diethyl sebacate had a positive response at day 2, as 10% in pet, and on day 3, with 1 and 10 % in pet; further patch testing was with diethyl sebacate was positive for this patient but negative for others	167
39 yr old male	1 mo history of tinea cruris after use of a cream; he developed contact dermatitis	patch testing with the cream in 5% diethyl sebacate was positive	171
60 yr old male	developed pruritic eczematoid eruptions after 1 yr use of a cream	positive patch test with 5% diethyl sebacate in petrolatum, to the cream, and to cetyl alcohol	170
49 yr -old female	contact dermatitis to a hand cream	positive patch test to cream; further testing revealed only diethyl sebacate gave a positive reaction	168
Diisopropyl Sebacate			
22 yr old male	reaction after 2 mos use of a cream containing 27% diisopropyl sebacate	positive patch testing to diisopropyl sebacate, alone and in combination, was reported; an open test on the forearm with diisopropyl sebacate produced red papules at 48 h	173
Diethylhexyl Sebacate			
	patient was sensitized to 3 other sebacate esters	10% in petrolatum was not irritating	172
Esterase Metabolites			
Stearyl Alcohol (metabolite of distearyl succinate)			
3 individuals	2 had urticarial-type reactions	1 reaction was thought due to impurities in stearyl alcohol	174

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APPENDIX I – ESTERASE METABOLITE SUMMARY DATA

Decyl Alcohol – metabolite of Decyl Succinate

Clinical Irritation and Sensitization

Tested in at a concentration of 3% in petrolatum, decyl alcohol produced no irritation in a 48 h closed-patch test in 25 human subjects.[185]

Methanol – metabolite of Dimethyl Succinate, Dimethyl Glutarate, Dimethyl Adipate

Absorption, Distribution, Metabolism, and Excretion

Methanol is further metabolized to formaldehyde and then to formic acid. The CIR Expert Panel concluded that formic acid is safe where used in cosmetic formulations as a pH adjustor with a 64 ppm limit for the free acid.[201] The main toxicological risks in humans are severe metabolic acidosis with increased anion gap, typically following oral exposure resulting in > 100 mg/L of formate in the urine.[202] The acidosis and the formic acid metabolite are believed to play a central role in both the central nervous system toxicity and the ocular toxicity.A study to determine the formate levels that resulted from exposure of human volunteers to 200 ppm of methanol for 4 h was conducted. Human volunteers (n=27; age 20-55 y) were exposed to 200 ppm methanol (the Occupational Safety and Health Administration [OSHA] Permissible Exposure Limit) for 4 h and to water vapor for 4 h in a double-blind, random study.[203] Urine samples were collected at 0, 4 and 8 h and blood samples were collected from the subjects before they entered the chamber, every 15 min for the first hour, every 30 min from the first to the third hour and at 4 h. Urine and serum samples were analyzed for formate (LOD 0.5 mg/L). Twenty-six of 27 enrolled subjects completed the study (11 females and 15 males). The researchers did not find any statistically significant differences in serum or urine formate levels between the two exposure conditions at any time point. At the end of the 4 h methanol exposure, formate concentrations of 14.28 ± 8.90 and 7.14 ± 5.17 mg/L were measured in serum and urine, respectively. Under control conditions, formate concentrations of 12.68 ± 6.43 (p=0.38; n=26) and 6.64 ± 4.26 (p=0.59; n=25) mg/L were measured in serum and urine respectively. After 8 h (4 h of no exposure) the serum concentrations were not statistically different with 12.38 ± 6.53 mg/L under methanol exposure conditions and 12.95 ± 8.01 (P=0.6; n=26) under control conditions. Urine formate concentrations after 8 h were 6.08 ± 3.49 and 5.64 ±3.70 mg/L (p=0.6; n=25) in exposed and control conditions, respectively, and were not statistically significantly different.[182]

Clinical Irritation and Sensitization

Methyl Alcohol caused primary irritation to the skin; prolonged and repeated contact with Methyl Alcohol resulted in defatting and dermatitis. In one occupational study, 3.2% of 274 metalworkers with dermatitis had positive results to a patch test of 30% Methyl Alcohol. Typical allergic responses observed after contact with alcohols were eczematous eruption and wheal and flare at the exposure sites. Eczema and erythema were reported after the consumption of alcoholic beverages by persons sensitized to ethyl alcohol. Five percent Methyl Alcohol caused a slight positive (+) reaction in a closed patch test for allergic contact dermatitis, and concentrations of 7% and 70% caused (+++) reactions.[204]

Clinical Assessment of Safety

Clinical data show that Methyl Alcohol can cause severe metabolic acidosis, blindness, and death: toxicity was manifested earlier and at a lower dose compared to ethyl alcohol, but the comparative fatal dose was the same for both alcohols. All routes of exposure were toxicologically equivalent, as the alcohol distributed readily and uniformly throughout all tissues and organs. Individual susceptibilities to Methyl Alcohol varied, but typically, the ingestion of 80 to 150 ml of 80% Methyl Alcohol was fatal. Symptoms of Methyl Alcohol intoxication after ingestion were delayed for 12 to 18 hours; afterwards, the symptoms included headache, anorexia, weakness, fatigue, leg cramps, and/or pain and vertigo. Severe gastrointestinal pain, nausea, vomiting, diarrhea, mania, failed vision, and convulsions could occur. Chronic exposure to Methyl Alcohol could cause edema, granular degeneration, and necrosis of heart muscle fibers, as well as fatty degeneration of the heart muscle; sudden cardiac failure was associated with Methyl Alcohol intoxication. The liver and kidneys often had parenchymatous degeneration, and the liver had focal necrosis and fatty infiltration. Severe acidosis was necessary for the development of blindness. Similar symptoms were observed after percutaneous or inhalation exposure to Methyl Alcohol.[204]

Propyl Alcohol – metabolite of Dipropyl Adipate

Absorption, Distribution, Metabolism, and Excretion

Rats (strain/sex/number not specified) were exposed via inhalation to 2,000 ppm (8360 mg/m^3) for 90 min.[205] Propyl acetate was rapidly hydrolyzed to propyl alcohol. During the 90 min exposure period, blood levels of propyl alcohol were

between 2.6 and 7.7 fold greater than propyl acetate.[182]

Reproductive and Developmental Toxicity

The effects of propyl alcohol on fertility were investigated by exposing male Sprague-Dawley rats (18/group) to 0, 3500 or 7000 ppm (0, 8.61 or 17.2 mg/L) propyl alcohol vapor via inhalation 7 h/day, 7days/week for 62 days, prior to mating with unexposed virgin females.[206] Female Sprague-Dawley rats (15/group) were similarly exposed and mated with unexposed males. Following parturition, litters were culled to 4/sex and the pups fostered by unexposed dams. The pups were weaned on post natal day (PND) 25 and weighed on PND 7, 14, 21, 28 and 35. Male rats exposed to 7000 ppm exhibited a decrease in mating success with 2/16 producing a litter (1 male died as a result of a cage fight and 1 male did not mate). Mating success was not affected in 3500 ppm exposed males or in females. Six males from the 7000 ppm group were retained to determine if this effect was reversible. All 6 males successfully mated 15 weeks after exposure. The authors reported that weight gain was not affected in 7000 ppm exposed females (data not shown), but feed intake was decreased in this treatment group. Crooked tails were observed in 2-3 offspring in 2 of 15 litters from the 7000 ppm maternally exposed group. No other effects on female fertility were reported. No significant differences resulted between offspring of the 7000 ppm group and controls on several behavioral toxicology measures including the Ascent test, Rotorod test, Open Field test, activity test, running wheel activity, avoidance conditioning, and operant conditioning. Activity measures were significantly different between offspring of the 3500 ppm exposure group and controls.[182]

Clinical Irritation and Sensitization

A cumulative irritation study was conducted involving 20 male subjects, where the relative irritancy of free fatty acids of different chain lengths was evaluated.[191] Equimolar concentrations (0.5 M and 1.0 M) of even- and odd-numbered - straight chain saturated fatty acids were dissolved in propanol. Each Al-test® patch containing a fatty acid (0.5 M) was applied to the interscapular area of 10 subjects, and, similarly, each fatty acid was applied at a higher concentration (1.0 M) to the remaining 10 subjects. A control patch containing propanol was also applied to each subject. Patches remained in place for 24 h and reactions were scored 30 minutes after patch removal. This procedure was repeated daily for a total of 10 applications. In both groups of 10 subjects, there were no reactions to propanol.\

In an irritation study, wherein 116 healthy male subjects (21 to 55 years old) were patch tested with pelargonic acid at concentrations of 5%, 10%, 20%, and 39.9% in propanol, a propanol-treated control patch was used.[192] Dose response curves were developed. Patches (Al-test® discs) were saturated with 0.04 ml of a test solution and applied to the upper back for 48 h. Reactions were scored at 48 h and 96 h post-application. There were no reactions to propanol.

In an another irritation study, wherein 16 volunteers (10 females, 6 males; median age of 29.5 years) were patch tested (closed patches, Finn chambers) with 20% pelargonic acid in propanol (pH of 4.3), propanol was one of the controls used.[207] Patches were applied to the anterolateral surface of both upper arms for 24 h. Reactions were scored at 24, 48, and 96 h post-application according to the following scale: 0 (no reaction) to 3 (strong positive reaction: marked erythema, infiltration, possibly vesicles, bullae, pustules and/or pronounced crusting). There were no reactions to propanol.

A skin irritation study was conducted using 42 healthy, non-atopic male volunteers (mean age = 34 years; skin types: II [20 subjects], III [17 subjects], and IV [5 subjects]).[193] Pelargonic acid was patch-tested (Finn chambers, volar forearm) at the following concentrations (in propanol): 40% (12 subjects), 60% (32 subjects), 70% (32 subjects), and 80% (28 subjects), and propanol was used as a control. Each subject received between 3 and 10 patch tests. The patches remained in place for 48 h, and reactions were scored 1 h later according to the following scale: - (no visible reaction) to 4+ (intense erythema with bullous formation). There were no reactions to propanol.

In an irritation study, wherein 16 healthy subjects (ages not stated) were patch tested with pelargonic acid (20% in propanol), propanol was used as a control.[194] Closed patches (Finn chambers) containing the test substance were applied to the anterolateral surface of both upper arms. The patches were removed at 24 h post-application and reactions were scored at 24 h and 96 h post-application. There were no reactions to propanol.

In study conducted to investigate a possible seasonal variation in the skin response to pelargonic acid during the winter and summer, propanol was used as a control.[196] The study was conducted using 17 healthy volunteers (10 males, 7 females; mean age = 27 years). The test substance was applied (closed patch, Finn chamber) to each arm for 24 h. Reactions were scored at 30 min post-removal. Reactions were not observed at sites treated with propanol, water, or to which an empty chamber was applied.

Cetyl Alcohol – metabolite of Dicetyl Succinate and Dicetyl Adipate

Clinical Irritation and Sensitization

A topical tolerance study involving an 11.5% Cetyl Alcohol cream base was conducted with 80 male subjects, ranging in age from 21 to 52 years and in weight from 120 to 220 pounds. The preparations were applied five times daily (every 3 hours) for 10 days. One subject had erythema, folliculitis, and pustule formation (forearm site).[200]

A formulation containing 6.0% Cetyl Alcohol was tested for its skin irritation potential in 20 subjects according to the protocol stated above. The product did not induce skin irritation. In another study, the skin irritation potential of a cream containing 6.0% Cetyl Alcohol was evaluated in 12 female subjects (18-60 years old). The total irritation score (all panelists) for the 21 applications was 418, indicating mild cumulative irritation.[200]

The skin irritation and sensitization potential of a product containing 8.4% Cetyl Alcohol was evaluated in 110 female subjects. Fourteen days after scoring of the tenth application site, a challenge patch was applied to each subject and removed after 48 h; sites were scored after patch removal. The product did not induce primary irritation or sensitization.[200]

The sensitization potential of a cream containing 3.0% Cetearyl Alcohol was evaluated in 25 subjects (18-25 years old). Following a 10-day non-treatment period, occlusive challenge patches were applied to new sites and removed after 48 h. Sensitization reactions were not observed in any of the subjects.[200]

Photosensitization

The photosensitization potential of a lipstick product containing 4.0% Cetyl Alcohol was evaluated in 52 subjects. The experimental procedure was not stated. Photosensitization reactions were not noted in any of the subjects. In another study, a skin care preparation containing 1.0% Cetyl Alcohol did not induce photosensitization in the 407 subjects tested. The experimental procedure was not stated.[200]

Isopropyl Alcohol – metabolite of Diisopropyl Adipate and Diisopropyl Sebacate

Absorption, Distribution, Metabolism, and Excretion

Male rabbits (3/group; strain not specified) were treated by different routes of exposure to compare the absorption and metabolism of isopropyl alcohol.[208] Groups 1 and 2 were treated via gavage with the equivalent of 2 and 4 ml/kg absolute isopropyl alcohol, respectively, as a 35% isopropanol/water solution. Groups 3 and 4 were treated via whole-body inhalation for 4 h (towels soaked with isopropyl alcohol were place in the inhalation chamber and replenished at ½ hour intervals to maintained a saturated environment; no exact concentration given), with Group 3 animals receiving an additional dermal exposure in the form of a towel soaked with 70% isopropyl alcohol applied to the animals' chests and Group 4 animals having plastic barriers on their chests and towels prepared the same way as in Group 3 applied on top of the plastic barriers. The alcohol on the towels was replenished at half hour intervals throughout the duration of the experiment. Blood samples were taken at 0, 1, 2, 3 and 4 h. Samples were analyzed for isopropyl alcohol and the metabolite acetone.

Following gavage exposure to 2 or 4 ml/kg, maximum blood levels of 147 and 282 mg/dl, respectively, of isopropyl alcohol were measured. Concentrations of acetone rose steadily over the 4 h period and were 74 and 73 mg/dl following exposure to 2 or 4 ml/kg, respectively. The authors stated that the maximum levels of isopropyl alcohol observed in this experiment, correlated with inebriation and near coma in the animals. Following inhalation and dermal exposure, the concentration of isopropyl alcohol in the blood continued to rise and was 112 mg/dl at 4 h while the concentration of acetone was 19 at 4 h. Inhalation exposure with a plastic barrier between the soaked towel and the chest resulted in isopropyl alcohol and acetone blood levels of <10 mg/dl. The researchers concluded that isopropyl alcohol is absorbed by the dermal route but that prolonged dermal exposure (i.e. repeated sponging or soaking for several hours) would be required to produce significant toxicity.

Subchronic Inhalation Toxicity

Fischer 344 rats and CD-1 mice (10/sex/group) were exposed via inhalation to 0, 100, 500, 1500, or 5000 ppm (0, 246, 1230, 3690, or 12,300 mg/m^3) isopropyl alcohol for 6 h/day, 5 days/wk for 13 weeks.[209] Ataxia, narcosis, hypoactivity and the lack of a startle reflex were observed during exposure at 5000 ppm. Hypoactivity was observed in animals exposed to 1500 ppm isopropyl alcohol. At 13 weeks, no gross lesions were observed. Microscopic examination of control and 5000 ppm exposed animal tissues showed hyaline droplets within the kidneys of male rats only. The size and frequency of the droplets was increased in the treated group. The authors concluded that the NOAEL for this study was 500 ppm and the lowest-observable adverse effect level (LOAEL) was 1500 ppm based upon clinical signs and changes in hematology at 6 weeks.

To evaluate the neurobehavioral effects of isopropanol exposure, an additional 15 rats/sex were exposed (via inhalation) to 0, 500, 1500, or 5000 ppm (0, 1230, 3690, or 12,300 mg/m^3) for 6 h/day, 5 days/wk for 13 weeks. Isopropyl alcohol did not produce any changes to the parameters of the functional observations battery which was conducted at 1, 2, 4, 9

and 13 weeks. Clinical signs observed in mice, during the exposure, included ataxia, narcosis, hypoactivity and lack of a startle reflex at 5000 ppm. Narcosis, ataxia and hypoactivity were observed in animals exposed to 1500 ppm isopropyl alcohol. At 5000 ppm, increased body weight and increased rate of weight gain were observed in female mice. At 13 weeks, no gross lesions were observed and no treatment-related microscopic changes were observed. A 10% and 21% increase in relative liver weight was observed in female mice at 1500 and 5000 ppm, respectively. The authors concluded that the NOAEL for this study was 500 ppm and the LOAEL was 1500 ppm based on clinical signs and increased liver weights.

Ocular Irritation

Isopropyl alcohol has been labeled a severe ocular irritant based on rabbit ocular irritation tests.[210]

Reproductive and Developmental Toxicity

Female Sprague-Dawley rats (25/group) were exposed to 0, 400, 800, or 1200 mg/kg bw/day isopropyl alcohol via gavage on gestational days (GD) 6 through 15.[211] Female New Zealand white rabbits (15/group) were exposed to 0, 120, 240, or 480 mg/kg bw/day isopropyl alcohol via gavage on GD 6 through 18. Animals were observed for body weight, clinical effects and feed consumption and the fetuses examined for body weight, sex and visceral and skeletal alterations at GD 20 for rats and GD 30 for rabbits. In rats, 2 dams died at the 1200 mg/kg bw dose and 1 dam died at the 800 mg/kg bw dose. Maternal gestational weight gain was reduced at the highest dose tested. No other effects were observed on maternal reproductive health. Fetal body weights at the two highest doses were decreased statistically. No evidence of teratogenicity was observed at any dose. In rabbits, four does died at the 480 mg/kg bw dose. Treatment related clinical signs of toxicity were observed at the 480 mg/kg bw dose and included, cyanosis, lethargy, labored respiration and diarrhea. No treatment related findings were observed at GD 30. Decreased feed consumption and maternal body weights, at 480 mg/kg bw, were statistically significant. No other effects were observed on maternal reproductive health. No evidence of teratogenicity was observed in the rabbits at any dose. The authors determined NOAELs for both maternal and developmental toxicity of 400 mg/kg bw, each, in rats and 240 and 480 mg/kg bw, respectively, in rabbits.

Carcinogenicity

Fischer 344 rats and CD-1 mice (65/rats/sex/group; 55/mice/sex/group) were treated via inhalation with 0, 500, 2500, or 5000 ppm (0, 1230, 6150, or 12,300 mg/m^3) isopropyl alcohol for 6 h/day, 5 days/wk for 104 weeks in rats and 78 weeks in mice.[209] An additional 10/animals/sex/species were treated with these same concentrations of isopropyl alcohol for 6 h/day, 5 days/wk for 72 weeks in rats and 54 weeks in mice and underwent an interim evaluation. Another 10 mice/sex/group were treated according to the paradigm described above for 54 weeks and then allowed to recover before being killed at 78 weeks. Animals were observed and evaluated for body and organ weights, ophthalmology, and clinical and anatomic pathology.

In rats, increased mortality due to chronic renal disease was observed at 5000 ppm (both sexes) and at 2500 ppm (males only). Hypoactivity and lack of startle reflex were observed in 2500 ppm treated rats and hypoactivity, lack of startle reflex and narcosis were observed in 5000 ppm treated rats. With the exception of the ataxia, the clinical signs were transient and ceased when the exposure ended. Increases in body weight, body weight gain, and liver weights were observed in 2500 and 5000 ppm treated rats. Chronic renal disease was exacerbated in rats treated with isopropyl alcohol. Male rats had a concentration related increase in absolute and relative testes weights. At the interim euthanasia (after 72 weeks) male rats treated with 5000 ppm had an increased frequency of testicular seminiferous tubule atrophy upon microscopic evaluation. At the terminal euthanasia (104 weeks), male rats had a concentration dependent increase in the incidence of interstitial (Leydig) cell adenomas of the testes at all administered doses. No other tumor types were increased in rats under these treatment conditions as compared to controls.

In mice, no differences in mortality were observed between control and treated animals. Hypoactivity, lack of a startle reflex, narcosis, ataxia, and prostration were observed in 5000 ppm treated mice. Hypoactivity, lack of startle reflex and narcosis were observed in 2500 ppm treated mice. Increases in body weight, body weight gain, and liver weights were observed in 2500 and 5000 ppm treated mice. Male mice in all treatment groups had a decrease in relative testes weights, and female mice exposed to 5000 ppm isopropyl alcohol exhibited decreases in absolute and relative brain weights. At the terminal euthanasia (78 weeks) an increased incidence of minimal to mild renal tubular proteinosis was observed in males and females in all treatment groups. Male mice exposed to 2500 and 5000 ppm exhibited an increased incidence of dilation of the seminal vesicles. No neoplastic lesions were observed in male or female mice. The authors reported a NOAEL for toxic effects of 500 ppm for both rats and mice based on kidney and testicular effects.

IARC (International Agency for Research on Cancer) has determined that isopropyl alcohol is not classifiable as to its carcinogenicity to humans (Group 3).[182]

Clinical Irritation and Sensitization

According to unpublished data, a 80.74% spray concentrate caused did not exhibit any potential for dermal sensitization in 9 human subjects.[197]

According to unpublished HRIPT study on 109 test subjects, a 2.85% hair dye base formulation of isopropyl alcohol and a 1.95% isopropyl acetate caused no dermal sensitization in humans.[198]

The applicability of fluorescence confocal laser scanning microscopy for in situ imaging of irritant contact dermatitis caused by pelargonic acid using 12 healthy individuals (8 males, 4 males; 18 to 64 years old) was studied.[199] Using Finn chambers (occlusive patches), the flexor side of the right and left forearm was exposed to 60 µl of 10% (w/v) pelargonic acid in isopropanol solution and isopropanol vehicle. Isopropanol was used as a control. The Finn chambers were removed at 24 h post-application and reactions were scored according to the following scale: 0 (no visible reaction) to 4+ (intense erythema with bullous formation). Reactions were not observed at sites treated with isopropanol.

Hexyl Alcohol – metabolite of Dihexyl Adipate

Ocular Irritation

Undiluted hexyl alcohol has been labeled as highly irritating on rabbit ocular irritation tests.[212]

Dermal Sensitization

In a maximization test using guinea pigs, hexyl alcohol was not a sensitizer at 1% in petrolatum.[212]

Caprylic Alcohol – metabolite of Dicapryl Succinate, Dicapryl Adipate and Dicaprylyl/Capryl Sebacate

Dermal Irritation – Animals

Caprylic alcohol applied full strength to intact or abraded rabbit skin produced a mild irritation.[185]

Clinical Irritation and Sensitization

Tested in at a concentration of 2% in petrolatum, caprylic alcohol produced no irritation in a 48 h closed-patch test in 25 human subjects.[185]

Isobutyl Alcohol – metabolite of Diisopropyl Adipate and Diisopropyl Sebacate

Subchronic Inhalation Toxicity

Rats (10/sex/group) were exposed via inhalation to isobutyl alcohol vapor concentrations of approximately 0, 770, 3100, or 7700 mg/m3, for 6 h/day, 5 days/week, for 14 weeks.[213] The functional observational battery was conducted along with endpoints of motor activity, neuropathology and scheduled-controlled operant behavior. A slight reduction in responsiveness to external stimuli was observed in all treated groups during exposure. This effect resolved upon cessation of exposure to isobutyl alcohol.

Ethylhexyl Alcohol – metabolite of Diethylhexyl Succinate, Diethylhexyl Adipate and Diethylhexyl Sebacate

Absorption, Distribution, Metabolism, and Excretion

In vitro dermal absorption rates were determined for ethylhexyl alcohol in rats and humans. In rats, the rate was 0.22 $mg/cm^2/h$ and in the human it was 0.038 $mg/cm^2/h$.[214] Accordingly, the human rate of ethylhexyl alcohol absorption was 5.78 times slower than in the rate in the rat.

Acute Dermal Toxicity

In three different acute dermal toxicity studies on rabbits with ethylhexyl alcohol, the LD50 values reported were 2380, greater than 2600 and greater than 5000 mg/kg body weight.[214]

Repeated Dose Dermal Toxicity

Rats (10) were dosed 2 ml/kg body weight/day (1600mg/kg/day) via single application on shaved backs.[214] Absolute and relative thymus weights, liver granulomas, brochiectasis in the lung, renal tubular epithelial necroses, edematous heart and testes, and spermatogenesis, all decreased.

Rats (10/sex) were dosed 0, 500, or 1000 mg/kg body weight/day (5 days occlusive, 2 days untreated, 4 days treated).[214] 500

and 1000 mg treated rats exhibited minimal exfoliation, decreased spleen weight and increased serum triglycerides in females.

Ocular Irritation

Instillation of 20 µg of ethylhexyl alcohol into the conjunctival sac of rabbits caused moderately severe irritation of the cornea.[212]

Dermal Irritation – Animals

Ethylhexyl alcohol was applied under occlusion to the skin of 3 male rabbits for 4 hours and found to be irritating.[212] In another study with rabbits, 0.5 ml of ethylhexyl alcohol was applied under occlusion on intact skin for 1, 2, 4, and 24 hours. Irritation was considered high, and effects seen after 7 days were not reversible.

Reproductive and Developmental Toxicity

A group of female rats was exposed for 7 h per day to 850 mg/m^3 of ethylhexyl alcohol on gestation days 1-19.[212] Dams were sacrificed at day twenty. Ethylhexyl alcohol reduced maternal feed intake, but did not produce any malformations.

The estrogenic activity of 2-ethylhexanoic acid was examined using an E-SCREEN assay using T47D human breast cancer cells.[215] Weak estrogenic activity was observed. (Additional details were not provided.)

Genotoxicity

In vitro, ethylhexyl alcohol was negative in a number of Ames assays, a liquid suspension assay, mouse lymphoma assay, and unscheduled DNA synthesis assay.[118,139,140,143,151] In a ^{3}H-thymidine assay, there was a dose-dependent inhibition of ^{3}H-thymidine into replicating DNA, with a dose-dependent increase in the ratio of acid-soluble DNA incorporated into the thymidine.[140] The urine of rats dosed orally with 1000 mg/kg bw ethylhexyl alcohol was not mutagenic.[150] In vivo, ethylhexyl alcohol was not genotoxic in a mouse micronucleus test or a transformation assay.[151]

Carcinogenicity

B6C3F1 mice (50/sex/group) were administered 0, 50, 200, or 750 mg/kg body weight/day via gavage, 5 days/week for 18 months.[214] At the 750 mg dose, weak hepatocellular carcinoma increased in females, body weight gain decreased and mortality increased. F344 rats (50/sex/group) were administered 0, 50, 150, or 500 mg/kg body weight/day via gavage, 5 days/week for 24 months. Rats dosed at 150 mg and greater were characterized with body weight gain decrease, lethargy and unkemptness. At 500 mg, mortality in females was at 52%.

Clinical Irritation and Sensitization

Tested in at a concentration of 4% in petrolatum, ethylhexyl alcohol produced no irritation in a 48 h occlusive-patch test in 29 male volunteers.[214] In a maximization study, ethylhexyl alcohol did not induce any sensitization reactions.

MEHA, Mono-(2-Ethyl-5-Hydroxyhexyl)Adipate, Mono-(2-Ethyl-5-Oxohexyl)Adipate - metabolites of Diethylhexyl Adipate

Genotoxicity

MEHA was not mutagenic in an Ames assay at concentrations of ≤1000[86] or 10,000 µg/plate.[139] Mono-2(ethyl-5-hydroxyhexyl)adipate and mono-(2-ethyl-5-oxohexyl)adipate, were not mutagenic in an Ames assay at concentrations of ≤1000 µg/plate.[86]

Isooctyl Alcohol – metabolite of Diisooctyl Adipate and Diisooctyl Sebacate

Subchronic Oral Toxicity

In a subchronic gavage toxicity study of a mixture of C7-9, branched alkyl alcohols in rats, a NOEL of 125 mg/kg bw/day and a lowest-observed effect level of 250 mg/kg bw/day were determined.[118]

Reproductive and Developmental Toxicity

In an oral gavage developmental toxicity study of a mixture of C7-9, branched alkyl alcohols in rats, a maternal NOAEL of 500 mg/kg bw and a fetal NOAEL of 1000 mg/kg bw were reported.[212]

Genotoxicity

A mixture of C7-9, branched alkyl alcohols were not mutagenic in in vitro bacterial and mammalian cell assays.[212]

Carcinogenicity

Ethylhexyl alcohol was not oncogenic in rats dosed, via gavage, with 0, 50, 150, or 500 mg/kg bw, in an aqueous vehicle with 0.005% Cremophor EL.[212]

Isononyl Alcohol – metabolite of Diisononyl Adipate

Reproductive and Developmental Toxicity

In an oral gavage developmental toxicity study of a mixture of C8-10, branched alkyl alcohols in rats, maternal and fetal NOAEL values were each reported to be 144 mg/kg bw. [212]

Isodecyl Alcohol – metabolite of Diisodecyl Adipate

Reproductive and Developmental Toxicity

In an oral gavage developmental toxicity study of a mixture of C9-11, branched alkyl alcohols in rats, a maternal NOAEL of 158 mg/kg bw and a fetal NOAEL of 790 mg/kg bw were reported. [212]

Isostearyl Alcohol – metabolite of Diisostearyl Glutarate, Diisostearyl Adipate and Isostearyl Sebacate

Clinical Irritation and Sensitization

The skin irritation potential of lsostearyl Alcohol was evaluated in 19 male and female subjects (18-65 years old) at a concentration of 25.0% in petrolatum. The test substance did not induce skin irritation in any of the subjects (Primary Irritation Index = 0.05). In three similar studies, three different lipstick products containing 25.0, 27.0, and 28.0% Isostearyl Alcohol, respectively, were tested according to the same protocol. The three products did not induce skin irritation.[200]
The irritation and sensitization potential of Isostearyl Alcohol (25% v/v in 95.0% isopropyl alcohol) was evaluated in 12 male subjects (21-60 years old). Challenge applications were made to original and adjacent sites 2 weeks after removal of the last induction patch. Three of 12 subjects had slight erythema during induction, and there was no evidence of sensitization.[200]

The sensitization potential of a pump spray antiperspirant containing 5.0% Isostearyl Alcohol was evaluated using 148 male and female subjects. The product was applied via an occlusive patch to the upper arm for a total of nine induction applications (3 times/week for 3 weeks). Each patch remained for 24 h, and sites were scored immediately before subsequent applications. During the challenge phase, a patch was applied to the induction site and to a new site on the opposite arm of each subject. Reactions were scored 48 and 96 h after application. Ten of the twelve subjects with reactions suggestive of sensitization were re-challenged with the product 2 months later. Patches remained for 24 h, and sites were scored at 48 and 96 h post-application. Six subjects had reactions during the re-challenge. Four of the six subjects were then tested with 5.0% Isostearyl Alcohol in solution with ethanol 6 weeks after scoring of the first rechallenge; all had positive responses. Negative responses were reported when the product (without lsostearyl Alcohol) and 100.0% ethanol each were tested. In a second study, the same product was applied to 60 male and female subjects (same protocol). Five of the subjects had positive responses after the first challenge. One of the five was re-challenged with 5.0% Isostearyl Alcohol in ethanol solution, and a positive reaction was observed.[200]

Isopropanol

Comedogenicity

An LD_{Lo} of 2-4 ml/kg of isopropyl alcohol has been reported in adults and 6 ml/kg (9 ml/kg 70% isopropyl alcohol) was reported to induce coma in children.

Final Report

Plant-Derived Fatty Acid Oils as Used in Cosmetics

March 4, 2011

The 2011 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Christina Burnett and Monice Fiume, Scientific Analysts/Writers, CIR.

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ABSTRACT

The CIR Expert Panel assessed the safety of 244 Plant-Derived Fatty Acid Oils as used in cosmetics. Oils are used in a wide variety of cosmetic products for their skin conditioning, occlusive, emollient, and moisturizing properties. Since many of these oils are edible, and their systemic toxicity potential low, the review of the Panel focused on their potential dermal effects. The Expert Panel concluded that the 244 Plant-Derived Fatty Acid Oils are safe as used in cosmetics.

INTRODUCTION

Oils derived from edible vegetables, fruits, seeds, and tree and ground nuts have been safely consumed by humans for millennia. While nuts and some fruits and vegetables themselves may cause allergic reactions in certain individuals, the refined oils derived from these plants generally pose no significant safety concern following oral exposure, and their general biology is well characterized due to extensive use in food materials. Most of the ingredients in this report are mixtures of triglycerides containing fatty acids and fatty acid derivatives, the safety of which in cosmetics has been established. This safety assessment focused solely on the basic chemistry, manufacturing/production, uses, and irritation and sensitization data available on these oils as they are used in cosmetic ingredients.

Various oils have been used on the skin since antiquity. Initially used for anointing in religious ceremonies, oils and their components have also been long used on the skin for cosmetic purposes. They are used in a wide variety of cosmetic products for their skin conditioning, occlusive, emollient, moisturizing and other properties. The full list of ingredients in this report, which includes oils, hydrogenated oils, unsaponifiables, oil fatty acids, and salts of the fatty acids, is found in Table 1. While a large number of oils derived from plants are included in this literature review, there is a commonality in that they all are mixtures of triglycerides containing fatty acids and fatty acid derivatives, the safety of which in cosmetics have been established.

In preparing this report, numerous inconsistencies were noted with both taxonomic and INCI naming conventions. For example, this report includes the macadamia nut ingredients, Macadamia Integrifolia Seed Oil and Macadamia Ternifolia Seed Oil, which are described in the International Cosmetic Ingredient Dictionary and Handbook.[1] The species *M. integrifolia* is currently the only species of macadamia nut that is used for oil production. The name *M. ternifolia* is an old naming convention for the edible nut that is currently used to describe a non-cultivated, inedible species.[2,3] Macadamia Integrifolia Seed Oil and Macadamia Ternifolia Seed Oil are the same ingredient. Similar naming conflicts have been discovered with Triticum Vulgare (Wheat) Germ Oil and Triticum Aestivum (Wheat) Germ Oil, Orbignya Oleifera Seed Oil and Orbignya Speciosa Kernel Oil, and Moringa Pterygosperma Seed Oil and Moringa Oleifera Seed Oil, with these pairs being synonyms for each other. The shea plant also has two species names, *Butyrospermum parkii* and *Vitellaria paradoxa*. Only *B. parkii* (as Butyrospermum Parkii [Shea] Oil or Butter) is the current naming convention described by the cosmetics industry.

This report includes cosmetic ingredients that have been previously reviewed by the Cosmetic Ingredient Review Expert Panel. The ingredients, their conclusions, and published citations are found in Table 2. Previously reviewed fatty acids and glyceryl triesters are also found in Table 2.

CHEMISTRY

The group of ingredients characterized as fats and oils are the glyceryl esters of fatty acids (triglycerides) normally found in plants, including those which have been hydrogenated to reduce or eliminate unsaturation.[4] Figure 1 represents the general structure of fats and oils. The raw oil may include diglycerides, monoglycerides, free fatty acids, plant sterols, pigments, glucosides, proteins, natural antioxidants, vitamins and impurities.[5,6] The extent to which these components are removed during processing varies. The available information on chemical properties of oils in this report, including Food

Chemicals Codex specifications when provided, are found in Table 3.[7] The available fatty acid compositions for the oils in this report are found in Table 4.

The percentage of chemical constituents in individual oil types is dependent on the region where the oilseed plant is grown, individual cultivars, and plant genetics.[6] This is especially true with rapeseed, where the erucic acid content varies from 1% to 58.6%. Low erucic acid rapeseed oil is also known as canola oil.

The nutritional content of these oils varies with oil type. For example, sunflower oil contains high levels of vitamins A, D, and K, while palm oil is a rich source of vitamins A and E. Crude sunflower oil also has the highest content of vitamin E in the form of α-tocopherol amongst vegetable oils.[6]

Vegetable Oil and Hydrogenated Vegetable Oil are cosmetic labeling names for blends of plant-derived oils.[8] The composition of a blend is determined by the desired physical properties. Vegetable Oil and Hydrogenated Vegetable Oil may include, but are not limited to: Canola Oil, Brassica Campestris (Rapeseed) Oil, Carthamus Tinctorius (Safflower) Seed Oil, Helianthus Annuus (Sunflower) Seed Oil, Sesamum Indicum (Sesame) Seed Oil, Elaeis Guineensis (Palm) Oil, Elaeis Guineensis (Palm Kernel) Oil, Cocos Nucifera (Coconut) Oil, Gossypium Herbaceum (Cottonseed) Oil, Glycine Soja (Soybean) Oil, Zea Mays (Corn) Oil, Olea Europaea (Olive) Oil, Prunus Amygdalus Dulcis (Sweet Almond) Oil, and hydrogenated products of these oils.

Processing

The oil may be directly expressed from the source (seed or pulp) followed by solvent extraction. *Bailey's Industrial Oil and Fat Products* states that the removal of pigments and polar materials is mandatory for most cosmetic applications.[9] The process used for oil refining for foods may be adequate for this purpose, or additional steps may be required. Special refining methods to yield colorless and odorless oils are used by the cosmetic industry and include proprietary adsorption chromatography and supercritical fluid extractions.

The majority of the oils presented in this report are produced either from mechanical extraction or solvent extraction or a hybrid of both methods, known as prepress solvent extraction.[6] In solvent extraction, hexane is the most commonly used solvent, as it is economical and easily removed from the extracted oil. Seeds that are rich in oil can be cold pressed to extract oil without the use of solvents.[10]

After the initial extraction by methods such as solvent extraction, the crude (degummed) oil is often refined.[6] The first step is treating the oil with caustic soda to neutralize free fatty acids, hydrolyze phosphatides, and remove some colored pigments and unsaponifiable materials. Soap stock is usually a by-product of this step. The next step involves treating the neutralized oil with activated earth to further adsorb pigments. The last major step in refining oil is deodorizing, usually by a type of steam distillation, which is intended to remove all oxidative cleavage products that impart odor or flavor to the oil. Deodorization also removes tocopherols, sterols, and other minor constituents of free fatty acids and undesirable foreign materials. Figure 2 is a flowchart of the basic refinement process.

After deodorization, oils can be further processed by hydrogenation, which makes oil more resistant to oxidative and thermal damage, and by winterization, where oil is slowly cooled to promote formation of crystals that cause cloudiness, and then filtered to remove the crystals.

Cosmetic grade fatty acid plant oils may include a physical refining step that involves heating crude oil under vacuum.[10] This step allows for the removal of volatile components such as color compounds, odor compounds, and free fatty acids, which gives the refined oil a lighter color, less odor, and lower acid values.

Analytical Methods

Near infrared spectroscopy and gas chromatography have been used, respectively, to phenotype and analyze fatty acid profiles in shea fat (described as *Vitellaria paradoxa*, not *Butyrospermum parkii*).[11] The fatty acid composition of hazel seed oil (*Corylus avellana*, in crude form) has also been analyzed by gas chromatography.[12] The triacylglycerol and diacylglycerol composition oils from hazelnut, pistachio, almond, Brazil nut, and macadamia nuts have been characterized with high-performance liquid chromatography with atmospheric pressure chemical ionization and UV detection.[13] The triacylglycerol profile of Brazil nut oil has also been quantified using dry matrix-assisted laser desorption/ionization time-of flight mass spectrometry.[14]

Impurities

Proteins

Many edible fatty acid oils are derived from foods that are recognized as potent food allergens. It has been shown that an individual that is allergic to a food will generally not react to the refined oil, especially if the oil has been "hot-pressed" or has undergone more processing.[15,16] A prime example is Arachis Hypogaea (Peanut) Oil. Peanuts are extremely allergenic to a large population, but reaction to the oil is rare. In its safety assessment on Arachis Hypogaea (Peanut) Oil, the Expert Panel noted that the major concern associated with allergic reactions to peanuts is the protein.[17] The protein does not partition into the refined oil, and therefore the oil is safe for use in cosmetics. However, researchers have reported protein levels in processed oils. Halsey et al. reported that Lowry protein determinations of cold-pressed and refined sunflower oil found 2-8 µg/ml protein,[18] while Zitouni et al. reported trace amounts of protein in the refined oil.[19] Olszewski et al. found 0.1-0.2 µg protein per g of peanut oil,[20] while Ramazzotti et al. reported finding IgE responsive residual proteins in peanut oil extracts.[21] Porras et al. found soy protein in some samples of soy oil, but not others.[22] Awazuhara et al. reported 1.4-4.0 µg protein per 100 g of soy oil.[23] Although Paschke et al. found approximately 35 µg/l protein content in refined soybean oil, no IgE-binding activity was detectable.[24]

While the Panel has found a general lack of clinical effects for fatty acid oils already reviewed,[17,25-33] other groups have raised concerns. The European Medicines Agency (EMEA) Working Party on Herbal Medicinal Products concluded that soy and peanut products "should be treated as allergenic unless they have an analytically-monitored non-allergenic specification and a safe maximum daily dose."[34] The EMEA found that threshold concentrations for induction of a protein contact dermatitis were not available and recommended, "all medications for topical use containing soya or peanut products should be treated as allergenic."

Aflatoxin

Aflatoxins are metabolic products of the molds *Aspergillus flavus* and *Aspergillus parasiticus*. They are most often produced in stored agricultural crops (such as peanuts and other nut crops) when growth conditions and genetic requirements are favorable.[35-37] The International Agency for Research on Cancer (IARC) categorized aflatoxins as group 1 agents, "carcinogenic to humans".[38,39]

The United States government places the following limitations on peanuts to be considered "negative" for aflatoxin: $\leq$ 15 ppb for "peanuts which have been certified as meeting edible quality grade requirements" and $\leq$ 25 ppb for "non-edible quality categories" (7 CFR Sections 997.30 and 998.200).[40]

> A study reported that crude peanut oil (obtained by solvent extraction or hydraulic pressing) has reduced aflatoxin concentration compared to peanut kernels, and that subsequent processing (alkali refining and bleaching) reduces the concentration still further.[17] In one example, processed peanut oil from moldy peanuts (contaminated with 5500 ppb aflatoxin) had an aflatoxin concentration of < 1ppb. [From CIR assessment on Arachis Hypogaea (Peanut) Oil, 2001.][17]

In 50 samples of hazel nuts from Spain, all samples showed fungal contamination, but no aflatoxin contamination.[41] Of the 50 fungal strains identified, 25 were aflatoxigenic strains. In 20 hazel nut samples collected in Egypt, however, aflatoxin (25-175 µg/kg) was reported as a contaminant in 90% of samples. [From CIR assessment on Hazel Seed Oil, 2001.][42]

Aflatoxin contamination of raw and dried coconut copra has been reported.[33] Improper drying, handling, and storage greatly increase the possibility of contamination by aflatoxins growing on copra. Smoke drying of copra inhibited aflatoxin formation. [From CIR assessment on Cocos Nucifera (Coconut) Oil, 2008.][43]

Glycidol

Glycidol and glycidol fatty acid esters have been detected in refined fatty acid oils.[44-47]

USE

Cosmetic

There are 244 oil ingredients included in this safety assessment, 146 of which are reported to be used; 118 of the in-use ingredients have never been reviewed by CIR, while 28 have been reviewed previously. For the ingredients being reviewed for the first time, the frequency of use, as supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP),[48] and/or concentration of use, as supplied by industry in response to a Personal Care Products Council (Council) survey,[49-51] can be found in Table 5a. (Also included in Table 5a are three ingredients, Citrullus Vulgaris (Watermelon) Seed Oil, Macadamia Nut Oil, and Vaccinium Oxycoccos (Cranberry) Seed Oil, that do not have identifiable International Nomenclature Cosmetic Ingredient (INCI) names. While these ingredients are not part of this assessment, they are very similar to the oils that are identified and information on them is included in this report for completeness.) For the ingredients that have been reviewed previously, the current and historical[26-28,32,52-55] frequency and concentration of use is given in Table 5b. The 97 ingredients not currently reported to be used are listed in Table 5c. [48-51,56,57]

It should be noted that the names vegetable oil and hydrogenated vegetable oil, are used in cosmetic formulations, refer to a blend of plant-derived oils, and the composition of the blend varies.[8]

Of the oils included in this report, Butyrospermum Parkii (Shea) Butter has the most reported uses in cosmetic and personal care products, with a total of 1950; 1680 of those uses are in leave-on formulations. A recent survey of use concentrations for Butyrospermum Parkii (Shea) Butter reports a maximum use concentration of 60% in leave-on products as a cuticle softener, a manicuring application.[58] Helianthus Annuus (Sunflower) Seed Oil has the second greatest number of overall uses reported, with a total of 1414; 1054 of those uses are in leave-on formulations, having use concentrations up to 96%. Many other ingredients are used in an extensive number of formulations. For example, Prunus Amygdalus Dulcis (Sweet Almond) Oil, Olea Europaea (Olive) Fruit Oil, and Glycine Soja (Soybean) Oil have 1127, 915, and 912 uses, respectively. Most of the in-use ingredients have uses in both leave-on and rinse-off product types, many are used in products that are applied around the eye and some are used in a way they can possibly be ingested. Some are used in products that involve mucous membrane exposure, and a few are used in underarm deodorant formulations. Many of the products are used in formulations at relatively high concentrations. Olea Europaea (Olive) Fruit Oil is used at up to 100%, Persea Gratissima (Avocado) Oil is used at up to 98%, Helianthus Annuus (Sunflower) Seed Oil at up to 96%, and Glycine Soja (Soybean) Oil at 95%.

Oils are used in a wide variety of cosmetic products for their skin conditioning, occlusive, emollient, moisturizing and other properties.

Some of the oils included in this report are used in products that can be inhaled, and effects on the lungs that may be induced by aerosolized products containing these ingredients are of concern. The particle size of aerosol hair sprays and of

pump hair sprays is 38 μm and >80 μm, respectively, and is relatively large compared to respirable particle sizes (≤10 μm). Therefore, because of their size, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

None of the oils, hydrogenated oils, unsaponifiables, oil fatty acids, and salts of the fatty acids described in this report were restricted from use in any way under the rules governing cosmetic products in the European Union.[59]

Non-Cosmetic

The primary uses for plant-derived fatty acid oils are for cooking. Palm oil is the world's most widely consumed edible oil (41.7 million metric tons), followed by soybean oil, rapeseed oil, sunflower seed oil, cottonseed oil, peanut oil, palm kernel oil, coconut oil, ,and olive oil.[6,60] Non-food, non-cosmetic uses for edible fatty acid oils are found in Table 6.

ANIMAL TOXICOLOGY

Many of the fatty acid oils in this assessment are edible, and exposure to the oils from food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Consequently, their systemic toxicity potential is not addressed in this report. The safety focus of use of these oils as cosmetic ingredients is on the potential for irritation and sensitization.

CARCINOGENICITY

The safety of glycidol fatty acid esters in refined vegetable oils was assessed by IARC. Glycidol was determined to be a Group2A (probably carcinogenic to humans) chemical while glycidol fatty acid esters was determined to be a Group 3 (not classifiable as to carcinogenicity to humans) chemical.[46,47]

The Federal Institute for Risk Assessment in Germany released a summary of their initial evaluation of the assessment of levels of glycidol fatty acid esters detected in refined vegetable fats.[45] While acknowledging that the levels of glycidol that may be released from glycidol fatty acid esters are not known, the evaluation noted that glycidol is classified as probably carcinogenic to humans. The evaluation was based on findings of the German Chemical and Veterinary Test Agency (CVUA) that noted that glycidol is converted to 3-chloropropanediol and it appeared to be the 3-chloropropanediol that was detected in the vegetable fat.[44] The levels of 3-chloropropanediol were negligible at the crude oil, degummed, neutralized, and bleached stages, but levels were significant at the deodorized stage.

Anacardium Occidentale (Cashew) Seed Oil

The modulatory effect of Anacardium Occidentale (Cashew) Seed Oil on antioxidant potential was investigated in female Swiss albino mice in a 120 day skin papillomagenesis study.[61] The mice were divided into 4 groups of 15 and 1 group of 10 (vehicle control). Test groups were as follows: Group I was the vehicle control, receiving 0.1 ml acetone; Group II was the positive control, receiving a single dose of 7,12-dimethylbenz(a)anthracene (DMBA) (0.005 mg/0.05 ml acetone) followed by applications of 2% croton oil 3 times a week until study termination; Group III received a single dose of DMBA followed by applications of 2.5% cashew nut kernel oil 3 times a week until study termination; Group IV received a single dose of DMBA followed by applications of 5% cashew nut kernel oil 3 times a week until study termination; and Group V was 5% cashew nut kernel oil applied until study termination. The oil was applied to the clipped dorsal scapular region that was 2 cm in diameter. Body weights were recorded at regular intervals. Skin papillomas greater than 1 mm in diameter at the application sites were recorded weekly and included in the data analysis if they persisted for more than 2 weeks. The positive control group yielded expected results (86% tumor incidence). No tumors were observed in the vehicle control or the other test groups. The authors concluded that cashew nut kernel oil did not exhibit any solitary carcinogenic activity.

IRRITATION AND SENSITIZATION

Dermal Effects

Non-Human

Dermal irritation and sensitization studies were performed in animals on a number of the plant-derived fatty acid oils, and the results were mostly negative in all of the studies. These studies are summarized in Table 7a. Photosensitization data, when available, are also included in Table 7a. None of the tested oils were phototoxic. Summary statements of non-human dermal studies from previous CIR reports on oils are provided in Table 7b.

Human

Plant-derived fatty acid oils are commonly believed to be safe for use on the skin.[9] de Groot notes that no documentation exists to show that high quality edible lipids cause adverse reactions in normal individuals (except for potential comedogenicity).[62] Very few reports of adverse reactions to cosmetic use of edible fatty acid oils have been reported.

Many plant-derived fatty acid oils are derived from foods that are recognized as potent food allergens. The allergic reactions are thought to be caused by the proteins present in the food. It has been shown that an individual that is allergic to a food will generally not react to the refined oil, especially if the oil has been "hot-pressed" or has undergone more processing.[15,16] In its safety assessment on Arachis Hypogaea (Peanut) Oil, the CIR Expert Panel noted that while peanuts are extremely allergenic to a large population, reaction to the oil is rare. Because the major concern associated with allergic reactions to peanuts is the protein[17] which does not partition into the refined oil; therefore the oil is safe for use in cosmetics. Crevel et al. also concluded that chemically refined peanut oil is safe for the majority of peanut allergic individuals.[16] They stated that "as peanut is acknowledged to be one of the most potent food allergens, it is reasonable to extrapolate the conclusions drawn up for peanut oil to other edible oils." However, they concede that validated analytical methodology for establishing the protein content of oil is needed.

In support of the conclusions stated earlier, Crevel et al. also examined the allergenicity of some other oils. Very few instances of allergic reactions to other major edible fatty acid oils have been reported. Even sesame oil, which differs from the other oils in that it is used as a flavorant and, therefore, is not as refined and is expected to contain significantly more protein that the other edible fatty acid oils, has had very few reports of allergic reaction. Additional studies demonstrating safety are summarized later in this section.[18,63]

A large number of clinical irritation and sensitization studies were made available on many of the oils, primarily in formulation, and these studies are summarized in Table 8a. All of the data indicated that the oils were not irritants or sensitizers. Summary statements of human dermal studies, including phototoxicity/photosensitization data, from previous CIR reports on oils are provided in Table 8b.

Mucosal Irritation

Non-Human

Ocular irritation studies were performed using animals on a number of plant-derived fatty acid oils. While the majority of the oils were non-irritating to mildly irritating, Crambe Abyssinica Seed Oil was an ocular irritant and Linum Usitatissimum (Linseed) Seed Oil was moderately irritating. Available ocular irritation studies are summarized in Table 9a. Summary statements of ocular irritation studies from previous CIR reports on oils are provided in Table 9b.

Human

In clinical ocular irritation studies, formulations containing Linum Usitatissimum (Linseed) Oil and Ribes Nigrum (Black Currant) Seed Oil did not produce adverse reactions, and were considered safe for contact lens wearers. These studies are also summarized in Table 9a.

CLINICAL USE

Clinical Trials/Case Studies

Case studies reporting various results have been summarized in Table 10 for a number of the oils included in this report.

SUMMARY

The report addresses the safety of Plant-Derived Fatty Acid Oils. These oils, which are derived from vegetable and fruit plants, are composed of mono-, di-, and, primarily, triglycerides, free fatty acids and other minor components, including natural antioxidants and fat-soluble vitamins. The percentage of chemical constituents and nutritional content of individual oil types is dependent on region where the oil plant is grown, individual cultivars, and plant genetics. Oils used in cosmetics are likely produced in the same manner as those used in the food industry. Oils may be expressed through mechanical or solvent extraction. The oils may undergo further refining, such as neutralizing, bleaching, and deodorizing, to remove pigments, odors, unsaponifiable materials, and other undesirables.

Individuals who have food allergies to a plant protein rarely exhibit allergic reactions when exposed to refined oils of the same plant. Data evaluation by the CIR Expert Panel regarding method of manufacture indicates that protein constituents do not partition into the refined oils. The CIR Expert Panel also has found a general lack of clinical effects for fatty acid oils that they have already reviewed; however, other researchers have raised concerns about the presence of residual proteins in oils, such as peanut and soy.

Glycidol fatty acid esters are possible impurities in refined vegetable oils. While the amount of glycidol that may be present with glycidol fatty acid esters is not known, the IARC has noted that glycidol is probably carcinogenic to humans and that glycidol fatty acid esters are not classifiable as to carcinogenicity in humans. Peanuts and soy may contain aflatoxins, metabolic products of certain molds that are carcinogenic to humans.

Of the oils described in this report, Butyrospermum Parkii (Shea) Butter has the most reported uses in cosmetic and personal care products with a total of 1950 and is used at a maximum concentration of 60%. Oils are used in a wide variety of cosmetic products, including use in hair spray and other aerosolized products. None of the oils, or the related counterparts, described in this report are restricted from use in the European Union.

Anacardium Occidentale (Cashew) Seed Oil was not a tumor promoter in a DMBA skin test system.

The safety focus of use of these oils as cosmetic ingredients is on the potential for irritation and sensitization. Undiluted, technical grade, Arachis Hypogaea (Peanut) Oil was moderately irritating to rabbits and guinea pig skin, and 5% aq. solutions of a bar soap containing 13% sodium cocoate had irritation scores of 1.6-4.0/8 in animal studies. However, the remaining animal and clinical irritation and/or sensitization studies conducted on a large number of the oils included in this report, primarily in formulation, did not report any significant irritation or sensitization reactions, indicating that refined oils derived from plants are not dermal irritants or sensitizers.

The phototoxic potential of formulations containing Butyrospermum Parkii (Shea) Butter and Elaeis Guineensis (Palm) Oil and of Oryza Sativa (Rice) Bran and (Rice) Germ Oil, neat, was evaluated in animal studies, and the phototoxic

potential of Cocos Nucifera (Coconut) Oil, Sodium Cocoate, Prunus Amygdalus Dulcis (Sweet) Almond Oil, and Oryza Sativa (Rice) Bran Oil was examined clinically. None of these ingredients were phototoxic.

The comedogenicity of Corylus Avellana (Hazel) Seed Oil was evaluated using rabbits, and a slight difference in the number and size of the pilosebaceous follicles and a slight excess of sebum and a dilation of the follicles was observed. In clinical testing with an eye mask containing 0.2% Ribes Nigrum (Black Currant) Seed Oil (undiluted), the formulation was non-comedogenic.

The ocular irritation potential of a number of the oils, mostly in formulation, was evaluated in testing using animals or alternative assays. The majority of the test results did not report significant ocular irritation. A lotion containing 1.5% Elaeis Guineensis (Palm) Oil was moderately irritating to rabbit eyes, and a mascara containing 9.4% Linum Usitatissimum (Linseed) Seed Oil was moderately irritating in an alternative assay.

In human testing, a mascara containing 9.4% Linum Usitatissimum (Linseed) Seed Oil did not produce ocular irritation or adverse effects in contact lenses wearers or subjects with sensitive eyes. An eye mask containing 0.2% Ribes Nigrum (Black Currant) Seed Oil (undiluted) was tested and considered safe for contact lens wearers.

DISCUSSION

Plant-derived fatty acid oils, oils which have been hydrogenated to reduce or eliminate unsaturation, fatty acid salts, and oil unsaponifiables were reviewed by the CIR Expert Panel. Most of theses ingredients in this report are mixtures of triglycerides containing fatty acids and fatty acid derivatives, the safety of which in cosmetics has been established. Upon review of these ingredients, the Panel expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulations.

Additionally, the Panel considered the safety of glycidol and glycidol fatty acid esters in refined vegetable oils. While the Panel recognizes that these impurities may be carcinogenic, absorption through the skin would be very low and likely does not pose a significant hazard. Nonetheless, suppliers should take steps to eliminate or reduce the presence of glycidol and glycidol fatty acid esters in plant-based fatty acid oils that are used in cosmetic products. Aflatoxins, which are potent carcinogens, may be present in moldy nuts and coconut copra, but are not found in oils expressed from these nuts and copra. The Panel adopted the U.S. Department of Agriculture designation of $\leq$15 ppb as corresponding to “negative” aflatoxin content.

Certain of the plant-derived oils are used in cosmetic products that may be inhaled during their use. In practice, however, the particle sizes produced by the cosmetic aerosols are not respirable.

The Panel discussed the relationship between food allergies and exposure to refined oils. Individuals who have food allergies to a plant protein rarely exhibit allergic reactions when exposed to refined oils of the same plant. The Panel has found a general lack of clinical effects for plant-derived fatty acid oils already reviewed.

Fatty acid composition data were available for the majority of the oils included in this review and the Panel agreed that the composition data, in combination with the available data on method of manufacture, impurities, safety test data, a long history of safe use in foods, and an absence of adverse reactions in clinical experience, was a sufficient basis for determining safety. The Expert Panel did note that vegetable oil is a blend of a number of different oils, and that a specific composition of vegetable oil was not available. The Expert Panel determined that the safety of vegetable oil as used in cosmetic formulations has been established, providing that the blend contains oils for which the fatty acid composition is known.

Addtionally, while data on the fatty acid composition of Fragaria Vesca (Strawberry) Seed Oil and Fragaria Virginiana (Strawberry) Seed Oil were not available, data were available for Fragaria Ananassa (Strawberry) Seed Oil and Fragaria Chiloensis (Strawberry) Seed Oil. In that the fatty acid compositions of Fragaria Ananassa and Fragaria Chiloensis (Strawberry) Seed Oil were similar to each other, it was assumed that Fragaria Vesca and Fragaria Virginiana (Strawberry) Seed Oils would also have similar fatty acid compositions.

The Expert Panel also noted that arachidonic acid is a fatty acid constituent of Lycium Barbarum Seed Oil, Oryza Sativa (Rice) Germ Oil, and Sclerocarya Birrea Seed Oil. Although a previously published CIR evaluation concluded that insufficient data exist to support the safety of arachidonic acid in cosmetic products, the Panel was of the opinion that the concentration of use of these ingredients was sufficiently low that the amount of free arachidonic acid from these oils would not warrant concern.

Finally, the conclusion reached by the Panel on the safety of the plant-derived fatty acid oils supersedes the 2001 conclusion of insufficient data for Corylus Americana and Corylus Avellana (Hazel) Seed Oil.

CONCLUSION

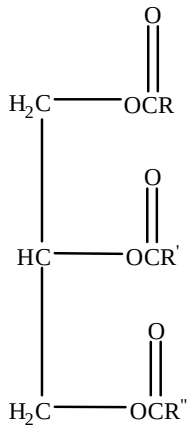
The CIR Expert Panel concluded that the 244 plant-derived fatty acid oils included in this review are safe in the present practices of use and concentration described in this safety assessment. Were the ingredients not in current use (as indicated by *) to be used in the future, the expectation is that they would be used in product categories and concentrations comparable to others in these groups. The ingredients found safe are:

Actinidia Chinensis (Kiwi) Seed Oil
Adansonia Digitata Oil
Adansonia Digitata Seed Oil*
Aleurites Moluccanus Bakoly Seed Oil*
Aleurities Moluccana Seed Oil
Amaranthus Hypochondriacus Seed Oil*
Anacardium Occidentale (Cashew) Seed Oil
Arachis Hypogaea (Peanut) Oil
Arctium Lappa Seed Oil*
Argania Spinosa Kernel Oil
Astrocaryum Murumuru Seed Butter
Avena Sativa (Oat) Kernel Oil
Babassu Acid*
Bassia Butyracea Seed Butter*
Bassia Latifolia Seed Butter
Bertholletia Excelsa Seed Oil
Borago Officinalis Seed Oil
Brassica Campestris (Rapeseed) Oil Unsaponifiables*
Brassica Campestris (Rapeseed) Seed Oil
Brassica Napus Seed Oil*
Brassica Oleracea Acephala Seed Oil*
Brassica Oleracea Italica (Broccoli) Seed Oil
Butyrospermum Parkii (Shea) Butter
Butyrospermum Parkii (Shea) Butter Unsaponifiables
Butyrospermum Parkii (Shea) Oil
Camelina Sativa Seed Oil
Camellia Japonica Seed Oil
Camellia Kissi Seed Oil
Camellia Oleifera Seed Oil
Camellia Sinensis Seed Oil
Canarium Indicum Seed Oil*
Canola Oil
Canola Oil Unsaponifiables
Carica Papaya Seed Oil
Carthamus Tinctorius (Safflower) Seed Oil
Carya Illinoensis (Pecan) Seed Oil*
Caryocar Brasiliense Fruit Oil
Chenopodium Quinoa Seed Oil
Citrullus Lanatus (Watermelon) Seed Oil
Citrus Aurantifolia (Lime) Seed Oil*
Citrus Aurantifolia (Lime) Seed Oil Unsaponifiables*
Citrus Aurantium Dulcis (Orange) Seed Oil*
Citrus Aurantium Dulcis (Orange) Seed Oil Unsaponifiables*
Citrus Grandis (Grapefruit) Seed Oil*
Citrus Grandis (Grapefruit) Seed Oil Unsaponifiables*
Citrus Limon (Lemon) Seed Oil*
Citrus Paradisi (Grapefruit) Seed Oil
Coconut Acid
Cocos Nucifera (Coconut) Oil
Cocos Nucifera (Coconut) Seed Butter*
Coix Lacryma-Jobi (Job's Tears) Seed Oil*
Corn Acid*
Corylus Americana (Hazel) Seed Oil
Corylus Avellana (Hazel) Seed Oil
Cottonseed Acid*
Crambe Abyssinica Seed Oil
Cucumis Sativus (Cucumber) Seed Oil
Cucurbita Pepo (Pumpkin) Seed Oil
Cynara Cardunculus Seed Oil*
Elaeis (Palm) Fruit Oil*
Elaeis Guineensis (Palm) Butter*
Elaeis Guineensis (Palm) Kernel Oil
Elaeis Guineensis (Palm) Oil
Elaeis Oleifera Kernel Oil
Euterpe Oleracea Fruit Oil
Fragaria Ananassa (Strawberry) Seed Oil*
Fragaria Chiloensis (Strawberry) Seed Oil*
Fragaria Vesca (Strawberry) Seed Oil*
Fragaria Virginiana (Strawberry) Seed Oil*
Garcinia Indica Seed Butter
Gevuina Avellana Seed Oil
Gevuina Avellana Oil

Glycine Soja (Soybean) Oil
Glycine Soja (Soybean) Oil Unsaponifiables
Gossypium Herbaceum (Cotton) Seed Oil
Guizotia Abyssinica Seed Oil*
Helianthus Annuus (Sunflower) Seed Oil
Helianthus Annuus (Sunflower) Seed Oil Unsaponifiables
Hippophae Rhamnoides Fruit Oil
Hippophae Rhamnoides Oil
Hippophae Rhamnoides Seed Oil*
Hydrogenated Adansonia Digitata Seed Oil*
Hydrogenated Apricot Kernel Oil
Hydrogenated Apricot Kernel Oil Unsaponifiables*
Hydrogenated Argania Spinosa Kernel Oil*
Hydrogenated Avocado Oil
Hydrogenated Black Currant Seed Oil*
Hydrogenated Camelina Sativa Seed Oil*
Hydrogenated Camellia Oleifera Seed Oil
Hydrogenated Canola Oil
Hydrogenated Coconut Acid
Hydrogenated Coconut Oil
Hydrogenated Cottonseed Oil
Hydrogenated Cranberry Seed Oil*
Hydrogenated Evening Primrose Oil
Hydrogenated Grapefruit Seed Oil*
Hydrogenated Grapefruit Seed Oil Unsaponifiables*
Hydrogenated Grapeseed Oil
Hydrogenated Hazelnut Oil*
Hydrogenated Kukui Nut Oil*
Hydrogenated Lime Seed Oil*
Hydrogenated Lime Seed Oil Unsaponifiables*
Hydrogenated Macadamia Seed Oil*
Hydrogenated Meadowfoam Seed Oil*
Hydrogenated Olive Oil
Hydrogenated Olive Oil Unsaponifiables
Hydrogenated Orange Seed Oil*
Hydrogenated Orange Seed Oil Unsaponifiables*
Hydrogenated Palm Acid*
Hydrogenated Palm Kernel Oil
Hydrogenated Palm Oil
Hydrogenated Passiflora Edulis Seed Oil*
Hydrogenated Peach Kernel Oil*
Hydrogenated Peanut Oil
Hydrogenated Pistachio Seed Oil*
Hydrogenated Pumpkin Seed Oil*
Hydrogenated Punica Granatum Seed Oil*
Hydrogenated Rapeseed Oil*
Hydrogenated Raspberry Seed Oil
Hydrogenated Rice Bran Oil*
Hydrogenated Rosa Canina Fruit Oil*
Hydrogenated Safflower Seed Oil*
Hydrogenated Sesame Seed Oil*
Hydrogenated Shea Butter
Hydrogenated Soybean Oil
Hydrogenated Sunflower Seed Oil
Hydrogenated Sweet Almond Oil
Hydrogenated Sweet Almond Oil Unsaponifiables*
Hydrogenated Vegetable Oil
Hydrogenated Wheat Germ Oil*
Hydrogenated Wheat Germ Oil Unsaponifiables*
Irvingia Gabonensis Kernel Butter
Juglans Regia (Walnut) Seed Oil
Limnanthes Alba (Meadowfoam) Seed Oil
Linseed Acid
Linum Usitatissimum (Linseed) Seed Oil
Luffa Cylindrica Seed Oil
Lupinus Albus Oil Unsaponifiables*
Lupinus Albus Seed Oil
Lycium Barbarum Seed Oil
Macadamia Integrifolia Seed Oil
Macadamia Ternifolia Seed Oil
Magnesium Cocoate
Mangifera Indica (Mango) Seed Butter
Mangifera Indica (Mango) Seed Oil
Morinda Citrifolia Seed Oil*
Moringa Oleifera Seed Oil
Moringa Pterygosperma Seed Oil
Oenothera Biennis (Evening Primrose) Oil
Olea Europaea (Olive) Husk Oil*
Olea Europaea (Olive) Oil Unsaponifiables
Olea Europaea (Olive) Fruit Oil
Olive Acid*
Orbignya Cohune Seed Oil
Orbignya Oleifera Seed Oil
Orbignya Speciosa Kernel Oil
Oryza Sativa (Rice) Bran Oil
Oryza Sativa (Rice) Germ Oil
Oryza Sativa (Rice) Seed Oil*
Palm Acid
Palm Kernel Acid
Passiflora Edulis Seed Oil
Peanut Acid*
Perilla Ocymoides Seed Oil
Persea Gratissima (Avocado) Butter
Persea Gratissima (Avocado) Oil
Persea Gratissima (Avocado) Oil Unsaponifiables
Pistacia Vera Seed Oil
Plukenetia Volubilis Seed Oil
Potassium Babassuate*
Potassium Cocoate
Potassium Cornate*
Potassium Hydrogenated Cocoate*
Potassium Hydrogenated Palmate*
Potassium Olivate
Potassium Palm Kernelate
Potassium Palmate
Potassium Peanutate
Potassium Rapeseedate*
Potassium Safflowerate*
Potassium Soyate*
Prunus Amygdalus Dulcis (Sweet Almond) Oil
Prunus Amygdalus Dulcis (Sweet Almond) Oil Unsaponifiables*
Prunus Armeniaca (Apricot) Kernel Oil
Prunus Armeniaca (Apricot) Kernel Oil Unsaponifiables*
Prunus Avium (Sweet Cherry) Seed Oil
Prunus Domestica Seed Oil
Prunus Persica (Peach) Kernel Oil
Punica Granatum Seed Oil
Pyrus Malus (Apple) Seed Oil
Rapeseed Acid*
Ribes Nigrum (Black Currant) Seed Oil
Ribes Rubrum (Currant) Seed Oil*
Rice Bran Acid*
Rosa Canina Fruit Oil
Rubus Chamaemorus Seed Oil
Rubus Idaeus (Raspberry) Seed Oil
Safflower Acid*
Schinziophyton Rautanenii Kernel Oil
Sclerocarya Birrea Seed Oil
Sesamum Indicum (Sesame) Oil Unsaponifiables
Sesamum Indicum (Sesame) Seed Butter*
Sesamum Indicum (Sesame) Seed Oil
Silybum Marianum Seed Oil [Thistle]

Sodium Astrocaryum Murumuruate
Sodium Avocadoate
Sodium Babassuate
Sodium Cocoa Butterate*
Sodium Cocoate
Sodium Grapeseedate
Sodium Hydrogenated Cocoate*
Sodium Hydrogenated Palmate*
Sodium Macadamiaseedate*
Sodium Mangoseedate
Sodium Olivate
Sodium Palm Kernelate
Sodium Palmate
Sodium Peanutate*
Sodium Rapeseedate*
Sodium Safflowerate*
Sodium Sesameseedate
Sodium Soyate*
Sodium Sweet Almondate
Sodium Theobroma Grandiflorum Seedate*
Solanum Lycopersicum (Tomato) Fruit Oil
Solanum Lycopersicum (Tomato) Seed Oil
Soy Acid*
Sunflower Seed Acid*
Theobroma Cacao (Cocoa) Seed Butter
Theobroma Grandiflorum Seed Butter
Torreya Nucifera Seed Oil*
Triticum Aestivum (Wheat) Germ Oil*
Triticum Vulgare (Wheat) Germ Oil
Triticum Vulgare (Wheat) Germ Oil Unsaponifiables*
Vaccinium Corymbosum (Blueberry) Seed Oil*
Vaccinium Macrocarpon (Cranberry) Seed Oil
Vaccinium Myrtillus Seed Oil
Vaccinium Vitis-Idaea Seed Oil
Vegetable (Olus) Oil
Vitis Vinifera (Grape) Seed Oil
Wheat Germ Acid
Zea Mays (Corn) Germ Oil
Zea Mays (Corn) Oil
Zea Mays (Corn) Oil Unsaponifiables

FIGURES AND TABLES



-OCR, -OCR', and -OCR" may be the same or different fatty acid radicals.

Figure 1. **General structure of fats and oils**
(Reference[4])

-

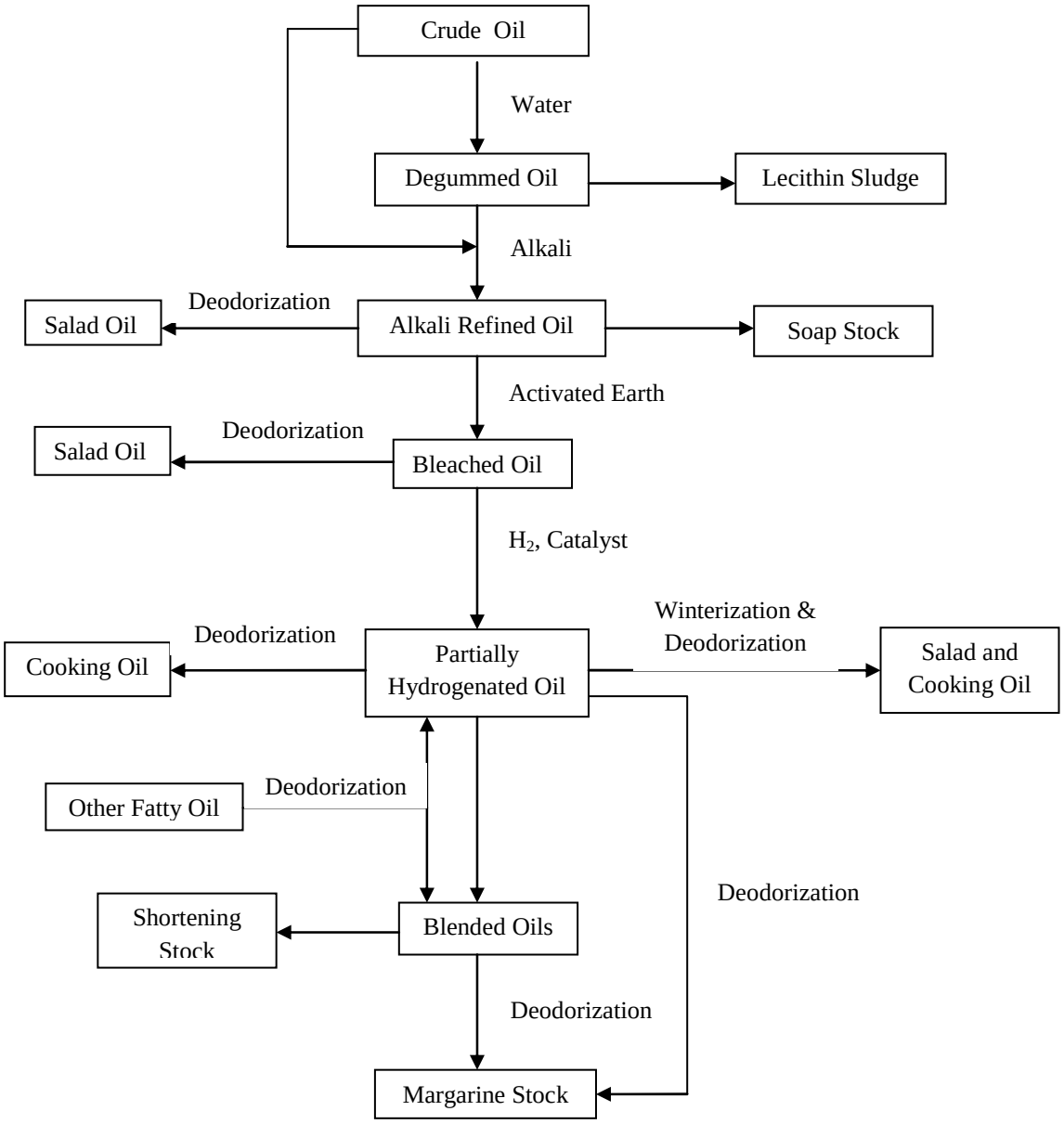


Figure 2. **Basic oil refinement flowchart**
(Reference.[6])

Table 1. **Plant-derived fatty acid oils**.

Actinidia Chinensis (Kiwi) Seed Oil
Adansonia Digitata Oil [Baobab]
  Adansonia Digitata Seed Oil
  Hydrogenated Adansonia Digitata Seed Oil
Aleurities Moluccana Seed Oil [Kukui] (CAS No. 8015-80-3)
  Hydrogenated Kukui Nut Oil
  Aleurites Moluccanus Bakoly Seed Oil
Amaranthus Hypochondriacus Seed Oil [Amaranth]
Anacardium Occidentale (Cashew) Seed Oil (CAS No. 8007-24-7)
Arachis Hypogaea (Peanut) Oil (CAS No. 8002-03-7)[a]
  Hydrogenated Peanut Oil (CAS No. 68425-36-5)
  Potassium Peanutate
  Sodium Peanutate
  Peanut Acid (CAS No. 91051-35-3)
Arctium Lappa Seed Oil [Burdock]
Argania Spinosa Kernel Oil [Argan]
  Hydrogenated Argania Spinosa Kernel Oil
Astrocaryum Murumuru Seed Butter [Murumuru]
  Sodium Astrocaryum Murumuruate
Avena Sativa (Oat) Kernel Oil
Bassia Butyracea Seed Butter
Bassia Latifolia Seed Butter [Mahwa]
Bertholletia Excelsa Seed Oil [Brazil]
Borago Officinalis Seed Oil [Borage] (CAS No. 225234-12-8)
Brassica Campestris (Rapeseed) Seed Oil
  Brassica Campestris (Rapeseed) Oil Unsaponifiables
  Hydrogenated Rapeseed Oil
  Rapeseed Acid
  Potassium Rapeseedate
  Sodium Rapeseedate
Brassica Napus Seed Oil [Rapeseed]
Brassica Oleracea Acephala Seed Oil [Kale]
Brassica Oleracea Italica (Broccoli) Seed Oil
Butyrospermum Parkii (Shea) Oil
  Butyrospermum Parkii (Shea) Butter (CAS No. 68920-03-6;194043-92-0)
  Butyrospermum Parkii (Shea) Butter Unsaponifiables
    (CAS No. 194043-92-0; 225234-14-0)
  Hydrogenated Shea Butter
Camelina Sativa Seed Oil [False Flax]
  Hydrogenated Camelina Sativa Seed Oil
Camellia Japonica Seed Oil
Camellia Kissi Seed Oil [Tea]
Camellia Oleifera Seed Oil [Tea Seed]
  Hydrogenated Camellia Oleifera Seed Oil
Camellia Sinensis Seed Oil
Canarium Indicum Seed Oil [Galip]
Canola Oil
  Canola Oil Unsaponifiables
  Hydrogenated Canola Oil
Carica Papaya Seed Oil [Papaya]
Carthamus Tinctorius (Safflower) Seed Oil
  Hydrogenated Safflower Seed Oil
  Potassium Safflowerate
  Sodium Safflowerate
  Safflower Acid
Carya Illinoensis (Pecan) Seed Oil
Caryocar Brasiliense Fruit Oil [Pequi]
Chenopodium Quinoa Seed Oil [Quinoa]
Citrullus Lanatus (Watermelon) Seed Oil
Citrus Aurantifolia (Lime) Seed Oil
  Citrus Aurantifolia (Lime) Seed Oil Unsaponifiables
  Hydrogenated Lime Seed Oil
  Hydrogenated Lime Seed Oil Unsaponifiables
Citrus Aurantium Dulcis (Orange) Seed Oil
  Citrus Aurantium Dulcis (Orange) Seed Oil Unsaponifiables
  Hydrogenated Orange Seed Oil
  Hydrogenated Orange Seed Oil Unsaponifiables
Citrus Grandis (Grapefruit) Seed Oil
  Citrus Grandis (Grapefruit) Seed Oil Unsaponifiables
  Hydrogenated Grapefruit Seed Oil
  Hydrogenated Grapefruit Seed Oil Unsaponifiables
Citrus Paradisi (Grapefruit) Seed Oil
Citrus Limon (Lemon) Seed Oil (CAS No. 85085-28-5)
Cocos Nucifera (Coconut) Oil (CAS No. 8001-31-8)
  Hydrogenated Coconut Oil (CAS No. 84836-98-6)
  Cocos Nucifera (Coconut) Seed Butter
  Magnesium Cocoate
  Potassium Cocoate (CAS No. 61789-30-8)
  Potassium Hydrogenated Cocoate
  Sodium Cocoate (CAS No. 61789-31-9)
  Sodium Hydrogenated Cocoate
  Coconut Acid (CAS No. 61788-47-4)
  Hydrogenated Coconut Acid (CAS No. 68938-15-8)
Coix Lacryma-Jobi (Job's Tears) Seed Oil
Corylus Americana (Hazel) Seed Oil
  Hydrogenated Hazelnut Oil
Corylus Avellana (Hazel) Seed Oil
Crambe Abyssinica Seed Oil [Abyssinian Mustard]
Cucumis Sativus (Cucumber) Seed Oil (CAS No. 70955-25-8)
Cucurbita Pepo (Pumpkin) Seed Oil (CAS No. 8016-49-7)
  Hydrogenated Pumpkin Seed Oil
Cynara Cardunculus Seed Oil [Artichoke] (CAS No. 923029-60-1)
Elaeis Guineensis (Palm) Oil (CAS No. 8002-75-3)
  Elaeis Guineensis (Palm) Kernel Oil (CAS No. 8023-79-8)

Table 1. Plant-derived Fatty Acid Oils

Hydrogenated Palm Kernel Oil (CAS No. 68990-82-9; 84540-04-5)
Elaeis (Palm) Fruit Oil
Hydrogenated Palm Oil (CAS No. 8033-29-2; 68514-74-9)
Elaeis Guineensis (Palm) Butter (CAS No. 8002-75-3)
Palm Kernel Acid
Potassium Palm Kernelate
Potassium Palmate
Potassium Hydrogenated Palmate
Sodium Palm Kernelate (CAS No. 61789-89-7)
Sodium Palmate (CAS No. 61790-79-2)
Sodium Hydrogenated Palmate
Palm Acid
Hydrogenated Palm Acid
Elaeis Oleifera Kernel Oil
Euterpe Oleracea Fruit Oil [Acai]
Fragaria Ananassa (Strawberry) Seed Oil
Fragaria Chiloensis (Strawberry) Seed Oil
Fragaria Vesca (Strawberry) Seed Oil
Fragaria Virginiana (Strawberry) Seed Oil
Garcinia Indica Seed Butter [Kokum]
Juglans Regia (Walnut) Seed Oil (CAS No. 8024-09-7)
Limnanthes Alba (Meadowfoam) Seed Oil (CAS No. 153065-40-8)
Hydrogenated Meadowfoam Seed Oil
Linum Usitatissimum (Linseed) Seed Oil (CAS No. 8001-26-1)
Linseed Acid (CAS No. 68424-45-3)
Luffa Cylindrica Seed Oil [Luffa]
Lupinus Albus Seed Oil [White Lupine]
Lupinus Albus Oil Unsaponifiables
Lycium Barbarum Seed Oil [Goji Berry]
Macadamia Integrifolia Seed Oil
Hydrogenated Macadamia Seed Oil
Macadamia Ternifolia Seed Oil (CAS No. 128497-20-1 or 129811-19-4)
Sodium Macadamiaseedate
Mangifera Indica (Mango) Seed Oil
Mangifera Indica (Mango) Seed Butter
Sodium Mangoseedate
Morinda Citrifolia Seed Oil [Noni]
Moringa Oleifera Seed Oil [Ben/Moringa]
Moringa Pterygosperma Seed Oil
Oenothera Biennis (Evening Primrose) Oil
Hydrogenated Evening Primrose Oil
Olea Europaea (Olive) Fruit Oil (CAS No. 8001-25-0)
Olea Europaea (Olive) Oil Unsaponifiables (CAS No. 156798-12-8)
Hydrogenated Olive Oil
Hydrogenated Olive Oil Unsaponifiables
Potassium Olivate (CAS No. 68154-77-8)
Sodium Olivate (CAS No. 64789-88-6)
Olea Europaea (Olive) Husk Oil
Olive Acid (CAS No. 92044-96-7)
Gevuina Avellana Oil [Chilean Hazel]
Gevuina Avellana Seed Oil
Glycine Soja (Soybean) Oil (CAS No. 8001-22-7)
Glycine Soja (Soybean) Oil Unsaponifiables (CAS No. 91770-67-1)
Hydrogenated Soybean Oil (CAS No. 8016-70-4)
Soy Acid (CAS No. 68308-53-2)
Potassium Soyate
Sodium Soyate
Gossypium Herbaceum (Cotton) Seed Oil (CAS No. 8001-29-4)
Hydrogenated Cottonseed Oil (CAS No. 68334-00-9)
Cottonseed Acid (CAS No. 68308-51-0)
Guizotia Abyssinica Seed Oil [Ramtil/Niger]
Helianthus Annuus (Sunflower) Seed Oil (CAS No. 8001-21-6)
Helianthus Annuus (Sunflower) Seed Oil Unsaponifiables
Hydrogenated Sunflower Seed Oil
Sunflower Seed Acid (CAS No. 84625-38-7)
Hippophae Rhamnoides Oil [Sea-Buckthorn]
Hippophae Rhamnoides Fruit Oil [Sea-Buckthorn]
Hippophae Rhamnoides Seed Oil [Sea-Buckthorn]
Irvingia Gabonensis Kernel Butter [Dika] (CAS No. 192230-28-7)
Orbignya Cohune Seed Oil [Cohune]
Orbignya Oleifera Seed Oil [Babassu] (CAS No. 91078-92-1)
Potassium Babassuate
Sodium Babassuate
Babassu Acid
Orbignya Speciosa Kernel Oil
Oryza Sativa (Rice) Bran Oil (CAS No. 68553-81-1; 84696-37-7)
Hydrogenated Rice Bran Oil
Oryza Sativa (Rice) Germ Oil
Oryza Sativa (Rice) Seed Oil
Rice Bran Acid (CAS No. 93165-33-4)
Passiflora Edulis Seed Oil [Passion Fruit] (CAS No. 87676-26-1)
Hydrogenated Passiflora Edulis Seed Oil
Perilla Ocymoides Seed Oil [Perilla]
Persea Gratissima (Avocado) Oil (CAS No. 8024-32-6)
Persea Gratissima (Avocado) Oil Unsaponifiables (CAS No. 91770-40-0)
Hydrogenated Avocado Oil
Persea Gratissima (Avocado) Butter
Sodium Avocadoate
Pistacia Vera Seed Oil [Pistachio] (CAS No. 90082-81-8; 129871-01-8)
Hydrogenated Pistachio Seed Oil
Plukenetia Volubilis Seed Oil [Sacha Inchi]
Prunus Amygdalus Dulcis (Sweet Almond) Oil
(CAS No. 8007-69-0; 90320-37-9)
Prunus Amygdalus Dulcis (Sweet Almond) Oil Unsaponifiables
Hydrogenated Sweet Almond Oil
Hydrogenated Sweet Almond Oil Unsaponifiables
Sodium Sweet Almondate
Prunus Armeniaca (Apricot) Kernel Oil (CAS No. 72869-69-3)

Table 1. Plant-derived Fatty Acid Oils

Prunus Armeniaca (Apricot) Kernel Oil Unsaponifiables
Hydrogenated Apricot Kernel Oil
Hydrogenated Apricot Kernel Oil Unsaponifiables
Prunus Avium (Sweet Cherry) Seed Oil
Prunus Domestica Seed Oil [Prune/Plum]
Prunus Persica (Peach) Kernel Oil (CAS No. 8002-78-6; 8023-98-1)
Hydrogenated Peach Kernel Oil
Punica Granatum Seed Oil [Pomegranate]
Hydrogenated Punica Granatum Seed Oil
Pyrus Malus (Apple) Seed Oil
Ribes Nigrum (Black Currant) Seed Oil (CAS No. 97676-19-2)
Hydrogenated Black Currant Seed Oil
Ribes Rubrum (Currant) Seed Oil
Rosa Canina Fruit Oil [Dog Rose]
Hydrogenated Rosa Canina Fruit Oil
Rubus Chamaemorus Seed Oil [Cloudberry]
Rubus Idaeus (Raspberry) Seed Oil
Hydrogenated Raspberry Seed Oil
Schinziophyton Rautanenii Kernel Oil [Mongongo]
Sclerocarya Birrea Seed Oil [Marula]
Sesamum Indicum (Sesame) Seed Oil (CAS No. 8008-74-0)
Sesamum Indicum (Sesame) Oil Unsaponifiables
Hydrogenated Sesame Seed Oil
Sesamum Indicum (Sesame) Seed Butter
Sodium Sesameseedate
Silybum Marianum Seed Oil [Thistle]
Solanum Lycopersicum (Tomato) Fruit Oil
Solanum Lycopersicum (Tomato) Seed Oil
Theobroma Cacao (Cocoa) Seed Butter (CAS No. 8002-31-1)
Sodium Cocoa Butterate
Theobroma Grandiflorum Seed Butter [Cupuacu] (CAS No. 394236-97-6)
Sodium Theobroma Grandiflorum Seedate
Torreya Nucifera Seed Oil [Kaya]
Triticum Vulgare (Wheat) Germ Oil (CAS No. 8006-95-9; 68917-73-7)
Triticum Aestivum (Wheat) Germ Oil
Triticum Vulgare (Wheat) Germ Oil Unsaponifiables
Hydrogenated Wheat Germ Oil Unsaponifiables
Hydrogenated Wheat Germ Oil
Wheat Germ Acid (CAS No. 68938-32-9)
Vaccinium Corymbosum (Blueberry) Seed Oil
Vaccinium Macrocarpon (Cranberry) Seed Oil
Hydrogenated Cranberry Seed Oil
Vaccinium Myrtillus Seed Oil [Bilberry] (CAS No. 1161921-09-0)
Vaccinium Vitis-Idaea Seed Oil [Ligonberry],
Vegetable (Olus) Oil
Hydrogenated Vegetable Oil
Vitis Vinifera (Grape) Seed Oil (CAS No. 8024-22-4)
Hydrogenated Grapeseed Oil
Sodium Grapeseedate
Zea Mays (Corn) Oil (CAS No. 8001-30-7)
Zea Mays (Corn) Oil Unsaponifiables
Zea Mays (Corn) Germ Oil
Potassium Cornate (CAS No. 61789-23-9)
Corn Acid (CAS No. 68308-50-9)

[a] Previously reviewed ingredients are in ***bold and italics***.

Table 2. **Previously reviewed oil and fatty acid ingredients.**

Ingredients	Publication Date	Conclusion
Oil Ingredients		
Arachis Hypogaea (Peanut) Oil (CAS No. 8002-03-7) Hydrogenated Peanut Oil (CAS No. 68425-36-5) Peanut Acid (CAS No. 91051-35-3)	IJT 20(S2):65-77, 2001	Safe
Carthamus Tinctorius (Safflower) Seed Oil (CAS No. 8001-23-8)	JACT 4(5):171-197, 1985; Re-reviewed, not reopened IJT 25(2):1-89, 2006	Safe
Cocos Nucifera (Coconut) Oil (CAS No. 8001-31-8) Coconut Acid (CAS No. 61788-47-4) Hydrogenated Coconut Acid (CAS No. 68938-15-8) Hydrogenated Coconut Oil (CAS No. 84836-98-6) Magnesium Cocoate Potassium Cocoate (CAS No. 61789-30-8) Potassium Hydrogenated Cocoate Sodium Cocoate (CAS No. 61789-31-9) Sodium Hydrogenated Cocoate	JACT 5(3):103-121, 1986; CIR Final Report, 2008	Safe
Corylus Americana (Hazel) Seed Oil Corylus Avellana (Hazel) Seed Oil	IJT 20 (S1):15-20, 2001	Insufficient data
Elaeis Guineensis (Palm) Oil (CAS No. 8002-75-3) Elaeis Guineensis (Palm) Kernel Oil (CAS No. 8023-79-8) Hydrogenated Palm Oil (CAS No. 8033-29-2; 68514-74-9) Hydrogenated Palm Kernel Oil (CAS No. 68990-82-9; 84540-04-5)	IJT 19(S2):7-28, 2000	Safe
Gossypium Herbaceum (Cotton) Seed Oil (CAS No. 8001-29-4) Cottonseed Acid (CAS No. 68308-51-0) Hydrogenated Cottonseed Oil (CAS No. 68334-00-9)	IJT 20(S2):21-29, 2001	Safe
Oryza Sativa (Rice) Bran Oil (CAS No. 68553-81-1; 84696-37-7) Oryza Sativa (Rice) Germ Oil Rice Bran Acid (CAS No. 93165-33-4)	IJT 25(S2):91-120, 2006	Safe
Prunus Amygdalus Dulcis (Sweet Almond) Oil (CAS No. 8007-69-0)	JACT 2(5):85-99, 1983; Re-reviewed, not reopened IJT 24 (S1):1-102, 2005	Safe
Sesamum Indicum (Sesame) Seed Oil (CAS No. 8008-74-0) Hydrogenated Sesame Seed Oil Sesamum Indicum (Sesame) Oil Unsaponifiables Sodium Sesameseedate	JACT 12(3):261-277, 1993; Amended Final Report, 2009	Safe
Zea Mays (Corn) Oil (CAS No. 8001-30-7) Zea Mays (Corn) Germ Oil Zea Mays (Corn) Oil Unsaponifiables Corn Acid (CAS No. 68308-50-9) Potassium Cornate (CAS No. 61789-23-9)	Final Report, 2008	Safe
Persea Gratissima (Avocado) Oil (CAS No. 8024-32-6)	JEPT 4(4):93-103, 1980; Re-reviewed, not reopened IJT 22(1):1-35, 2003	Safe
Triticum Vulgare (Wheat) Germ Oil (CAS No. 8006-95-9; 68917-73-7)	JEPT 4(4):33-45, 1980; Re-reviewed, not reopened IJT 22(1):1-35, 2003	Safe
Fatty Acids		
Arachidonic Acid (CAS No. 506-32-1)	JACT 12 (5):481-559, 1993	Insufficient data
Hydroxystearic Acid (CAS No. 106-14-9)	IJT 18(S1):1-10, 1999	Safe
Lauric Acid (CAS No. 143-07-7) Myristic Acid (CAS No. 544-63-8) Oleic Acid (CAS No. 112-80-1) Palmitic Acid (CAS No. 57-10-3) Stearic Acid (CAS No. 57-11-4)	JACT 6(3):321-401, 1987; Re-reviewed, not reopened IJT 25(2):1-89, 2006	Safe

Table 2. **Previously reviewed oil and fatty acid ingredients.**

Ingredients	Publication Date	Conclusion
Glyceryl Triesters		
Trilaurin		
Triarachidin		
Tribehenin		
Tricaprin		
Tricaprylin		
Trierucin		
Triheptanoin		
Triheptylundecanoin		
Triisononanoin		
Triisopalmitin		
Triisostearin		
Trilinolein	IJT 20 (S4):61-94, 2001	Safe
Trimyristin		
Trioctanoin		
Triolein		
Tripalmitin		
Tripalmitolein		
Triricinolein		
Tristearin		
Triundecanoin		
Glyceryl Triacetyl Hydroxystearate		
Glyceryl Triacetyl Ricinoleate		
Glyceryl Stearate Diacetate		

Table 3. **Chemical properties for plant-derived fatty acid oils.**

Properties and Constituents [a]	Actinidia Chinensis (Kiwi) Seed Oil[64]	Adansonia Digitata Oil [65,66]	Aleurites Moluccana Seed Oil [Kukui][67-70]	Anacardium Occidentale (Cashew) Seed Oil[71]	Arachis Hypogaea (Peanut) Oil[6,67,72-75]	Argania Spinosa Kernel Oil[76,77]	Astrocaryum Murumuru Seed Butter[6,78]
Appearance		Pale yellow	Clear yellow liquid		Light yellow	Yellow	Pale brown waxy solid at room temperature
Specific gravity			0.920-0.930 (20◦C)		0.912-0.920 (20◦C)	0.908-0.918 (20◦C)	0.890-0.910 (25◦C)
Refractive index			1.470-1.480 (20◦C)		1.46-1.475 (20◦C)		
Iodine value		65-95	130-175		74-107	95	15 max
Saponification value		190-210	185-210		180-208		270-350
Peroxide value (meq/kg)	44.37	5.0-10	5.0 max	0.22	0.39, 5.0 max	10.0 max	20.0 max
Melting point (◦C)							25-37
Unsaponifiable matter (%)			0.3 - 1		≤1.0		
Free fatty acids (%)	1.2	2.0 max as oleic acid	0.1-4		0.2-2.08		12.56 as oleic acid
Titer (◦C)					26-32		
Acid value					0.5	3-4	

Properties and Constituents	Avena Sativa (Oat) Kernel Oil[79]	Bertholletia Excelsa Seed Oil[71,80]	Borago Officinalis Seed Oil[81,82]	Brassica Campestris (Rapeseed) Seed Oil[6]	Hydrogenated Rapeseed Oil[7]	Rapeseed Acid[83]	Canola Oil[7]
Appearance	Yellow		Clear, pale yellow-golden		White waxy solid		Light yellow oil
Specific gravity	0.914-0.932 (25◦C)	1.473	0.918-0.928 (20◦C)				
Refractive index	1.469-1.471 (25◦C)	0.914 (20◦C)	1.474-1.479 (20◦C)				1.465-1.467 (40°C)
Iodine value		74.2	130-155	81-112	4 max	119-120 g/100 g	110-126
Saponification value	176-186	192.4	184-194	168-192			
Peroxide value (meq/kg)	0.6-1.1	0.16	10.0 max		2.0 max		10 max
Melting point (◦C)							
Unsaponifiable matter (%)	3.7-4.3			0.5 - 2			1.5 max
Free fatty acids (%)	0.1-0.3			1	2.0 max as oleic acid		0.1% max as oleic acid
Titer (◦C)							
Acid value			1.0 max			197-200 mg KOH/g	

Table 3. Chemical properties for plant-derived fatty acid oils (continued).

Properties and Constituents [a]	Brassica Oleracea Acephala Seed Oil[84]	Brassica Oleracea Italica (Broccoli) Seed Oil[85]	Butyrospermum Parkii (Shea) Butter[6,67,86-89]	Butyrospermum Parkii (Shea) Oil[7]	Camellia Oleifera Seed Oil[90,91]	Canarium Indicum Oil[92,93]	Carica Papaya Seed Oil[94,95]
Appearance	Yellow	Golden	Grey, tallow-like	Pale yellow	Clear, pale yellow or "water white"	Cream to golden	Pale yellow
Specific gravity	0.9010 (20◦C)	0.910-0.918 (20◦C)	0.918 (15◦C)				
Refractive index	1.4741 (23◦C)	1.465-1.475 (20◦C)	1.468 (25◦C)			1.45-1.47	
Iodine value	61.2	90-120	45-77	28 - 43	80-94		65-100
Saponification value	123.06		165-190	185-195	188-196		
Peroxide value (meq/kg)			5.0 max	≤ 10	10.0 max	≤ 20	10.0 max
Melting point (◦C)			32-46; 28-42 (slip)				
Unsaponifiable matter (%)	1.6		3-13	≤ 1.5	1.5 max	≤ 1	
Free fatty acids (%)			1.0 max as oleic acid	≤ 0.1 as oleic acid		0.2	0.8-3
Titer (◦C)			49-54				
Acid value	2.1	1.5	1.5		1.0 max	≤ 10	

Properties and Constituents	Carthamus Tinctorius (Safflower) Seed Oil[7]	Carya Illinoensis (Pecan) Seed Oil[67,71,80]	Caryocar Brasiliense Fruit Oil [Pequi][83,96]	Citrullus Lanatus (Watermelon) Seed Oil[6,97]	Citrus Aurantifolia (Lime) Seed Oil[98,99]	Citrus Aurantium Dulcis (Orange) Seed Oil[100,101]	Citrus Paradisi (Grapefruit) Seed Oil[102,103]
Appearance	Light yellow oil		Yellow[96]	Pale to golden yellow liquid	Clear yellow	Clear, light yellow	Clear yellow
Specific gravity		0.924 (25◦C)		0.8930-0.9166		0.910-0.920 (20◦C)	
Refractive index		1.472		1.4668		1.466-1.475 (20◦C)	
Iodine value	135-150	100 - 105	48.65-74.80[96] 50-70 g/100 g[83]	113-123		90-110	80-125
Saponification value		190	160.15-202[96] 190-210 mg KOH/g	193-195		185-200	
Peroxide value (meq/kg)	10 max	0.15	0.99-5.22[96] ≤20[83]	≤ 5.0	5.0 max	5-10	5-10
Melting point (◦C)							
Unsaponifiable matter (%)	1.5 max	0.35-40					
Free fatty acids (%)	0.1 max as oleic acid		0.98-2.85 (mg KOH/g) [96]	< 5.0 as oleic acid		0.5 as oleic acid	
Titer (◦C)							
Acid value			10 mg KOH/g max[83]		1.0 max	0.8 max	1.0 max

Table 3. Chemical properties for plant-derived fatty acid oils (continued).

Properties and Constituents [a]	Cocos Nucifera (Coconut) Oil[6,7,104]	Cucurbita Pepo (Pumpkin) Seed Oil[105,106]	Elaeis Guineensis (Palm) Oil[6,7]	Elaeis Guineensis (Palm) Kernel Oil[6,7]	Fragaria Ananassa (Strawberry) Seed Oil[6,107,108]	Fragaria Chiloensis (Strawberry) Seed Oil[109,110]	Garcinia Indica Seed Butter [Kokum][111-113]
Appearance	White to light yellow-tan	Dark green	Pale yellow to deep orange in color	Nearly colorless	Light golden/yellow to yellow	Light yellow with some green	
Specific gravity	0.917 - 0.919 (25◦/15.5◦C)		0.921-0.925 (40◦C)		0.93-0.95	0.912-0.930	
Refractive index	1.448 - 1.450 (40◦C)		1.453-1.458 (40◦C)			1.465-1.485	1.4565-1.4575 (40◦C)
Iodine value	6-11	110-330	44-58	14-33		170-190	30-50
Saponification value	248-265	174-197	195-205	245-255		180-195	185-195
Peroxide value (meq/kg)	≤ 10	5.0 max	10 max	10 max	< 15	10 max	
Melting point (◦C)	22 - 26		25-50	25-30			37-43; 27 (slip)
Unsaponifiable matter (%)	≤ 0.5	1.5	0.2-0.8	1.5 max			1.5 max; 18-20; 32-40
Free fatty acids (%)	≤ 0.1% as oleic acid; ≤ 0.07% as lauric acid	1.5 as oleic acid	0.1 max as oleic acid; 0.09 as palmitic acid	0.1 max as oleic acid; 0.07 max as lauric acid		3	0.1-1
Titer (◦C)	20 - 24						
Acid value					18 max		

Properties and Constituents	Glycine Soja (Soybean) Oil[6,7]	Gossypium Herbaceum (Cotton) Seed Oil[6,7]	Guizotia Abyssinica Seed Oil[6]	Hazel Seed Oil*[72,114-116]	Helianthus Annuus (Sunflower) Seed Oil[6,7]	Sunflower Seed Acid[83]	Hippophae Rhamnoides Fruit Oil[117]
Appearance	Light amber oil	Dark red-brown oil	Pale yellow with a bluish tint		Light amber oil		Orange-red
Specific gravity			0.910-0.928	0.912-0.917 (15.5◦C); 0.905-0.925 (20◦C)	0.894-0.899 (60◦C)		0.90
Refractive index			1.467-1.471	1.467-1.474 (20◦C)	1.4597-1.4745 (25◦C)		
Iodine value	120.9-151.4	90-113	126-139	83-100	128-144	125-140 g/100 g	
Saponification value		180-198	180-195	180-200	188-194		
Peroxide value (meq/kg)	10 max	10 max		0.43; 10.0 max	10 max		10 max
Melting point (◦C)					0		
Unsaponifiable matter (%)	0.3-0.6	1.5 max	0.5-1	≤ 1.0	0.3-0.5		
Free fatty acids (%)	0.05-0.7	0.1 max as oleic acid	0.4-3	0.2 max as oleic acid	0.1 max as oleic acid		
Titer (◦C)							
Acid value				≤ 0.5		125-140 mg KOH/g	18 max

*Information mainly on Corylus Avellena.

Table 3. Chemical properties for plant-derived fatty acid oils (continued).

Properties and Constituents [a]	Hippophae Rhamnoides Seed Oil[118-120]	Irvingia Gabonenesis Kernel Butter[121]	Juglans Regia (Walnut) Seed Oil[67,72,80]	Linum Usitatissimum (Linseed) Seed Oil[6]	Macadamia Nut Oil[72,80,122-124]	Mangifera Indica (Mango) Seed Oil[6]	Moringa Oleifera Seed Oil[125-127]
Appearance	Orange				Pale to golden yellow	Pale yellow to ivory cream color	
Specific gravity	0.890-0.955 (20◦C)		0.917 (25◦C)	0.927-0.931 (20◦C)	0.911-0.918 (20◦C)	0.91	0.908 (20◦C); 0.8933 (24◦C)
Refractive index	1.4650-1.4825 (20◦C)		1.475 (25◦C)	1.4786-1.4815	1.466-1.470 (20◦C)	1.456	1.4566 (40◦C)
Iodine value	130-200		150 - 162	170-204	62-82	32-93	66.47
Saponification value	184-210		190 - 197	189-196	190-200	190-195	164.27; 192
Peroxide value (meq/kg)	5-10 max		0.37		0.36; 10.0 max		0.45; 10.0
Melting point (◦C)				0		34-43	18.93
Unsaponifiable matter (%)	1.0	0.13	0.5	0.5-1.5	1.5	0.8-2.9	0.58
Free fatty acids (%)	2.0 max; 18 max	0.30	0.2 - 2.5	5	0.5 max; 1.0 max as oleic acid		2.55 as oleic acid
Titer (◦C)							
Acid value	15				1		

Properties and Constituents	Oenothera Biennis (Evening Primrose) Oil[128,129]	Olea Europaea (Olive) Fruit Oil[6]	Olea Europaea(Olive) Husk Oil[130]	Olive Acid[83]	Oryza Sativa (Rice) Bran Oil[131,132]	Oryza Sativa (Rice) Bran Oil[131,132]	Passiflora Edulis Seed Oil [Passion Fruit]
Appearance	Light yellow	Almost colorless to yellow, greenish, or brown in color			Light golden yellow	Light golden yellow	Golden-orange
Specific gravity	0.920-0.930 (20◦C)	0.914-0.918			0.916-0.922 (15.5◦C)	0.916-0.922 (15.5◦C)	0.917 (20◦C)
Refractive index	1.475-1.480 (20◦C)	1.469-1.484			1.470-1.473 (20◦C)	1.470-1.473 (20◦C)	1.468-1.473 (20◦C)
Iodine value	145-165	64-88; refined 75-94		85-91 g/100 g	92-115	92-115	119.9-129.29[133]
Saponification value	180-195	185-212; refined 184-186			180-195	180-195	176-187.4
Peroxide value (meq/kg)	10.0 max	20 max (refined)	14.33		10.0 max	10.0 max	1.37-2.23
Melting point (◦C)							
Unsaponifiable matter (%)		0.6-1.2; 1.5 max refined					0.9-2.86
Free fatty acids (%)		0.6-1.4; 0.3 max refined			1.0 as oleic acid	1.0 as oleic acid	
Titer (◦C)							
Acid value	1-2			190-201 mg KOG/g			2.11-2.36

Table 3. Chemical properties for plant-derived fatty acid oils (continued).

Properties and Constituents [a]	Persea Gratissima (Avocado) Oil[6]	Pistacia Vera Seed Oil [71]	Plukenetia Volubilis Seed Oil[134]	Prunus Amygdalus (Sweet Almond) Oil[6,67,72,135-137]	Prunus Armeniaca (Apricot) Kernel Oil	Prunus Avium (Sweet Cherry) Seed Oil[138,139]
Appearance			Yellow-amber	Colorless to pale yellow liquid		Clear light yellow
Specific gravity	0.910-0.916		0.90-0.93 (20◦C)	0.911-0.920 (20◦C)	0.923[6]	0.905-0.925 (20◦C)
Refractive index	1.461-1.465		1.478-1.481 (20◦C)	1.467-1.473 (20◦C)	1.4672-1.4722[6]	1.463-1.480 (20◦C)
Iodine value	71-95		180-200	93 - 106	81-123[6]	90-115
Saponification value	177-198		180-210	183 - 197	191[6]	105-135
Peroxide value (meq/kg)		0.22	0-15	0.19		10.0 max
Melting point (◦C)						
Unsaponifiable matter (%)				0.4-1.0	0.4-1.4	
Free fatty acids (%)				1.0 max		0.5% max
Titer (◦C)					0-6[140]	
Acid value			0-2	0.5		1.0 max

Properties and Constituents	Prunus Domestica Seed Oil[141,142]	Prunus Persica (Peach) Kernel Oil[6,143]	Punica Granatum Seed Oil[144,145]	Pyrus Malus (Apple) Seed Oil[146]	Ribes Nigrum (Black Currant) Seed Oil[147-149]	Ribes Rubrum (Currant) Seed Oil[150]
Appearance		Pale yellow (refined)	Golden to dark yellow		Pale yellow or slightly greenish	Pale yellow or slightly greenish
Specific gravity		0.910-0.920 (20◦C) refined	0.935 (15.5◦C)	0.902-0.903 (25◦C)	0.92	0.92
Refractive index				1.465-1.466 (40◦C)		
Iodine value	90-108	90-115 (refined)	190-230	94.14-101.15	145-185	
Saponification value				179.01-197.25		
Peroxide value (meq/kg)	10.0 max	5.0 max (refined)	10.0 max	2.43-2.52	1-10	10 max
Melting point (◦C)						
Unsaponifiable matter (%)						
Free fatty acids (%)	2.0 max as oleic acid		1.4; 5.0 max as oleic acid		0.2	
Titer (◦C)						
Acid value				4.036-4.323	3; 18 max	18 max

Table 3. Chemical properties for plant-derived fatty acid oils (continued).

Properties and Constituents [a]	Rubus Chamaemorus Seed Oil[151]	Rubus Idaeus (Raspberry) Seed Oil[152-154]	Schinziophyton Rautanenii Kernel Oil[155]	Sclerocarya Birrea Seed Oil [Marula][156]	Solanum Lycopersicum (Tomato) Seed Oil[157]	Theobroma Cacao (Cocoa) Seed Butter[6]
Appearance	Yellow-red	Yellow or yellow-red	Light yellow		Clear golden yellow to darker red	
Specific gravity	0.92	0.92			0.9135-0.9357	0.950-0.998
Refractive index			1.4830	1.46	1.4577-1.4771	1.453-1.458
Iodine value		175-195		100.25	105-130.5	35-40
Saponification value		180-200		162.70	156-194.9	190-200
Peroxide value (meq/kg)	10 max	5.0 max; 10 max	10 mg/kg	4.58		
Melting point (◦C)				26-28		33.5
Unsaponifiable matter (%)				3.06		
Free fatty acids (%)		1.5 max as oleic acid				
Titer (◦C)						
Acid value	18 max	18 max		33.70		

Properties and Constituents	Vaccinium Corymbosum (Blueberry) Seed Oil[64,158,159]	Vaccinium Macrocarpon (Cranberry) Seed Oil[6,64,160-163]	Vaccinium Myrtillus Seed Oil[164]	Vaccinium Vitis-Idaea Seed Oil[165]	Vitis Vinifera (Grape) Seed Oil[6]	Zea Mays (Corn) Oil[166,167]
Appearance	Green with yellow tint or dark green /brown	Pale yellow to greenish; light green	Pale yellow to greenish	Pale yellow		Clear, bright golden yellow
Specific gravity		0.923	0.93	0.92	0.91-0.93	0.920-0.928 (15.5◦C)
Refractive index					1.470-1.476	1.472-1.476 (20◦C)
Iodine value	155-175	140-180			125-143	103-128
Saponification value		170-200			176-206	185-195
Peroxide value (meq/kg)	20-24.62	< 15; 10 max	10 max	10 max		10.0 max
Melting point (◦C)						
Unsaponifiable matter (%)						
Free fatty acids (%)	0.67; 2.0 as oleic acid	0.7; 1.0 as oleic acid				
Titer (◦C)						
Acid value		2.0 max; 18 max	18 max	18 max		0.2 max

Table 4. **Total fatty acid composition of plant-derived fatty acid oils (%).**

Fatty Acids	Actinidia Chinensis (Kiwi) Seed Oil[64]	Adansonia Digitata Oil [Baobab] [65,66]	Aleurites Moluccana Seed Oil [Kukui][67,69,70]	Amaranthus Hypochondriacus Seed Oil [Amaranth][168]	Anacardium Occidentale (Cashew) Seed Oil[71]	Arachis Hypogaea (Peanut) Oil[6,73,74]	Arctium Lappa Seed Oil[169]	Argania Spinosa Kernel Oil [Argan][76,77]	Astrocaryum Murumuru Seed Butter [Murumuru][78]	Avena Sativa (Oat) Kernel Oil[79,170]
Caproic (C6)										
Caprylic (C8)									1.85	
Capric (C10)									1.85	
Lauric (C12)*	0.02								47.46	
Myristic (C14)	0.03				0.07		0.01		26	0.2-0.3
Myristoleic (C14:1)										
Palmitic (C16)	5.96	18-30	5-8	19 - 20	9.9	5-16	7.27	10-15	6.28	13.9-18.82
Palmitoleic (C16:1)		1	0.5		0.4		0.01			0.1-0.4
Heptadecanoic (C17:0)					0.1					
Stearic (C18)	3.09	2-8	0.1-6.7	3	8.7	1-6.5	32.56	5-6.5	2.65	0.8-2.79
Oleic (C18:1)	14.6	30-40	10-35	22 - 26	57.2	33.3-76	50.21	45-55	12.56	31.4-51.26
Linoleic (C18:2)	17.55	24-34	35-50	46 - 50	20.8	8-47.5	3.18	28-36	2.87	22.8-43.1
Linolenic (C18:3)	57.4	1-3	24-40		0.2	0-0.6				0.64-2.1
Arachidic (C20)	0.34		1.5		1	0.17-3	0.22			
Eicosenoic (C20:1)			1		0.3	0.33-3	0.33			0.5-1
Eicosadienoic (C20:2)										
Arachidonic (C20:4)										
Behenic (C22)					0.4	1-5				
Erucic (C22:1)					0.3	0.5				
Docosadienoic (C22:2)										
Docosahexaenoic (C22:6)										
Lignoceric (C24)						0.2-3	0.49			
Others						<C16:0 = 0.4	heptadecenoic=0.02; nonadecadienoic acid=2.99; heneicosanoic acid =1.07; dicosanoic acid=0.43			Arachidic (C20) + Eicosadienoic (C20:2)=0.1-0.3; C18:1, n-11=0.9-1.3

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Bassia Butyracea Seed Butter[a,111]	Bassia Latifolia Seed Butter [Mahwa][b,111]	Bertholletia Excelsa Seed Oil [Brazil][71]	Borago Officinalis Seed Oil [Borage] [81,82]	Brassica Campestris (Rapeseed) Seed Oil[6]	Rapeseed Acid[83]	Brassica Napus Seed Oil [Rapeseed][171]	Hydrogenated Rapeseed Oil[7]	Canola Oil[7]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)									
Myristic (C14)			0.06			≤0.5		< 1.0	<0.2
Myristoleic (C14:1)									
Palmitic (C16)	60.8	23.7-24.7	13.5	9-13	1.5 - 3	≤8	2	3-5.0	<6.0
Palmitoleic (C16:1)			0.3			≤2			<1.0
Heptadecanoic (C17:0)			0.2						
Stearic (C18)	3.2	19.3-29.9	11.8	3-5	0.7 - 1.3	≤3	1	38-42	<2.5
Oleic (C18:1)	30.9	36.3-43.3	29.1	10-22	12.1 - 57.4	54-70	21	1	>50
Linoleic (C18:2)	4.9	11.6-15.8	42.8	33-46	11.4 - 22.1	18-24	20	< 1.0	<40.0
Linolenic (C18:3)			0.2	18-25	8.3 - 12.5	5-10	2		<14
Arachidic (C20)			0.5			≤6	1	8-10.0	<1.0
Eicosenoic (C20:1)			0.2	2-6	5.6 - 3.1			< 1.0	<2.0
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)			0.1					42-50	<0.5
Erucic (C22:1)			0.3	1-3.5	1 - 58.6		53	< 1.0	<2.0
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)							2	1-2.0	<0.2
Others				α-Linolenic (C18:3) = 0.4%; γ-Linolenic = 1-3.5%		<C14 = ≤0.5; >C18:3 = ≤5; >C20 = ≤6			<C14 = <0.1; C24:1 = <0.2

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Brassica Oleracea Acephala Seed Oil [Kale][84]	Brassica Oleracea Italica (Broccoli) Seed Oil[85]	Butyrospermum Parkii (Shea) Oil[7]	Butyrospermum Parkii (Shea) Butter[6,86-88]	Camelina Sativa Seed Oil [False Flax][172]	Camellia Japonica Seed Oil[173]	Camellia Kissi Seed Oil[173]	Camellia Oleifera Seed Oil [Tea Seed][90,91]	Camellia Sinensis Seed Oil[173]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)									
Myristic (C14)				0.5					
Myristoleic (C14:1)									
Palmitic (C16)	4.4	0-5	3.8-4.1	3-9	7.8	7.9		6.1-15	8-10
Palmitoleic (C16:1)						0.16			
Heptadecanoic (C17:0)									
Stearic (C18)	0.7	0-5	41.2-56.8	30-50	2.96	2.46		0.8-2	1.5-3.5
Oleic (C18:1)	11.3	10-20	34.0-46.9	38-50	16.77	84.99	80	72-87	78-86
Linoleic (C18:2)	12.6	10-20	3.7-6.5	3-8	23.08	3.76		5.3-14.3	7-10
Linolenic (C18:3)	10.2	5-10		0.5 max	31.2				0.2-0.8
Arachidic (C20)	8.2		1-2	2.5-3		0.49			
Eicosenoic (C20:1)	0.4	5-10			11.99				
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)									
Erucic (C22:1)	51.8	40-50			2.8				
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)									
Others					3.4				

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Canarium Indicum Oil [Galip][92,93]	Carica Papaya Seed Oil [Papaya][94,95]	Carthamus Tinctorius (Safflower) Seed Oil[32,174]	Carya Illinoensis (Pecan) Seed Oil[67,71]	Caryocar Brasiliense Fruit Oil [Pequi][c,83,96]	Chenopodium Quinoa Seed Oil [Quinoa][175]	Citrullus Lanatus (Watermelon) Seed Oil[97]	Citrus Aurantifolia (Lime) Seed Oil[98,99]	Citrus Aurantium Dulcis (Orange) Seed Oil[100,101]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)	≤ 2								
Myristic (C14)	≤ 2			Trace	0.5	0.2		1	
Myristoleic (C14:1)									
Palmitic (C16)	28-38	8-18	2	3-4.3	34.4-44.3	9.9 - 11	8.0 - 13.0	20-30	14-22
Palmitoleic (C16:1)	≤ 2	2		0.1	1.3	0.1	< 1.0		
Heptadecanoic (C17:0)	≤ 2			0.1					
Stearic (C18)	10-20	2-6		1.8-2	0.66-1.8	0.7 - 0.8	8.0 - 12.0	3-8	2-6
Oleic (C18:1)	30-40	60-77	26	40.6-79	54.55-57.4	22 – 50.2	15.0 - 30.0	20-38	26-35
Linoleic (C18:2)	12-22	3-25	68	16-50.3	0.84-2.8	1.2 – 56	55.0 - 65.0	30-45	35-45
Linolenic (C18:3)		0.8	Trace	0.7	0.18-1.0	0.7 – 7	< 1.0	5-15	2-6
Arachidic (C20)			Trace	Trace		0.7	< 1.0	2	0.5
Eicosenoic (C20:1)		2		1.2			< 1.0		
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)				0.2			< 1.0		
Erucic (C22:1)				0.3					
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)							< 2.0		
Lignoceric (C24)									
Others	Others = ≤ 2	α-Linolenic (C18:3) = 2%;					< 1.0		

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Citrus Grandis (Grapefruit) Seed Oil[102,103]	Citrus Limon (Lemon) Seed Oil[176]	Citrus Paradisi (Seed) Oil[177]	Cocos Nucifera (Coconut) Oil[33]	Coix Lacryma-Jobi (Job's Tears) Seed Oil[178]	Corylus Americana (Hazel) Seed Oil[171]	Corylus Avellana (Hazel) Seed Oil[12,114-116]	Crambe Abyssinica Seed Oil [Abyssinian Mustard][171,179]	Cucumis Sativus (Cucumber) Seed Oil[180]	Cucurbita Pepo (Pumpkin) Seed Oil[105,106]
Caproic (C6)				0-1						
Caprylic (C8)				5-9						
Capric (C10)				6-10				<0.01-0.11		
Lauric (C12)	1.5		2.95	44-52				<0.01-0.14		
Myristic (C14)	1		1.01	13-19			≤0.2	<0.01-0.43		
Myristoleic (C14:1)								<0.01-0.09		
Palmitic (C16)	18-30	18.8	36.25	8-11	16.0	6	4-9	0.81-5.55	9-13	10-16
Palmitoleic (C16:1)				0-1			0.2-1	<0.01-0.77		
Heptadecanoic (C17:0)		0.08					≤0.1			
Stearic (C18)	2-8	3.5	5.95	1-3	trace	3	1-6	0.6-10.42	6-9	3-7
Oleic (C18:1)	20-38	30.1	18.34	5-8	53	76	66-85	12.8-23.13	14-20	18-38
Linoleic (C18:2)	30-48	33.4	29.26	Trace-2.5	30.5	15	7-25	9.08-15.86	60-68	40-62
Linolenic (C18:3)	2-6	13.5	3.58		trace		≤0.6	3.27-9.43	<1	1
Arachidic (C20)		0.3	0.38				≤0.5	<0.01-1.19		
Eicosenoic (C20:1)		0.03	0.84				≤0.5	<0.01-6		
Eicosadienoic (C20:2)								<0.01-0.21		
Arachidonic (C20:4)								<0.01		
Behenic (C22)		0.08					≤0.3	<0.01-2.59		
Erucic (C22:1)							Trace-0.01	48.86-60		
Docosadienoic (C22:2)										
Docosahexaenoic (C22:6)								<0.01-1.34		
Lignoceric (C24)		0.2	C12:1=1.44				0.01	<0.01-1.85		
Others		C23:0 = <0.01; C26:0 = 0.01					C17:1 = ≤ 0.1	C20:3 = <0.01-0.19; C20:5 = <0.01-1.91		

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Cynara Cardunculus Seed Oil [Artichoke][181]	Elaeis Guineensis (Palm) Oil[26]	Elaeis Guineensis (Palm) Kernel Oil[26]	Elaeis Oleifera Kernel Oil[182]	Euterpe Oleracea Fruit Oil [Acai][183]	Fragaria Ananassa (Strawberry) Seed Oil[64,107,108]	Fragaria Chiloensis (Strawberry) Seed Oil[110]	Garcinia Indica Seed Butter [Kokum][d,121,184]	Gevuina Avellana Oil [Chilean Hazel][185]
Caproic (C6)			0.3	0.1					
Caprylic (C8)			4.4	0.9					
Capric (C10)			3.7	0.8					
Lauric (C12)		0.2	48.3	29.3					
Myristic (C14)		1.1	15.6	25.7		0.05			
Myristoleic (C14:1)									
Palmitic (C16)	12	44	7.8	10.1	22	4.32	3-5	2-8	1.9
Palmitoleic (C16:1)		0.1			2		0-0.2		22.7
Heptadecanoic (C17:0)									
Stearic (C18)	3	4.5	2	1.8	2	1.68	1-2	50-67.4	0.5
Oleic (C18:1)	25	39.2	15.1	26.4	60	10-20	15-18	27-42	39.4
Linoleic (C18:2)	60	10.1	2.7	4.5	12	28.5 – 50	40-46	0.5-2	5.6
Linolenic (C18:3)		0.4			Trace	25-40	30-36		0.1
Arachidic (C20)		0.4			2.5	0.71	0-0.2	0.7	1.4
Eicosenoic (C20:1)							0-0.2		3.1
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)									2.2
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)									0.5
Others			0.2	0.4		5.5 - 8.5	C18:3 w6=0-0.1		C18:1Δ12 = 6.2; C20:1Δ15 = 6.6; ; C22:1Δ17 = 7.9; ; C22:1Δ19 = 1.6

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Glycine Soja (Soybean) Oil[6]	Gossypium Herbaceum (Cotton) Seed Oil[27]	Guizotia Abyssinica Seed Oil [Ramtil/Niger][6]	Helianthus Annuus (Sunflower) Seed Oil[6]	Sunflower Seed Acid[83]	Hippophae Rhamnoides Fruit Oil[e,117,186]	Hippophae Rhamnoides Seed Oil[119,120,186]	Irvingia Gabonenesis Kernel Butter[121,121]	Juglans Regia (Walnut) Seed Oil[187]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)								35-51.1	
Myristic (C14)		2			≤2	0.4-0.6		36.8-58	
Myristoleic (C14:1)						0.2			
Palmitic (C16)		21	5.0-13	5.0 - 7.2	6-11	24-42	5-11.3	3.9-5	3-7
Palmitoleic (C16:1)						24-42	4.4		
Heptadecanoic (C17:0)									
Stearic (C18)		Trace	2.0-11	2.0 - 6.5	3-7	0.9-2.1	2-5	0.4-0.7	0.5-3
Oleic (C18:1)	11.5 - 60.0	30	6.0-40	14.7 - 37.2	19-31	3-30	11-30	0.6-2.7	9-30
Linoleic (C18:2)	0000	45	45-77	51.5 - 73.5	57-66		28-45	0.60	57-76
Linolenic (C18:3)	2.9 - 12.1			Trace - 0.3	≤1	1.7-6.8	24.9-38	1.3	2-16
Arachidic (C20)		Trace		0.3 - 1	≤3				
Eicosenoic (C20:1)									
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)									
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)			2 max						
Others					>C20 = ≤3	Vakccenic C18:1(n-7) = 7.3-7.5; α-Linoleic C18:2 = 4.1-5.5	Vakccenic C18:1(n-7) = 3.2; α-Linoleic C18:2 = 34.1; Others = 3 max		

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued)[a]

Fatty Acids	Limnanthes Alba (Meadowfoam) Seed Oil[6]	Linum Usitatissimum (Linseed) Seed Oil[6]	Luffa Cylindrica Seed Oil[188]	Lupinus Albus Seed Oil[189]	Lycium Barbarum Seed Oil[190]	Macadamia Integrifolia Seed Oil[f,2,122-124]	Mangifera Indica (Mango) Seed Oil[g,6]	Morinda Citrifolia Seed Oil[191]	Moringa Oleifera Seed Oil [Ben/Moringa] [125,126,192]	Oenothera Biennis (Evening Primrose) Oil[128,129]
Caproic (C6)										
Caprylic (C8)								1.44		
Capric (C10)										
Lauric (C12)						0.1-1.4				
Myristic (C14)			0.1			0.7-1.5			Trace	
Myristoleic (C14:1)										
Palmitic (C16)		5.5	12.2	14.44-21.57		6-12	5-8	9.0	5-9.3	4-10
Palmitoleic (C16:1)			0.1	0.36-1.03		12-25		0.12	1.5-3	
Heptadecanoic (C17:0)								0.13		
Stearic (C18)		3.5	0.1	1.37-3.91	3	0.5-8	33-48	4.07	3-8	2-4
Oleic (C18:1)		19.1	19.6	42.78-52.87	19.1	50-67	35-50	17.45	65-80	5-12
Linoleic (C18:2)		15.3	59.7	9.20-17.23	68.3	1.5-5	4.0-8	59.45	1.5-5	60-85
Linolenic (C18:3)		57		4.81-9.02	2.8	0.5-1.9		0.27	1-1.5	
Arachidic (C20)				1.61-2.30		1.5-5	1-7	0.51	2-5	
Eicosenoic (C20:1)	52 - 77			3.86-5.30		1.5-3.1		0.2	2.5-4	
Eicosadienoic (C20:2)										
Arachidonic (C20:4)					0.68					
Behenic (C22)				4.75-5.99		0.3-1			8-8.6	
Erucic (C22:1)	8.0 - 29			0.51-1.47		1			3	
Docosadienoic (C22:2)	7.0 - 20									
Docosahexaenoic (C22:6)										
Lignoceric (C24)									Trace	
Others										α-Linolenic (C18:3) = 1% γ-Linolenic = 7-12%

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Olea Europaea (Olive) Oil[6]	Olea Europaea (Olive) Husk Oil[130]	Olive Acid[83]	Orbignya Cohune Seed Oil [Cohune][6]	Orbignya Oleifera Seed Oil [Babassu][6]	Orbignya Speciosa Kernel Oil[193]	Oryza Sativa (Rice) Bran Oil[132]	Oryza Sativa (Rice) Germ Oil[28]	Passiflora Edulis Seed Oil [Passion Fruit][133]
Caproic (C6)									
Caprylic (C8)				7.5	4 to 8	2-10			
Capric (C10)				6.5	4 to 8	2-12			
Lauric (C12)				46.5	44 - 47	35-50			
Myristic (C14)	Trace		≤1.0	16	15 - 20	12-25		6.92[28]	0.03
Myristoleic (C14:1)									
Palmitic (C16)	7.5 - 20	14.96	9-15	9.5	6 to 9	4-15	14	9.28	8.57
Palmitoleic (C16:1)	0.3 - 3.5	2.18	≤2					4.41[28]	0.23
Heptadecanoic (C17:0)			≤0.5						
Stearic (C18)	0.5 - 3.5	1	2-5	3	3 to 5	1-7	2	7.91[28]	1.66
Oleic (C18:1)	53 - 86	64.08	69-78	10	10 to 12	5-20	45	17.81[28]	16.25
Linoleic (C18:2)	3.5 - 20	16.09	8-14	1	1 to 3	<3	34	16.22[28]	72.69
Linolenic (C18:3)	0 - 1.5	0.71	≤3.5				1	15.56[28]	0.26
Arachidic (C20)	Trace							3.08[28]	
Eicosenoic (C20:1)									
Eicosadienoic (C20:2)									
Arachidonic (C20:4)								5.48[28]	
Behenic (C22)	Trace								
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)	Trace								
Others								Arachidontrienoic = 5.21[28]	Unspecified other fatty acids = 0.31

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Perilla Ocymoides Seed Oil [Perilla][6]	Persea Gratissima (Avocado) Oil[6]	Pistacia Vera Seed Oil [Pistachio][71]	Plukenetia Volubilis Seed Oil [Sacha Inchi][194]	Prunus Amygdalus (Sweet Almond) Oil[6,67,135-137,195]	Prunus Armeniaca (Apricot) Kernel Oil[140]	Prunus Avium (Sweet) Cherry Seed Oil[h,138,139]	Prunus Domestica Seed Oil [Prune/Plum][141,142]
Caproic (C6)								
Caprylic (C8)								
Capric (C10)								
Lauric (C12)								
Myristic (C14)			0.09	0.02	1			
Myristoleic (C14:1)								
Palmitic (C16)		13-17	7.4	4.72	4-9	4.6-6	4-10	4-9
Palmitoleic (C16:1)		3 - 5.1	0.7	0.04	0.8	1-2		1
Heptadecanoic (C17:0)				0.12	0.2			
Stearic (C18)			0.9	3.33	2-3	0.5-1.2	1-4	3
Oleic (C18:1)	14-23	67-72	58.2	10.46	62-86	58-65.7 (total 18:1) 29-33	23-55	60-80
Linoleic (C18:2)	16	10 to 12	30.3	37.64	20-30	28.5 (undef. 18:2)	30-55	15-25
Linolenic (C18:3)	63-70		0.4	48.96	0.4	05-1.0 (undef 18:3)	13	1
Arachidic (C20)			0.6	0.09	0.2	0.2	2	
Eicosenoic (C20:1)			0.6	0.3	0.3			
Eicosadienoic (C20:2)								
Arachidonic (C20:4)								
Behenic (C22)			0.3		0.2			
Erucic (C22:1)			0.6		0.1			
Docosadienoic (C22:2)								
Docosahexaenoic (C22:6)								
Lignoceric (C24)								
Others				C17:1 = 0.06; gamma C18:3 = 0.24;Others = 0.02	<C16:0 = 0.1	Oleic/Linoleic = 90-93%	Eleostearic (C18:3 conj) = 10%	

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Prunus Persica (Peach) Kernel Oil[143]	Punica Granatum Seed Oil [Pomegranate][144,145]	Pyrus Malus (Apple) Seed Oil[146]	Ribes Nigrum (Black Currant) Seed Oil[147-149]	Ribes Rubrum (Currant) Seed Oil[150,196]	Rosa Canina Seed Oil [Dog Rose][176,197]	Rubus Chamaemorus Seed Oil[151]	Rubus Idaeus (Raspberry) Seed Oil[64,152-154]
Caproic (C6)								
Caprylic (C8)								
Capric (C10)								
Lauric (C12)								
Myristic (C14)						0.11-0.21		0.07
Myristoleic (C14:1)								
Palmitic (C16)	2.0 - 7	1-10	6.51-6.60	6-10	4.6-4.8	1.71-4.6		2-2.43
Palmitoleic (C16:1)			0-0.05			0.24-1.01		
Heptadecanoic (C17:0)						0.04		
Stearic (C18)	0.5 - 3.5	1-5	1.75-1.96	1-4	2-3	1.69-2.47		0.9-1
Oleic (C18:1)	55 - 70	3-12	37.49-38.55	9-16	17.1-17.8	14.71-21.7	13-19	8-13
Linoleic (C18:2)	22 - 33	2-12	50.70-51.40	40-54	36-48	47.9-54.41	40-52	47-63
Linolenic (C18:3)	≤ 1		0.19-0.30	11-18	15-30	16.42-21.8	27-38	25-40
Arachidic (C20)			1.49-1.54	1		1.0-2.61		0.37
Eicosenoic (C20:1)			0.51-0.56	3		0.3		
Eicosadienoic (C20:2)						0.07		
Arachidonic (C20:4)								
Behenic (C22)			0-0.40	1		0.1-0.64		
Erucic (C22:1)				1				
Docosadienoic (C22:2)								
Docosahexaenoic (C22:6)								
Lignoceric (C24)						0.04		
Others		Punicic (C18:3conj) = 60-80; Other C18:3conj = 18%		C18:3 (n-6) = 11-18 C18:4 (n-3) = 2-5	C18:1n-7 = 0.5-0.6; C18:3n-6 = 5.6-12; C18:4n-3 = 2-5; Others = 0-0.3	C17:1 = 0.01; C21:0= 0.01, C23:0 = 0.03		

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Schinziophyton Rautanenii Kernel Oil[155]	Sclerocarya Birrea Seed Oil [Marula][156,198]	Sesamum Indicum (Sesame) Seed Oil[25,55]	Silybum Marianum Seed Oil [Thistle][199]	Solanum Lycopersicum (Tomato) Seed Oil[157]	Solanum Lycopersicum (Tomato) Fruit Oil[i,200]	Theobroma Cacao (Cocoa) Seed Butter[6]	Theobroma Grandiflorum Seed Butter [Cupuacu][201]
Caproic (C6)		1.41						
Caprylic (C8)								
Capric (C10)								
Lauric (C12)					Trace-0.3			
Myristic (C14)		2.12	<0.5		1.5-2.3			Trace
Myristoleic (C14:1)					Trace			
Palmitic (C16)	8	9-12; 22.56	7.0 - 12.0	9.4	16.9-23.4	47	24-29	7.2
Palmitoleic (C16:1)		0.05 - 0.15	<0.5		3.3-6.8			0.1
Heptadecanoic (C17:0)								0.2
Stearic (C18)	9	5-8; 50.76	3.5 - 6.0	6.6	4.0-9.5	3	34-36	30.8
Oleic (C18:1)	15	4.13; 70 - 78	35 - 50	21.3	18.3-29.7	30	30-40	43.9
Linoleic (C18:2)	37	4.0 - 7.0	35 - 50	53.3	37.6-42.8	12	2.4	4.6
Linolenic (C18:3)	25	0.1 - 0.6	<1.0	trace	Trace-0.7			Trace
Arachidic (C20)		0.3 - 0.7	<1.0	3.8	0.8-1.3			11
Eicosenoic (C20:1)		0.1 - 0.5	<0.5	0.5				
Eicosadienoic (C20:2)								
Arachidonic (C20:4)		8.46						
Behenic (C22)		5.14	<0.5	2.4	Trace-0.7			
Erucic (C22:1)		0.1 - 0.5						
Docosadienoic (C22:2)								
Docosahexaenoic (C22:6)								
Lignoceric (C24)		4.13		0.7				
Others		Butyric = 0.35%	Trace of components below C14			Other (C14 + C20) = 8		

Table 4. Total fatty acid composition of plant-derived fatty acid oils (%) (continued).

Fatty Acids	Torreya Nucifera Seed Oil [Kaya][202]	Triticum Vulgare (Wheat) Germ Oil[30,52]	Vaccinium Corymbosum (Blueberry) Seed Oil[64,158,159]	Vaccinium Macrocarpon (Cranberry) Seed Oil[64,160-163]	Vaccinium Myrtillus Seed Oil [Bilberry][164,203]	Vaccinium Vitis-Idaea Seed Oil [Lingonberry][165,203]	Vitis Vinifera (Grape) Seed Oil[6]	Zea Mays (Corn) Oil[53,166,167]	Zea Mays (Corn) Oil[53,166,167]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)			0.02	0.14					
Myristic (C14)	Trace		0.09	0.08	2.2-2.5	1.6-2.6		0.1 - 1.7	0.1 - 1.7
Myristoleic (C14:1)									
Palmitic (C16)	6.03	11.0 - 16	3-8	4-6	4.8-7.4	4.4-6.7	7-9.5	8-16.5	8-16.5
Palmitoleic (C16:1)	Trace							0.2 - 1.6	0.2 - 1.6
Heptadecanoic (C17:0)	Trace								
Stearic (C18)	2.51	1.0 - 6	0.5-3.5	1-1.25	2.2-2.5	1.2-1.9	3.5-5.5	0-4.5	0-4.5
Oleic (C18:1)	30.35	8.0 - 30	15-25	15-25.3	17.4-23	10-25	14-44	19 – 49	19 – 49
Linoleic (C18:2)	51.26	44 - 65	35-45	32-42	35-47.5	30-46.8	46-74	34-66	34-66
Linolenic (C18:3)	0.23	4.0 - 10	22-38	30-40	23.1-40	25.2-55		0-2	0-2
Arachidic (C20)			0.25	0.07				1	1
Eicosenoic (C20:1)	0.28							1	1
Eicosadienoic (C20:2)	0.98								
Arachidonic (C20:4)									
Behenic (C22)									
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)									
Others	C18:1 Δ11 = 0.57; C18:3 Δ5,9,12 = 0.08; C20:2 Δ 5,11 = 0.79; C20:3 Δ5,11,14 = 6.68; Others = 0.24	0 - 1.2 C20-22 Saturated acids		α-Linolenic (C18:3) = 34-35%					

[a]As Bassia Butyracea seed fat. [b]As Bassia Latifolia seed fat or Madhuca Indica seed fat. [c]As Caryocar Brasiliense pulp oil. [d]As Garcinia Indica seed fat. [e]As Hippophae pulp oil.
[f]Macadamia Integrifolia and Macadamia Ternifolia are synonyms; information is being reported under the more common name. [g]As mango kernel fat. [h]As cherry kernel oil. [i]With palm oil.

Table 5a. **Frequency and concentration of use according to duration and exposure - ingredients not previously reviewed by the CIR**

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses[48]	Conc of Use (%)	No. of Uses[48]	Conc of Use (%)	No. of Uses[48]	Conc of Use (%)
	Actinidia Chinensis (Kiwi) Seed Oil		**Adansonia Digitata Oil**		**Aleurities Moluccana Seed Oil**		**Anacardium Occidentale (Cashew) Seed Oil**		**Argania Spinosa Kernel Oil**		**Astrocaryum Murumuru Seed Butter**	
Totals*	**7**	**0.1**	**6**	**0.01**	**141**	**0.00001-5**	**10**	**0.002-1**	**100**	**0.001-10**	**192**	**0.001-7**
Duration of Use												
Leave-On	*5*	*NR*	*4*	*0.01*	*87*	*0.00002-5*	*9*	*0.04-1*	*87*	*0.001-10*	*171*	*0.001-7*
Rinse-Off	*2*	*0.1*	*2*	*NR*	*54*	*0.00001-3*	*1*	*0.002*	*13*	*0.001-2*	*21*	*0.001-0.2*
Exposure Type												
Eye Area	NR	NR	NR	NR	6	0.0001-0.005	NR	NR	11	0.1-1	21	0.06-0.5
Possible Ingestion	1	NR	NR	0.01	1	0.01	NR	NR	9	0.1-1	22	1-7
Inhalation	1	NR	NR	NR	15	0.1	NR	NR	NR	0.01	NR	NR
Dermal Contact	5	NR	5	0.01	76	0.00001-5	9	0.002-1	88	0.001-10	178	0.001-7
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.001	NR	NR
Hair - Non-Coloring	2	0.1	1	NR	58	0.00002-0.1	1	NR	8	0.01-1	11	0.001-0.2
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.07-0.1	3	NR
Nail	NR	NR	NR	NR	4	NR	NR	NR	2	0.001-0..1	NR	NR
Mucous Membrane	NR	NR	NR	NR	5	0.00001-0.4	NR	NR	2	0.001-2	3	NR
Bath Products	NR	NR	NR	NR	6	0.01-0.3	NR	NR	1	0.05	NR	NR
Baby Products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Sodium Astrocaryum Murumuruate		**Avena Sativa (Oat) Kernel Oil**		**Bassia Latifolia Seed Butter**		**Bertholletia Excelsa Seed Oil**		**Borago Officinalis Seed Oil**		**Brassica Campestris (Rapeseed) Seed Oil**	
Totals	**NR**	**0.002-0.005**	**43**	**0.01-3**	**22**	**0.001-2**	**55**	**0.0003-0.5**	**180**	**0.001-1**	**27**	**0.007-17**
Duration of Use												
Leave-On	*NR*	*0.002*	*37*	*0.1-3*	*17*	*0.001-0.05*	*18*	*0.0003-0.5*	*160*	*0.001-1*	*23*	*0.007-17*
Rinse-Off	*NR*	*0.002-0.005*	*6*	*0.001-0.1*	*5*	*0.001-2*	*37*	*0.01-0.2*	*20*	*0.001-0.01*	*4*	*0.1-1*
Exposure Type												
Eye Area	NR	NR	NR	0.2	4	0.01	1	NR	7	0.001-0.5	2	NR
Possible Ingestion	NR	NR	NR	2	NR	NR	NR	NR	NR	0.01	1	9
Inhalation	NR	NR	NR	NR	NR	NR	1	NR	3	0.1	NR	NR
Dermal Contact	NR	0.002-0.005	41	0.001-3	22	00.01-2	29	0.0003-0.5	168	0.001-1	27	0.007-17
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	2	0.1	NR	0.001-0.5	12	0.03-0.2	10	NR	NR	0.1
Hair - Coloring	NR	NR	NR	NR	NR	NR	14	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	0.002	2	0.01-0.1	5	NR	7	0.01	4	0.001-0.01	1	NR
Bath Products	NR	NR	1	NR	NR	NR	3	NR	1	NR	NR	NR
Baby Products	NR	NR	6	0.1	NR	NR	NR	NR	3	NR	NR	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Hydrogenated Rapeseed Oil		**Brassica Oleracea Italica (Broccoli) Seed Oil**		**Butyrospermum Parkii (Shea) Oil**		**Butyrospermum Parkii (Shea) Butter**		**Butyrospermum Parkii (Shea) Butter Unsaponifiables**		**Hydrogenated Shea Butter**	
Totals	**1**	**0.3-4**	**NR**	**0.001-3**	**22**	**0.01-15**	**1950**	**0.0005-60**	**38**	**0.06-3**	**4**	**1**
Duration of Use												*1*
Leave-On	*NR*	*0.3-4*	*NR*	*3*	*16*	*0.01-15*	*1680*	*0.001-60*	*35*	*0.06-3*	*2*	*1*
Rinse-Off	*1*	*NR*	*NR*	*0.001-0.5*	*22*	*0.6-1*	*270*	*0.0005-30*	*3*	*NR*	*2*	*1*
Exposure Type												
Eye Area	NR	2	NR	NR	1	NR	108	0.1-8	7	0.2-0.7	NR	NR
Possible Ingestion	NR	NR	NR	NR	NR	15	128	0.5-26	2	3-Jan	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	17	0.001-3	NR	NR	NR	NR
Dermal Contact	1	0.3-4	NR	NR	22	0.6-15	1724	0.001-45	33	0.06-3	4	1
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	2	1	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	210	0.0005-3	5	2	NR	NR
Hair - Coloring	NR	NR	NR	0.001-3	NR	NR	4	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	0.01-1	7	0.01-60	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	3	0.6	101	0.003-5	NR	NR	NR	NR
Bath Products	NR	NR	NR	NR	3	1	13	1	NR	NR	2	NR
Baby Products	NR	NR	NR	NR	NR	NR	24	0.01-5	NR	NR	NR	NR

	Camelina Sativa Seed Oil		**Camellia Japonica Seed Oil**		**Camellia Kissi Seed Oil**		**Camellia Oleifera Seed Oil**		**Hydrogenated Camellia Oleifera Seed Oil**		**Camellia Sinensis Seed Oil**	
Totals	**76**	**0.002-1**	**NR**	**0.01-0.2**	**47**	**0.1-10**	**25**	**0.003-3**	**1**	**NR**	**12**	**0.1**
Duration of Use												
Leave-On	*61*	*0.002-1*	*NR*	*0.01-0.2*	*34*	*0.1-10*	*23*	*0.003-3*	*1*	*NR*	*8*	*0.1*
Rinse-Off	*15*	*1*	*NR*	*0.1*	*13*	*0.1-3*	*2*	*0.01-0.1*	*NR*	*NR*	*4*	*0.1*
Exposure Type												
Eye Area	NR	0.05	NR	0.01	4	0.1	NR	2	NR	NR	NR	NR
Possible Ingestion	34	0.05-0.5	NR	0.1	1	0.1	3	3	NR	NR	1	0.1
Inhalation	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	47	0.002-1	NR	0.01-0.2	36	0.1-10	23	0.003-3	1	NR	10	0.1
Deodorant (Underarm)	NR	NR	NR	0.01	NR	NR	NR	NR	NR	NR	NR	0.1
Hair - Non-Coloring	29	1	NR	0.1	11	0.1-1	2	2	NR	NR	2	0.1
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	0.1	1	0.1	NR	0.01-0.1	NR	NR	2	0.1
Bath Products	NR	NR	NR	NR	1	0.3	NR	0.05	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Canola Oil		Canola Oil Unsaponifiables		Hydrogenated Canola Oil		Carica Papaya Seed Oil		Caryocar Brasiliense Fruit Oil		Chenopodium Quinoa Seed Oil	
Totals	**132**	**0.0002-73**	**NR**	**0.001**	**3**	**NR**	**NR**	**0.1**	**31**	**0.0005-0.2**	**1**	**0.3**
Duration of Use												
Leave-On	*112*	*0.002-73*	*NR*	*NR*	*2*	*NR*	*NR*	*0.1*	*29*	*0.0005-2*	*1*	*NR*
Rinse-Off	*20*	*0.02-33*	*NR*	*0.0001*	*1*	*NR*	*NR*	*NR*	*2*	*NR*	*NR*	*0.3*
Exposure Type												
Eye Area	3	0.002-0.03	NR	NR	NR	NR	NR	NR	12	NR	NR	NR
Possible Ingestion	62	0.3-70	NR	NR	NR	NR	NR	NR	12	0.2	NR	NR
Inhalation	1	0.0002-17	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	113	0.0002-73	NR	NR	3	NR	NR	0.1	30	0.0005-0.2	NR	NR
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	19	0.006-24	NR	0.001	NR	NR	NR	NR	1	NR	1	NR
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.3
Nail	NR	5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	2	0.02-1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Bath Products	1	1-33	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Citrullus Lanatus (Watermelon) Seed Oil		Citrullus Vulgaris (Watermelon) Seed Oil**		Citrus Limon (Lemon) Seed Oil		Citrus Paradisi (Grapefruit) Seed Oil		Crambe Abyssinica Seed Oil		Cucumis Sativus (Cucumber) Seed Oil	
Totals	**1**	**2**	**5**	**NR**	**6**	**6**	**NR**	**0.01-20**	**6**	**NR**	**6**	**NR**
Duration of Use												
Leave-On	*1*	*2*	*3*	*NR*	*5*	*5*	*NR*	*0.08-20*	*5*	*NR*	*5*	*NR*
Rinse-Off	*NR*	*NR*	*2*	*NR*	*1*	*1*	*NR*	*0.01-1*	*1*	*NR*	*1*	*NR*
Exposure Type												
Eye Area	NR	NR	NR	NR	NR	1	NR	NR	NR	NR	1	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	5	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	NR	2	NR	NR	NR	NR
Dermal Contact	1	2	5	NR	6	5	NR	2-5	6	NR	5	NR
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	1	NR	0.01-20	NR	NR	1	NR
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	9	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	1	NR	1	NR	NR	NR	1	NR	NR	NR
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Cucurbita Pepo (Pumpkin) Seed Oil		**Palm Kernel Acid**		**Potassium Palm Kernelate**		**Potassium Palmate**		**Sodium Palm Kernelate**		**Sodium Palmate**	
Totals	**18**	**0.003-0.1**	**72**	**0.2-12**	**7**	**0.3-30**	**5**	**0.3-3**	**194**	**12-44**	**212**	**3-68**
Duration of Use												
Leave-On	*17*	*0.003-0.1*	*3*	*NR*	*NR*	*NR*	*NR*	*NR*	*10*	*NR*	*7*	*NR*
Rinse-Off	*1*	*NR*	*69*	*0.2-12*	*7*	*0.3-30*	*5*	*0.3-3*	*184*	*12-44*	*205*	*3-68*
Exposure Type												
Eye Area	1	0.003	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Possible Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Inhalation	1	NR	NR	NR	NR	NR	NR	NR	1	NR	1	NR
Dermal Contact	18	0.003-0.1	71	0.2-12	7	0.3-30	5	0.3-3	194	12-44	212	3-68
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	64	0.2-3	1	0.3-30	2	0.3-3	173	16-44	189	3-68
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR	3	NR	1	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	4	NR	3	NR

	Palm Acid		**Elaeis Oleifera Kernel Oil**		**Euterpe Oleracea Fruit Oil**		**Garcinia Indica Seed Butter**		**Gevuina Avellana Oil**		**Glycine Soja (Soybean) Oil**	
Totals	**33**	**1-17**	**5**	**NR**	**29**	**0.00001-0.5**	**30**	**0.1-2**	**5**	**0.002-0.2**	**912**	**0.0002-95**
Duration of Use												
Leave-On	*1*	*NR*	*NR*	*NR*	*19*	*0.00001-0.5*	*27*	*0.1-2*	*5*	*0.04-0.2*	*718*	*0.0005-95*
Rinse-Off	*32*	*1-17*	*5*	*NR*	*10*	*0.05*	*3*	*NR*	*NR*	*0.002-0.01*	*194*	*0.0002-95*
Exposure Type												
Eye Area	NR	NR	NR	NR	2	0.5	1	NR	NR	NR	53	0.04-2
Possible Ingestion	NR	NR	NR	NR	1	0.002	3	0.1-2	NR	NR	103	0.6-4
Inhalation	1	NR	NR	NR	1	NR	NR	NR	NR	NR	6	0.03-0.5
Dermal Contact	33	1-17	NR	NR	14	0.00001-0.5	30	0.1-2	4	0.002-0.2	800	0.0005-93
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.01-0.5
Hair - Non-Coloring	NR	NR	2	NR	15	NR	NR	NR	NR	NR	97	0.0002-95
Hair - Coloring	NR	NR	3	NR	NR	NR	NR	NR	NR	NR	5	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	6	0.02-95
Mucous Membrane	31	1-4	NR	NR	3	NR	1	NR	NR	NR	70	0.01-52
Bath Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	19	0.1-78
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	21	2

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Glycine Soja (Soybean) Oil Unsaponifiables		**Hydrogenated Soybean Oil**		**Helianthus Annuus (Sunflower) Seed Oil**		**Helianthus Annuus (Sunflower) Seed Oil Unsaponifiables**		**Hydrogenated Sunflower Oil**		**Hippophae Rhamnoides Oil**	
Totals	**12**	**0.0001-0.2**	**36**	**0.001-42**	**1414**	**0.000007-96**	**10**	**0.005-2**	**NR**	**6-35**	**15**	**0.2-0.7**
Duration of Use												
Leave-On	*12*	*0.0001-0.2*	*33*	*0.001-39*	*1054*	*0.0002-96*	*10*	*0.005-2*	*NR*	*6-35*	*10*	*0.2-0.7*
Rinse-Off	*NR*	*NR*	*3*	*0.05-42*	*360*	*0.000007-92*	*NR*	*0.002*	*NR*	*15-35*	*5*	*0.2*
Exposure Type												
Eye Area	NR	NR	4	0.03-7	64	0.0005-19	2	0.02	NR	7	NR	NR
Possible Ingestion	NR	NR	3	0.1-39	260	0.08-41	NR	NR	NR	6	NR	NR
Inhalation	NR	NR	NR	NR	3	0.0002-85	NR	NR	NR	NR	NR	NR
Dermal Contact	12	0.0001-0.2	34	0.01-39	707	0.0002-96	10	0.005-2	NR	6-35	1	0.2-0.7
Deodorant (Underarm)	NR	NR	NR	NR	1	0.0003-4	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	1	0.1	179	0.000007-92	NR	NR	NR	NR	6	NR
Hair - Coloring	NR	NR	NR	NR	85	0.03-35	NR	NR	NR	15-35	NR	NR
Nail	NR	NR	NR	0.001-25	8	0.05-30	NR	NR	NR	NR	8	NR
Mucous Membrane	NR	NR	NR	0.05-6	52	0.0003-4	NR	0.002	NR	NR	1	0.2
Bath Products	NR	NR	NR	5-42	11	0.005-75	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	18	0.2	NR	NR	NR	NR	NR	NR

	Hippophae Rhamnoides Fruit Oil		**Irvingia Gabonensis Kernel Butter**		**Juglans Regia (Walnut) Seed Oil**		**Limnanthes Alba (Meadowfoam) Seed Oil**		**Linum Usitatissimum (Linseed) Seed Oil**		**Linseed Acid**	
Totals	**7**	**0.004-2**	**109**	**0.003-0.4**	**15**	**0.00003-0.2**	**316**	**0.002-74**	**102**	**0.001-10**	**3**	**NR**
Duration of Use												
Leave-On	*7*	*0.004-2*	*109*	*0.003-0.4*	*12*	*0.01-0.2*	*225*	*0.002-74*	*52*	*0.002-10*	*3*	*NR*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*3*	*0.00003-0.1*	*91*	*0.01-2*	*50*	*0.001-0.4*	*NR*	*NR*
Exposure Type												
Eye Area	1	NR	2	NR	1	NR	30	0.1-20	3	0.01	NR	NR
Possible Ingestion	NR	NR	64	0.003-0.3	NR	NR	67	0.6-26	NR	0.01	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	1	0.1-3	3	NR	NR	NR
Dermal Contact	6	2	108	0.003-0.4	15	0.003-0.2	211	0.002-74	58	0.003-4	3	NR
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.05-0.1	NR	NR
Hair - Non-Coloring	NR	NR	1	NR	NR	0.00003-0.1	47	0.1-1	42	0.001-0.1	NR	NR
Hair - Coloring	NR	NR	NR	NR	NR	NR	46	0.2-2	NR	NR	NR	NR
Nail	1	0.004	NR	NR	NR	NR	NR	0.5	2	0.002-0.05	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	4	0.001-0.6	5	0.003-0.4	NR	NR
Bath Products	NR	NR	NR	NR	2	NR	2	0.5-0.9	1	0.02-0.2	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	1	NR	2	NR	1	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Luffa Cylindrica Seed Oil		**Lupinus Albus Seed Oil**		**Lycium Barbarum Seed Oil**		**Macadamia Integrifolia Seed Oil**		**Macadamia Ternifolia Seed Oil**		**Macadamia Nut Oil****	
Totals	**21**	**0.01**	**1**	**NR**	**2**	**NR**	**41**	**0.00006-5**	**533**	**0.0003-30**	**208**	**NS**
Duration of Use												
Leave-On	*21*	*NR*	*1*	*NR*	*2*	*NR*	*25*	*0.00006-5*	*482*	*0.001-30*	*191*	*NS*
Rinse-Off	*NR*	*0.01*	*NR*	*NR*	*NR*	*NR*	*16*	*0.006-3*	*51*	*0.0003-10*	*17*	*NS*
Exposure Type												
Eye Area	1	NR	NR	NR	1	NR	3	0.1	16	0.1-15	22	NS
Possible Ingestion	9	NR	NR	NR	1	NR	4	1	33	0.1-30	11	NS
Inhalation	NR	NR	NR	NR	NR	NR	NR	0.5	12	0.007-16	2	NS
Dermal Contact	21	0.01	1	NR	2	NR	36	0.00006-5	493	0.001-30	170	NS
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NS
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	12	0.01-0.03	33	0.0003-16	9	NS
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	3	0.02	NR	NS
Nail	NR	NR	NR	NR	NR	NR	NR	3	1	0.001-0.5	NR	NS
Mucous Membrane	NR	0.01	NR	NR	NR	NR	10	2	12	0.02-10	NR	NS
Bath Products	NR	NR	NR	NR	NR	NR	1	0.5	2	1-10	1	NS
Baby Products	NR	NR	NR	RN	NR	NR	NR	NR	4	NR	NR	NS

	Mangifera Indica (Mango) Seed Oil		**Mangifera Indica (Mango) Seed Butter**		**Sodium Mangoseedate**		**Moringa Oleifera Seed Oil**		**Moringa Pterygosperma Seed Oil**		**Oenothera Biennis (Evening Primrose) Oil**	
Totals	**72**	**0.003-6**	**175**	**0.0005-3**	**1**	**NR**	**NR**	**0.001**	**15**	**0.003-3**	**150**	**0.00002-58**
Duration of Use												
Leave-On	*64*	*0.003-6*	*134*	*0.01-5*	*NR*	*NR*	*NR*	*0.001*	*13*	*0.004-3*	*113*	*0.00002-58*
Rinse-Off	*8*	*0.05-0.2*	*41*	*0.0005-0.5*	*1*	*NR*	*NR*	*NR*	*2*	*0.003*	*37*	*0.002-0.2*
Exposure Type												
Eye Area	13	5	6	0.02	NR	NR	NR	NR	4	3	4	0.00002-0.5
Possible Ingestion	7	0.03-6	25	1-5	NR	NR	NR	NR	1	NR	14	0.1-15
Inhalation	1	NR	2	0.02	NR	NR	NR	NR	NR	NR	2	NR
Dermal Contact	60	0.003-6	147	0.0005-5	1	NR	NR	0.001	11	0.003-3	109	0.00002-58
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.2
Hair - Non-Coloring	12	0.05-0.2	12	0.02-0.5	NR	NR	NR	NR	1	0.02	37	0.05-0.1
Hair - Coloring	NR	0.05	16	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.5	NR	NR	NR	NR	NR	NR	4	0.001-3
Mucous Membrane	2	0.1	10	0.0005-0.5	1	NR	NR	NR	NR	0.003	4	0.1-0.2
Bath Products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	2	0.2
Baby Products	NR	NR	3	NR	NR	NR	NR	NR	NR	NR	3	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Hydrogenated Evening Primrose Oil		Olea Europaea (Olive) Fruit Oil		Olea Europaea (Olive) Oil Unsaponifiables		Hydrogenated Olive Oil		Hydrogenated Olive Oil Unsaponifiables		Potassium Olivate	
Totals	**14**	**NR**	**915**	**0.0005-100**	**77**	**0.0001-3**	**50**	**0.0005-12**	**2**	**0.05-5**	**3**	**NR**
Duration of Use												
Leave-On	*14*	*NR*	*617*	*0.001-100*	*68*	*0.0001-3*	*36*	*0.1-12*	*2*	*0.05-5*	*NR*	*NR*
Rinse-Off	*NR*	*NR*	*298*	*0.0005-94*	*9*	*0.04-0.3*	*14*	*0.0005-0.1*	*NR*	*NR*	*3*	*NR*
Exposure Type												
Eye Area	1	NR	26	0.004-17	12	0.02-0.4	13	0.1-3	NR	0.3-2	NR	NR
Possible Ingestion	NR	NR	26	0.7-26	1	0.08	7	0.1-12	NR	NR	NR	NR
Inhalation	NR	NR	6	0.2-5	NR	3	NR	NR	NR	NR	NR	NR
Dermal Contact	14	NR	711	0.0005-100	67	0.0001-3	34	0.0005-12	2	0.05-5	3	NR
Deodorant (Underarm)	NR	NR	3	0.02-0.1	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	190	0.006-94	6	0.02-0.3	11	0.01-0.1	NR	NR	NR	NR
Hair - Coloring	NR	NR	NR	0.2-0.5	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	5	1-40	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	121	0.0005-3	4	NR	1	0.0005	NR	NR	1	NR
Bath Products	NR	NR	14	0.9-17	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	9	0.2	NR	0.04	NR	0.4	NR	NR	NR	NR

	Sodium Olivate		Orbignya Cohune Seed Oil		Orbignya Oleifera Seed Oil		Sodium Babassuate		Orbignya Speciosa Kernel Oil		Passiflora Edulis Seed Oil	
Totals	**16**	**4-18**	**1**	**NR**	**161**	**0.0009-27**	**NR**	**8**	**8**	**0.5-0.9**	**62**	**0.0007-3**
Duration of Use												
Leave-On	*5*	*NR*	*NR*	*NR*	*118*	*0.0009-4*	*NR*	*NR*	*1*	*0.9*	*53*	*0.003-5*
Rinse-Off	*11*	*4-18*	*1*	*NR*	*43*	*0.01-27*	*NR*	*8*	*7*	*0.5*	*9*	*0.0007-0.005*
Exposure Type												
Eye Area	NR	NR	NR	NR	7	0.5-0.6	NR	NR	NR	NR	3	0.8
Possible Ingestion	NR	NR	NR	NR	57	0.001-2	NR	NR	NR	NR	14	0.6-3
Inhalation	NR	NR	NR	NR	5	0.02-2	NR	NR	NR	NR	3	NR
Dermal Contact	16	4-18	NR	NR	110	0.0009-27	NR	8	NR	NR	49	0.003-3
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.003
Hair - Non-Coloring	NR	NR	1	NR	43	0.02-2	NR	NR	5	0.5-0.9	10	0.007-0.5
Hair - Coloring	NR	NR	NR	NR	8	NR	NR	NR	3	NR	3	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	9	4-18	NR	NR	5	27	NR	8	NR	NR	1	NR
Bath Products	NR	NR	NR	NR	2	0.01-0.1	NR	NR	NR	NR	NR	0.01-0.05
Baby Products	1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Perilla Ocymoides Seed Oil		**Persea Gratissima (Avocado) Oil Unsaponifiables**		**Hydrogenated Avocado Oil**		**Persea Gratissima (Avocado) Butter**		**Sodium Avocadoate**		**Pistacia Vera Seed Oil**	
Totals	**7**	**NR**	**63**	**0.2-6**	**11**	**0.5**	**15**	**NR**	**1**	**NR**	**158**	**0.003-1**
Duration of Use												
Leave-On	*5*	*NR*	*57*	*0.5-6*	*9*	*NR*	*15*	*NR*	*NR*	*NR*	*107*	*0.08-0.2*
Rinse-Off	*2*	*NR*	*6*	*0.2*	*2*	*0.5*	*NR*	*NR*	*1*	*NR*	*51*	*0.003-1*
Exposure Type												
Eye Area	2	NR	9	0.5	NR	NR	NR	NR	NR	NR	7	NR
Possible Ingestion	NR	NR	2	3	2	NR	11	NR	NR	NR	6	NR
Inhalation	NR	NR	4	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	5	NR	56	0.2-3	8	NR	15	NR	1	NR	133	0.003-0.2
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	2	NR	2	6	3	0.5	NR	NR	NR	NR	16	0.05-1
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	3	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR	1	NR	19	NR
Bath Products	NR	NR	4	NR	NR	NR	NR	NR	NR	NR	8	NR
Baby Products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	3	NR

	Plukenetia Volubilis Seed Oil		**Hydrogenated Sweet Almond Oil**		**Sodium Sweet Almondate**		**Prunus Armeniaca (Apricot) Kernel Oil**		**Hydrogenated Apricot Kernel Oil**		**Prunus Avium (Sweet Cherry) Seed Oil**	
Totals	**13**	**0.05-0.6**	**21**	**0.5**	**4**	**15**	**588**	**0.00001-89**	**2**	**NR**	**2**	**0.01-0.02**
Duration of Use												
Leave-On	*12*	*0.05-0.6*	*13*	*0.5*	*4*	*NR*	*449*	*0.0001-40*	*2*	*NR*	*NR*	*NR*
Rinse-Off	*1*	*NR*	*8*	*0.5*	*NR*	*15*	*139*	*0.00001-89*	*NR*	*NR*	*2*	*0.01-0.02*
Exposure Type												
Eye Area	1	NR	NR	NR	NR	NR	25	0.002-18	NR	NR	NR	NR
Possible Ingestion	3	0.6	1	NR	NR	NR	38	0.001-5	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	5	0.0009-1	NR	NR	NR	NR
Dermal Contact	13	0.6	15	0.5	4	15	486	0.00001-18	2	NR	2	0.01-0.02
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	1	0.003-0.1	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	6	0.5	NR	NR	78	0.0001-89	NR	NR	NR	NR
Hair - Coloring	NR	NR	NR	NR	NR	NR	10	0.1	NR	NR	NR	NR
Nail	NR	0.05	NR	NR	NR	NR	10	0.002-40	NR	NR	NR	NR
Mucous Membrane	NR	NR	1	NR	NR	15	24	0.01-9	NR	NR	2	0.01-0.02
Bath Products	NR	NR	1	NR	NR	NR	8	4	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	7	NR	NR	NR	NR	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Prunus Domestica Seed Oil		**Prunus Persica (Peach) Kernel Oil**		**Punica Granatum Seed Oil**		**Pyrus Malus (Apple) Seed Oil**		**Ribes Nigrum (Black Currant) Seed Oil**		**Rosa Canina Fruit Oil**	
Totals	**NR**	**0.04**	**22**	**0.003-22**	**46**	**0.001-1**	**8**	**NR**	**53**	**0.000001-0.3**	**121**	**0.001-19**
Duration of Use												
Leave-On	*NR*	*NR*	*16*	*0.05-22*	*44*	*0.001-1*	*8*	*NR*	*45*	*0.000001-0.3*	*106*	*0.001-19*
Rinse-Off	*NR*	*0.04*	*6*	*0.003-6*	*2*	*0.001-0.1*	*NR*	*NR*	*8*	*0.05*	*15*	*0.001-0.5*
Exposure Type												
Eye Area	NR	NR	NR	NR	2	NR	NR	NR	2	0.08	17	0.1-0.5
Possible Ingestion	NR	NR	NR	0.04-22	30	1	1	NR	7	0.03-0.1	7	0.001-2
Inhalation	NR	NR	NR	2	NR	NR	NR	NR	NR	NR	1	NR
Dermal Contact	NR	0.04	18	0.003-22	46	0.001-1	8	NR	43	0.000001-0.3	109	0.008-19
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	4	NR	NR	NR	NR	NR	5	NR	9	0.001-0.5
Hair - Coloring	NR	NR	NR	0.1	NR	0.1	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	0.001	NR	NR	5	0.2	1	0.1-2
Mucous Membrane	NR	NR	1	NR	2	0.001	NR	NR	2	NR	3	0.001
Bath Products	NR	NR	1	0.1-1	NR	NR	NR	NR	NR	NR	1	0.5
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Rubus Chamaemorus Seed Oil		Rubus Idaeus (Raspberry) Seed Oil		Schinziophyton Rautanenii Kernel Oil		Sclerocarya Birrea Seed Oil		Silybum Marianum Seed Oil		Solanum Lycopersicum (Tomato) Fruit Oil	
Totals	**3**	**0.1**	**10**	**0.1-5**	**6**	**NR**	**29**	**1**	**NR**	**0.5**	**NR**	**0.01-1**
Duration of Use												
Leave-On	*3*	*0.1*	*8*	*0.1-5*	*4*	*NR*	*23*	*1*	*NR*	*0.5*	*NR*	*0.001-1*
Rinse-Off	*NR*	*NR*	*2*	*NR*	*2*	*NR*	*6*	*1*	*NR*	*NR*	*NR*	*NR*
Exposure Type												
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.01
Possible Ingestion	NR	NR	1	NR	NR	NR	6	NR	NR	NR	NR	0.001
Inhalation	NR	NR	NR	NR	NR	NR	2	NR	NR	NR	NR	NR
Dermal Contact	3	0.1	8	0.1-5	3	NR	23	1	NR	0.5	NR	0.001-1
Deodorant (Underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	3	NR	6	1	NR	NR	NR	NR
Hair - Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	2	NR	NR	NR	2	NR	NR	NR	NR	NR
Bath Products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Solanum Lycopersicum (Tomato) Seed Oil		**Theobroma Cacao (Cocoa) Seed Butter**		**Theobroma Grandiflorum Seed Butter**		**Triticum Vulgare (Wheat) Germ Oil Unsaponifiables**		**Wheat Germ Acid**		**Vaccinium Macrocarpon (Cranberry) Seed Oil**	
Totals	**1**	**NR**	**442**	**0.000002-37**	**153**	**0.00005-7**	**17**	**0.2**	**16**	**NR**	**21**	**0.002-2**
Duration of Use												
Leave-On	*1*	*NR*	*367*	*0.000002-37*	*119*	*0.00005-7*	*17*	*0.2*	*3*	*NR*	*18*	*0.002-2*
Rinse-Off	*NR*	*NR*	*75*	*0.0001-2*	*34*	*0.001-1*	*NR*	*NR*	*13*	*NR*	*3*	*0.003-0.1*
Exposure Type												
Eye Area	NR	NR	11	0.0002-9	21	0.1-2	1	NR	NR	NR	2	NR
Possible Ingestion	NR	NR	33	37	49	7	NR	NR	NR	NR	NR	0.3
Inhalation	NR	NR	2	0.4	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	1	NR	417	0.000002-37	141	0.00005-7	17	0.2	NR	NR	17	0.002-2
Deodorant (Underarm)	NR	NR	NR	0.001-1	NR	0.1	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	24	0.01-2	9	0.001-1	NR	NR	16	NR	4	0.01-0.1
Hair - Coloring	NR	NR	NR	0.1	3	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.1-1	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	35	0.02-2	19	0.05-0.1	NR	NR	NR	NR	1	0.003-0.1
Bath Products	NR	NR	4	0.1-1	4	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	8	0.01	NR	NR	NR	NR	NR	NR	NR	NR

	Vaccinium Myrtillus Seed Oil		**Vaccinium Oxycoccos (Cranberry) Seed Oil****		**Vaccinium Vitis-Idaea Seed Oil**		**Vegetable (Olus) Oil**		**Hydrogenated Vegetable Oil**		**Vitis Vinifera (Grape) Seed Oil**	
Totals	**33**	**0.01-0.1**	**4**	**NS**	**9**	**NR**	**165**	**0.0005-31**	**457**	**0.0004-60**	**465**	**0.001-43**
Duration of Use												
Leave-On	*32*	*0.01-0.12*	*3*	*NS*	*9*	*NR*	*135*	*0.0005-11*	*439*	*0.0005-60*	*368*	*0.001-41*
Rinse-Off	*1*	*NR*	*1*	*NS*	*NR*	*NR*	*30*	*0.002-31*	*18*	*0.0004-8*	*97*	*0.001-43*
Exposure Type												
Eye Area	NR	NR	NR	NS	NR	NR	11	0.01-11	102	0.008-49	14	0.01-5
Possible Ingestion	29	0.01	NR	NS	NR	NR	74	0.03-11	216	0.8-60	34	0.03-7
Inhalation	NR	NR	NR	NS	NR	NR	1	0.0005-0.02	1	3	6	0.001-7
Dermal Contact	33	0.01-0.1	4	NS	1	NR	143	0.0005-31	450	0.005-60	401	0.001-41
Deodorant (Underarm)	NR	NR	NR	NS	NR	NR	NR	---	NR	NR	NR	0.001-0.2
Hair - Non-Coloring	NR	NR	NR	NS	NR	NR	2	0.02-2	2	0.0005-0.09	46	0.01-0.3
Hair - Coloring	NR	NR	NR	NS	NR	NR	18	---	NR	0.0004-1	10	43
Nail	NR	NR	NR	NS	8	NR	1	2	1	0.2	8	0.001-35
Mucous Membrane	NR	NR	NR	NS	NR	NR	1	0.03-2	2	2-4	21	0.001-7
Bath Products	NR	NR	NR	NS	NR	NR	2	0.002-0.02	NR	0.5	8	0.01-2
Baby Products	NR	NR	NR	NS	NR	NR	1	---	NR	NR	5	NR

Table 5a. Frequency and concentration of use according to duration and exposure. - ingredients not previously reviewed by the CIR (continued)

	No. of Uses	Conc of Use (%)	No. of Uses	Conc of Use (%)
	Hydrogenated Grapeseed Oil		**Sodium Grapeseedate**	
Totals	**7**	**0.3-0.5**	**4**	**NR**
Duration of Use				
Leave-On	*4*	*0.3-0.5*	*4*	*NR*
Rinse-Off	*3*	*0.5*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Possible Ingestion	1	0.5	NR	NR
Inhalation	NR	NR	NR	NR
Dermal Contact	5	0.5	NR	NR
Deodorant (Underarm)	NR	NR	NR	NR
Hair - Non-Coloring	1	NR	4	NR
Hair - Coloring	NR	NR	NR	NR
Nail	1	0.3	NR	NR
Mucous Membrane	1	NR	NR	NR
Bath Products	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR

*Note - Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses.

NR - not reported to the VCRP or Council

NS - not surveyed

**not listed as an INCI name; included because of similarity

Table 5b. **Current and historical frequency and concentration of use according to duration and type of exposure - previously reviewed ingredients**

	# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)	
	Arachis Hypogaea (Peanut) Oil				**Hydrogenated Peanut Oil**				**Carthamus Tinctorius (Safflower) Seed Oil**				**Cocos Nucifera (Coconut) Oil**			
data year	*1998*	*2010*	*1984*	*2010*	*1998*	*2010*	*1998*	*2010*	*2002*	*2010*	*2003*	*2010*	*2007*	*2010*	*2008*	*2010*
Totals*	**22**	**74**	**mostly ≤25; >50 (1 use)**	**0.0001-30**	**19**	**12**	******	**2-5**	**142**	**508**	**0.00005-84**	**NS**	**626**	**798**	**0.0001-80**	**NS**
Duration of Use																
Leave-On	*14*	*59*	**	*0.0001-1*	*19*	*12*	**	*2-5*	*114*	*402*	*0.00005-84*	*NS*	*243*	*409*	*0.005-80*	*NS*
Rinse Off	*8*	*15*	**	*0.0002-30*	*NR*	*NR*	**	*NR*	*28*	*106*	*0.001-72*	*NS*	*383*	*389*	*0.0001-16*	*NS*
Exposure Type																***NS***
Eye Area	NR	4	**	NR	NR	NR	**	NR	5	15	1-6	NS	7	25	0.01-80	NS
Possible Ingestion	3	NR	**	NR	NR	NR	**	2	18	83	0.1-60	NS	19	44	0.2-51	NS
Inhalation	NR	2	**	NR	NR	NR	**	NR	3	5	5	NS	7	10	0.01-26	NS
Dermal Contact	19	53	**	0.0001-1	19	12	**	2-5	113	395	0.001-72	NS	380	548	0.005-80	NS
Deodorant (underarm)	NR	NR	**	NR	NR	NR	**	NR	NR	NR	NR	NS	NR	NR	0.1-16	NS
Hair - Non-Coloring	3	21	**	25-30	NR	NR	**	NR	28	79	0.00005-27	NS	97	176	0.0001-13	NS
Hair-Coloring	NR	NR	**	NR	NR	NR	**	NR	NR	20	1	NS	145	69	NR	NS
Nail	NR	NR	**	NR	NR	NR	**	NR	1	32	84	NS	2	5	0.005-2	NS
Mucous Membrane	4	2	**	NR	NR	NR	**	NR	NR	31	NR	NS	12	161	0.0005-16	NS
Bath Products	NR	NR	**	NR	NR	NR	**	NR	NR	3	7	NS	141	15	0.004-23	NS
Baby Products	NR	NR	**	NR	NR	NR	**	NR	NR	6	10	NS	12	15	0.010-0.3	NS

	Hydrogenated Coconut Oil				**Magnesium Cocoate**				**Potassium Cocoate**				**Sodium Cocoate**			
data year	*2007*	*2010*	*2008*	*2010*	*2007*	*2010*	*2008*	*2010*	*2007*	*2010*	*2008*	*2010*	*2007*	*2010*	*2008*	*2010*
Totals	**62**	**105**	**0.001-50**	**NS**	**11**	**9**	**NR**	**NS**	**24**	**40**	**0.003-40**	**NS**	**230**	**340**	**1-52**	**NS**
Duration of Use																
Leave-On	*55*	*79*	*0.001-50*	*NS*	*NR*	*NR*	*NR*	*NS*	*4*	*NR*	*28*	*NS*	*12*	*16*	*NR*	*NS*
Rinse-Off	*7*	*26*	*0.001-38*	*NS*	*11*	*9*	*NR*	*NS*	*20*	*40*	*0.03-40*	*NS*	*218*	*324*	*1-52*	*NS*
Exposure Type																
Eye Area	9	7	0.2-22	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Possible Ingestion	6	10	0.7-29	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Inhalation	NR	NR	0.3	NS	NR	NR	NR	NS	NR	NR	NR	NS	1	NR	NR	NS
Dermal Contact	3	102	0.001-25	NS	11	9	NR	NS	22	38	0.3-40	NS	175	269	1-52	NS
Deodorant (underarm)	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Hair - Non-Coloring	3	3	0.001-2	NS	NR	NR	NR	NS	2	2	15	NS	55	71	2	NS
Hair-Coloring	NR	NR	0.5-0.6	NS	NR	NR	NR	NS	NR	NR	0.003	NS	NR	NR	NR	NS
Nail	NR	NR	0.8-25	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Mucous Membrane	NR	18	1-17	NS	NR	NR	NR	NS	NR	8	0.3	NS	1	238	1-2	NS
Bath Products	1	NR	0.5-39	NS	NR	NR	NR	NS	11	NR	0.3-40	NS	149	3	1-52	NS
Baby Products	1	1	2-50	NS	NR	NR	NR	NS	NR	NR	NR	NS	2	5	NR	NS

Table 5b. Current and historical frequency and concentration of use according to duration and type of exposure - previously reviewed ingredients (continued)

	# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)	
	Coconut Acid				**Hydrogenated Coconut Acid**				**Corylus Americana (Hazel) Seed Oil**				**Corylus Avellana (Hazel) Seed Oil**			
data year	*2007*	*2010*	*2008*	*2010*	*2007*	*2010*	*2008*	*2010*	*1998#*	*2010*	*1998*	*2010*	*1998#*	*2010*	*1997*	*2010*
Totals	**142**	**141**	**0.03-14**	**NS**	**NR**	**NR**	**6-10**	**NS**	**#**	**10**	******	**NR**	**85**	**150**	**≤100**	**0.005-98**
Duration of Use																
Leave-On	*18*	*17*	*NR*	*NS*	*NR*	*NR*	*6*	*NS*	*#*	*9*	**	*NR*	*74*	*131*	**	*0.005-98*
Rinse Off	*124*	*124*	*0.03-14*	*NS*	*NR*	*NR*	*10*	*NS*	*#*	*1*	**	*NR*	*11*	*19*	**	*0.005-5*
Exposure Type				***NS***				***NS***								
Eye Area	1	1	NR	NS	NR	NR	NR	NS	#	NR	**	NR	2	9	**	0.1
Possible Ingestion	NR	NR	NR	NS	NR	NR	NR	NS	#	NR	**	NR	NR	NR	**	14
Inhalation	NR	NR	NR	NS	NR	NR	NR	NS	#	NR	**	NR	NR	2	**	NR
Dermal Contact	140	140	0.04-14	NS	NR	NR	6-10	NS	#	10	**	NR	83	147	**	0.005-98
Deodorant (underarm)	NR	NR	NR	NS	NR	NR	NR	NS	#	NR	**	NR	NR	NR	**	NR
Hair - Non-Coloring	2	1	0.03-0.3	NS	NR	NR	NR	NS	#	NR	**	NR	1	2	**	NR
Hair-Coloring	NR	NR	NR	NS	NR	NR	NR	NS	#	NR	**	NR	NR	NR	**	NR
Nail	NR	NR	NR	NS	NR	NR	NR	NS	#	NR	**	NR	1	1	**	NR
Mucous Membrane	1	101	0.04-2	NS	NR	NR	NR	NS	#	1	**	NR	4	1	**	NR
Bath Products	93	NR	0.04-14	NS	NR	NR	NR	NS	#	NR	**	NR	2	2	**	NR
Baby Products	1	1	NR	NS	NR	NR	NR	NS	#	NR	**	NR	NR	1	**	NR

	Elaeis Guineensis (Palm) Oil				**Elaeis Guineensis (Palm) Kernel Oil**				**Hydrogenated Palm Kernel Oil**				**Hydrogenated Palm Oil**			
data year	*1997*	*2010*	*1997*	*2010*	*1997*	*2010*	*1997*	*2010*	*1997*	*2010*	*1997*	*2010*	*1997*	*2010*	*1997*	*2010*
Totals	**36**	**272**	******	**0.002-48**	**11**	**77**	******	**0.05-23**	**29**	**47**	******	**0.4-13**	**13**	**152**	******	**0.2-30**
Duration of Use																
Leave-On	*28*	*171*	**	*0.008-13*	*9*	*60*	**	*0.8-3*	*27*	*45*	**	*0.4-13*	*13*	*134*	**	*0.2-30*
Rinse-Off	*8*	*101*	**	*0.002-48*	*2*	*17*	**	*0.05-23*	*2*	*2*	**	*0.6-2*	*NR*	*18*	**	*2*
Exposure Type																
Eye Area	NR	12	**	0.04-2	NR	10	**	0.8	5	2	**	2-10	1	61	**	0.2-30
Possible Ingestion	NR	11	**	2	NR	6	**	NR	2	5	**	3-13	3	12	**	2-30
Inhalation	1	3	**	NR	NR	NR	**	NR	NR	1	**	NR	NR	NR	**	1
Dermal Contact	36	229	**	0.002-48	11	71	**	0.05-2	24	47	**	0.4-13	12	123	**	0.4-30
Deodorant (underarm)	NR	NR	**	NR	NR	NR	**	NR	NR	NR	**	NR	NR	NR	**	NR
Hair - Non-Coloring	NR	43	**	2-34	NR	6	**	0.9-23	NR	NR	**	NR	NR	NR	**	NR
Hair-Coloring	NR	NR	**	NR	NR	NR	**	NR	NR	NR	**	NR	NR	NR	**	NR
Nail	NR	NR	**	NR	NR	NR	**	3	NR	NR	**	NR	NR	NR	**	NR
Mucous Membrane	7	68	**	0.002-48	NR	10	**	0.05	2	2	**	0.9-2	NR	17	**	2
Bath Products	NR	NR	**	NR	NR	1	**	NR	NR	NR	**	NR	NR	NR	**	NR
Baby Products	1	2	**	NR	NR	NR	**	NR	NR	NR	**	NR	NR	NR	**	NR

Table 5b. Current and historical frequency and concentration of use according to duration and type of exposure - previously reviewed ingredients (continued)

	# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)		# of Uses		Conc. of Use (%)	
	Gossypium Herbaceum (Cotton) Seed Oil				**Hydrogenated Cottonseed Oil**				**Oryza Sativa (Rice) Bran Oil**				**Oryza Sativa (Rice) Germ Oil**			
data year	*1998*	*2010*	*1998*	*2010*	*1998*	*2010*	*1998*	*2010*	*2002*	*2010*	*2000-2003*	*2010*	*2002*	*2010*	*2000-2003*	*2010*
Totals	**4**	**83**		**0.004-32**	**272**	**362**	**	**0.001-24**	**39**	**371**	**0.1-39**	**0.0003-78**	**6**	**34**	**0.1**	**0.003-3**
Duration of Use																
Leave-On	*1*	*68*		*0.08-32*	*272*	*358*	**	*0.001-24*	*32*	*267*	*0.1-8*	*0.0003-78*	*5*	*29*	*0.1*	*0.003-3*
Rinse Off	*3*	*15*	**	*0.004-29*	*NR*	*4*	**	*0.01-0.1*	*7*	*104*	*0.2-39*	*0.005-6*	*1*	*5*	*NR*	*0.003-3*
Exposure Type																
Eye Area	NR	4		0.1-11	116	155	**	0.5-24	NR	5	0.1-1	0.5-0.8	NR	2	NR	0.01-1
Possible Ingestion	NR	9	**	0.2-1	151	NR	**	8-12	NR	17	0.1-1	0.1-8	NR	4	NR	0.1-3
Inhalation	NR	12	**	0.2	NR	NR	**	NR	NR	11	NR	0.1	NR	NR	NR	NR
Dermal Contact	4	78	**	0.004-29	156	356	**	0.001-24	36	321	0.1-39	0.0003-27	6	32	0.1	0.003-3
Deodorant (underarm)	NR	1	**	0.2	NR	NR	**	NR	NR	NR	NR	0.5	NR	NR	NR	0.003
Hair - Non-Coloring	NR	2	**	NR	NR	4	**	0.01-0.1	3	42	0.3	0.005-0.5	NR	NR	NR	NR
Hair-Coloring	NR	NR	**	NR	NR	NR	**	NR	NR	NR	NR	0.3	NR	NR	NR	NR
Nail	NR	1	**	0.5-32	NR	NR	**	NR	2	5	NR	0.02-78	NR	NR	NR	NR
Mucous Membrane	NR	7	**	0.004-0.01	NR	NR	**	NR	NR	48	1	0.0006-6	NR	1	NR	0.003-0.005
Bath Products	NR	NR	**	NR	NR	NR	**	NR	1	17	1-39	0.2	NR	1	NR	0.5
Baby Products	NR	NR	**	NR	NR	8	**	NR	NR	1	NR	NR	NR	NR	NR	NR

	Persea Gratissima (Avocado) Oil				**Prunus Amygdalus Dulcis (Sweet Almond) Oil**				**Sesamum Indicum (Sesame) Seed Oil**				**Sesamum Indicum (Sesame) Oil Unsaponifiables**			
data year	*2001*	*2010*	*2001*	*2010*	*2002*	*2010*	*2002*	*2010*	*2009*	*2010*	*2008*	*2010*	*2009*	*2010*	*2008*	*2010*
Totals	**188**	**883**	**0.001-23**	**0.0001-98**	**375**	**1127**	**0.004-76**	**0.0001-77**	**402**	**480**	**0.0001-73**	**NS**	**6**	**17**	**0.01-0.03**	**NS**
Duration of Use																
Leave-On	*40*	*657*	*0.001-23*	*0.0005-98*	*302*	*791*	*0.004-76*	*0.001-77*	*313*	*374*	*0.0001-73*	*NS*	*NR*	*17*	*0.01-0.03*	*NS*
Rinse-Off	*148*	*226*	*0.1-5*	*0.0001-15*	*73*	*336*	*0.01-2*	*0.0001-43*	*89*	*106*	*0.001-68*	*NS*	*NR*	*NR*	*NR*	*NS*
Exposure Type																
Eye Area	8	24	0.1-3	0.05-2	6	28	0.4	0.1-22	11	14	0.0008-10	NS	NR	NR	0.01	NS
Possible Ingestion	29	60	0.7-21	0.05-26	3	55	0.5	0.1-19	57	52	0.1-16	NS	NR	11	0.03	NS
Inhalation	2	11	0.02-3	0.01-8	3	18	1-3	0.5-39	5	5	2	NS	NR	NR	NR	NS
Dermal Contact	165	685	0.001-23	0.0005--98	323	986	0.04-11	0.001-46	346	414	0.0008-73	NS	6	17	0.01-0.03	NS
Deodorant (underarm)	NR	NR	NR	0.1	NR	2	0.004	0.02-1	NR	NR	NR	NS	NR	NR	NR	NS
Hair - Non-Coloring	11	189	0.002-3	0.0001-41	46	116	0.3-3	0.001-19	50	59	0.0001-30[a]	NS	NR	NR	NR	NS
Hair-Coloring	8	NR	NR	0.3	2	2	0.1	0.02	NR	NR	0.03-0.8[b]	NS	NR	NR	NR	NS
Nail	4	7	0.4-19	0.001-34	4	13	1-76	0.001-77	6	7	≤1-10	NS	NR	NR	NR	NS
Mucous Membrane	NR	43	0.1-5	0.002-3	19	93	0.5	<0.1-23	4	28	NR	NS	NR	NR	NR	NS
Bath Products	5	25	0.1-5	0.6-6	10	41	0.01-0.1	0.1-43	27	5	0.09-68	NS	NR	NR	NR	NS
Baby Products	NR	9	NR	NR	7	14	NR	2-3	1	3	6	NS	NR	NR	NR	NS

Table 5b. Current and historical frequency and concentration of use according to duration and type of exposure - previously reviewed ingredients (continued)

	# of Uses		*Conc. of Use (%)*		*# of Uses*		*Conc. of Use (%)*		*# of Uses*		*Conc. of Use (%)*		*# of Uses*		*Conc. of Use (%)*	
	Triticum Vulgare (Wheat) Germ Oil				**Zea Mays (Corn) Oil**				**Zea Mays (Corn) Oil Unsaponifiables**				**Zea Mays (Corn) Germ Oil**			
data year	*2001*	*2010*	*2001*	*2010*	*2007*	*2010*	*2006*	*2010*	*2007*	*2010*	*2006*	*2010*	*2007*	*2010*	*2006*	*2010*
Totals	**303**	**527**	**0.00002-18**	**0.0001-28**	**498**	**598**	**0.00003-14**	**NS**	**7**	**1**	**NR**	**NS**	**37**	**53**	**0.2-25**	**NS**
Duration of Use																
Leave-On	*80*	*373*	*0.00002-18*	*0.0001-28*	*241*	*361*	*0.00003-14*	*NS*	*6*	*1*	*NR*	*NS*	*25*	*34*	*3-25*	*NS*
Rinse Off	*223*	*154*	*0.00002-5*	*0.001-2*	*257*	*237*	*0.001-0.07*	*NS*	*1*	*NR*	*NR*	*NS*	*12*	*19*	*0.2-3*	*NS*
Exposure Type																
Eye Area	9	12	0.00004-3	0.0001-0.5	39	35	0.0008-0.2	NS	NR	NR	NR	NS	NR	NR	NR	NS
Possible Ingestion	33	29	0.1-3	0.3-5	29	30	0.003-10	NS	NR	NR	NR	NS	NR	NR	NR	NS
Inhalation	2	7	0.0002-0.01	0.0001-0.0005	1	1	0.001-0.1	NS	NR	NR	NR	NS	NR	NR	NR	NS
Dermal Contact	220	360	0.00002-18	0.0005-23	276	371	0.00003-14	NS	7	1	NR	NS	31	50	3-25	NS
Deodorant (underarm)	NR	NR	0.02	NR	1	4	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Hair - Non-Coloring	63	142	0.0001-2	0.0001-<1	38	40	0.0001-0.02	NS	NR	NR	NR	NS	4	3	0.2	NS
Hair-Coloring	12	20	0.1	0.01-0.2	182	183	0.004-0.007	NS	NR	NR	NR	NS	NR	NR	NR	NS
Nail	4	2	0.1-4	0.1-28	1	3	0.001-5	NS	NR	NR	NR	NS	NR	NR	NR	NS
Mucous Membrane	3	22	0.02-1	0.01-0.5	2	2	0.004-0.01	NS	NR	NR	NR	NS	4	3	3	NS
Bath Products	1	2	0.001-2	0.5	NR	NR	0.001-0.01	NS	NR	NR	NR	NS	3	4	NR	NS
Baby Products	1	9	0.5	NR	8	8	0.004	NS	NR	NR	NR	NS	2	4	NR	NS

*Note - Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

NR - not reported to the VCRP or the Council

NS - not surveyed; ingredients that were recently reviewed were not resurveyed for concentration of use

** concentration of use data were not given in the original report

- was not distinguished whether C. Americana or C. Avellana was reported; arbitrarily reported under C. Avellana (Hazel) Seed Oil for this table

[a] 15% after dilution.

[b] 0.4 after dilution.

Table 5c**. Ingredients with no reported use concentrations or uses.**

Adansonia Digitata Seed Oil
Aleurites Moluccanus Bakoly Seed Oil
Amaranthus Hypochondriacus Seed Oil
Arctium Lappa Seed Oil
Babassu Acid
Bassia Butyracea Seed Butter
Brassica Campestris (Rapeseed) Oil Unsaponifiables
Brassica Napus Seed Oil
Brassica Oleracea Acephala Seed Oil
Canarium Indicum Seed Oil
Carya Illinoensis (Pecan) Seed Oil
Citrus Aurantifolia (Lime) Seed Oil
Citrus Aurantifolia (Lime) Seed Oil Unsaponifiables
Citrus Aurantium Dulcis (Orange) Seed Oil
Citrus Aurantium Dulcis (Orange) Seed Oil Unsaponifiables
Citrus Grandis (Grapefruit) Seed Oil
Citrus Grandis (Grapefruit) Seed Oil Unsaponifiables
Cocos Nucifera (Coconut) Seed Butter
Coix Lacryma-Jobi (Job's Tears) Seed Oil
Corn Acid
Cottonseed Acid
Cynara Cardunculus Seed Oil
Elaeis (Palm) Fruit Oil
Elaeis Guineensis (Palm) Butter
Fragaria Ananassa (Strawberry) Seed Oil
Fragaria Chiloensis (Strawberry) Seed Oil
Fragaria Vesca (Strawberry) Seed Oil
Fragaria Virginiana (Strawberry) Seed Oil
Guizotia Abyssinica Seed Oil
Hippophae Rhamnoides Seed Oil
Hydrogenated Adansonia Digitata Seed Oil
Hydrogenated Apricot Kernel Oil Unsaponifiables
Hydrogenated Argania Spinosa Kernel Oil
Hydrogenated Black Currant Seed Oil
Hydrogenated Camelina Sativa Seed Oil
Hydrogenated Cranberry Seed Oil
Hydrogenated Grapefruit Seed Oil
Hydrogenated Grapefruit Seed Oil Unsaponifiables
Hydrogenated Hazelnut Oil
Hydrogenated Kukui Nut Oil
Hydrogenated Lime Seed Oil
Hydrogenated Lime Seed Oil Unsaponifiables
Hydrogenated Macadamia Seed Oil
Hydrogenated Meadowfoam Seed Oil
Hydrogenated Orange Seed Oil
Hydrogenated Orange Seed Oil Unsaponifiables
Hydrogenated Palm Acid
Hydrogenated Passiflora Edulis Seed Oil
Hydrogenated Peach Kernel Oil
Hydrogenated Pistachio Seed Oil
Hydrogenated Pumpkin Seed Oil
Hydrogenated Punica Granatum Seed Oil
Hydrogenated Raspberry Seed Oil
Hydrogenated Rice Bran Oil
Hydrogenated Rosa Canina Fruit Oil
Hydrogenated Safflower Seed Oil
Hydrogenated Sesame Seed Oil
Hydrogenated Sweet Almond Oil Unsaponifiables
Hydrogenated Wheat Germ Oil
Hydrogenated Wheat Germ Oil Unsaponifiables
Lupinus Albus Oil Unsaponifiables
Morinda Citrifolia Seed Oil
Olea Europaea (Olive) Husk Oil
Olive Acid
Oryza Sativa (Rice) Seed Oil
Peanut Acid
Potassium Babassuate
Potassium Cornate
Potassium Hydrogenated Cocoate
Potassium Hydrogenated Palmate
Potassium Peanutate
Potassium Rapeseedate
Potassium Safflowerate
Potassium Soyate
Prunus Amygdalus Dulcis (Sweet Almond) Oil Unsaponifiables
Prunus Armeniaca (Apricot) Kernel Oil Unsaponifiables
Rapeseed Acid
Ribes Rubrum (Currant) Seed Oil
Rice Bran Acid
Safflower Acid
Sesamum Indicum (Sesame) Seed Butter
Sodium Cocoa Butterate
Sodium Hydrogenated Cocoate
Sodium Hydrogenated Palmate
Sodium Macadamiaseedate
Sodium Peanutate
Sodium Rapeseedate
Sodium Safflowerate
Sodium Sesameseedate
Sodium Soyate
Sodium Theobroma Grandiflorum Seedate
Soy Acid
Sunflower Seed Acid
Torreya Nucifera Seed Oil
Triticum Aestivum (Wheat) Germ Oil
Triticum Vulgare (Wheat) Germ Oil Unsaponifiables
Vaccinium Corymbosum (Blueberry) Seed Oil

Table 6. **Examples of non-cosmetic uses of oils.**

Oil	**Use**[6,112,204-209]
Aleurities Moluccana Seed Oil [Kukui]	wood preservative, varnishes, paint oil, illumination, soap making, waterproofing paper, rubber substitute, insulating material
Arachis Hypogaea (Peanut) Oil	pharmaceutical, soap making, lubricants, emulsions for insect control, diesel engine fuel
Brassica Napus Seed Oil [Rapeseed]/Canola Oil	rubber additive · lubricants · fat liquoring of leather · varnishes and lacquers · textile chemicals · detergent additives · plasticizers · weed control
Butyrospermum Parkii (Shea) Oil	illumination
Camelina Sativa Seed Oil [False Flax]	drying oil · manufacturing of varnishes and paints
Citrullus Lanatus (Watermelon) Seed Oil	illumination
Cocos Nucifera (Coconut) Oil	lubricants, hydraulic fluid, paints, synthetic rubber, plastics, illumination
Elaeis Guineensis (Palm) Oil	crayon and candle manufacturing · tin plate industry
Elaeis Guineensis (Palm) Kernel Oil	detergent production · pharmaceutical · crayon and candle manufacturing · tin plate industry
Garcinia Indica Seed Butter [Kokum]	candle and soap making, sizing of cotton yarn, pharmaceutical
Guizotia Abyssinica Seed Oil [Niger/Ramtil]	paint · lubricant · pharmaceutical
Helianthus Annuus (Sunflower) Seed Oil	manufacturing of lacquers, copolymers, polyester films, modified resins, plasticizers, alkyl resins, other similar products
Juglans Regia (Walnut) Seed Oil	paints, soap making
Linum Usitatissimum (Linseed) Seed Oil	manufacturing of linoleum, cloth oil, printing and lithographic inks, core oils, linings, packings, oil-modified alkyd resins, caulking compounds, putties, leather-finishing compounds, lubricants, greases, polishes, pyrotechnic compositions · pigment binder in petrochemicals · concrete protector · stabilizer/plasticizer for vinyl plastics · industrial stains · jute textiles · drying oil in paints and varnishes
Mangifera Indica (Mango) Seed Butter	substitute for cocoa butter
Olea Europaea (Olive) Fruit Oil	textile industry · pharmaceutical
Orbignya Cohune Seed Oil	manufacturing of soaps, candles, and nightlights · cotton dyeing · ointment base · substitute for cocoa butter in food
Perilla Ocymoides Seed Oil [Perilla]	substitute for linseed oil in the manufacture of paints, varnishes, linoleum, oilclothes, and printing inks
Prunus Amygdalus Dulcis (Sweet Almond) Oil	pharmaceutical, energy source
Prunus Armeniaca (Apricot) Kernel Oil	pharmaceutical
Theobroma Cacao (Cocoa) Seed Butter	pharmaceutical
Vitis Vinifera (Grape) Seed Oil	substitute for linseed oil in the manufacture of paints, and varnishes

Table 7a. **Dermal effects – Non-Human studies**

Ingredient	Concentration	Animals	Procedure	Results	Reference
			Adansonia Digitata Seed Oil		
Adansonia Digitata (Baobab) Oil	100%		MatTek EpiDerm MTT viability assay; 100 µl of test material for 1-24 h	classified as non-irritating	210
			Arachis Hypogaea (Peanut) Oil		
Arachis Hypogaea (Peanut) Oil		Hartley and/or Himalayan guinea pigs	Single drops of a store-bought peanut oil were applied to clipped skin on the backs of 4 guinea pigs. Applications were made at 2-6 wk intervals, for a total of 7 applications over a 5-mo period. It appears that the test sites were not covered. The test sites were scored 24 h after application. Well-defined erythema was considered a positive reaction.	None of the animals had a positive reaction following the initial application. Two animals had positive reactions following application at wks 6 and 12, while one animal had a positive reaction following dosing at wk 12 only	17
			Butyrospermum Parkii (Shea) Butter		
Butyrospermum Parkii (Shea) Butter	not specified	3 male New Zealand White (NZW) rabbits	0.5 ml applied to the shaved dorso-lumbar region under an occlusive patch for 4 h	very slight erythema with or without edema was observed in 2 rabbits; resolved by day 3 or 4	211
Butyrospermum Parkii (Shea) Butter	induction: 75% challenge: 20 and 50%	10 female albino Hartley/Dunkin guinea pigs	maximization study with Freund's complete adjuvant (FCA) during induction	no evidence of delayed hypersensitivity	212
			Crambe Abyssinica Seed Oil		
Crambe Abyssinica Seed Oil	undiluted		dermal irritation study; details not provided	not a dermal irritant	213
			Hippophae Rhamnoides Seed Oil		
Hippophae Rhamnoides Seed Oil		albino rabbits, number not specified	0.5 ml applied under an occlusive patch for 4 h	no irritation	214
			Olea Europaea (Olive) Fruit Oil		
Olea Europaea (Olive) Fruit Oil		12 Harley and/or Himalayan guinea pigs	Single drops of a USP-grade olive oil that had been stored in its original metal container for 10 yrs were applied to a clipped area on the backs of 12 guinea pigs. (The composition of the oil was not determined.) Applications were made at 2-6 wk intervals over a period of 5 mos. Four guinea pigs were treated similarly using store-bought virgin olive oil.	None of the animals had a positive reaction following the initial application of either oil. With 10-yr-old olive oil, 11/12 of the animals had a positive reaction at some point. Some, but not all, of these guinea pigs reacted consistently following the first positive reaction; 2 animals had only 1 positive reaction; 2 guinea pigs in this group died by wk 16. In the group dosed with virgin olive oil, 1 animal had a positive reaction at wk 2 and 1 animal had a positive reaction at wks 4 and 6	215
		22 guinea pigs sensitive to the 10-yr-old USP olive oil	cross-reactivity to store-bought olive oil, another store-bought olive oil (not specified as virgin olive oil), corn oil, and peanut oil was determined. The 5 oils were applied simultaneously to the backs of the guinea pigs	18 of the animals reacted to the virgin olive oil, and 18 reacted to the other store-bought olive oil. (Overlap of these animals was not complete.) Cross-reactivity to corn or peanut oil was not observed.	
		8 sensitized and 4 non-sensitized guinea pigs	single drops of the unsaponifiable fraction of the 10-yr-old oil were applied	All of the sensitized animals reacted to the unsaponifiable fraction, while the non-sensitized animals did not.	
			Zea Mays (Corn) Oil		
corn oil, store-bought		6 Hartley and/or Himalayan guinea pigs	sensitization study, details not specified	0 of the animals had a positive reaction following the initial application; 2 animals had positive reactions following application at wks 4 and 6, while 1 animal had a positive reaction following application at wk 12	215

Table 7a. **Dermal effects – Non-Human studies**

Ingredient	Concentration	Animals	Procedure	Results	Reference
			PHOTOTOXICITY		
			Butyrospermum Parkii (Shea) Butter		
Butyrospermum Parkii (Shea) Butter	10 and 20% in acetone	10 Pirbright white guinea pigs	animals were treated with test compound, then irradiated with UV-B light for 80 seconds followed by UV-A light for 80 min	not phototoxic	216

Table 7b. Dermal effects – Non-Human studies- – summarized from previous CIR reports

Ingredient	Concentration	Animals	Procedure	Results	Reference
Arachis Hypogaea (Peanut) Oil					
Undiluted technical grade Arachis Hypogaea (Peanut) Oil was moderately irritating to rabbits and guinea pig skin and mildly irritating to rat skin following exposure; there was no indication that the test site was occluded. However, in a 48 h occlusive patch test using miniature swine, technical grade Arachis Hypogaea (Peanut) Oil was not irritating					17
Carthamus Tinctorius (Safflower) Oil					
Undiluted Carthamus Tinctorius (Safflower) Seed Oil was minimally irritating in a repeat open patch test using rabbits and was not a primary irritant or sensitizer in a maximization study using guinea pigs.					32
Cocos Nucifera (Coconut) Oil					
Undiluted Cocos Nucifera (Coconut) Oil was non- irritating to rabbit skin. In guinea pigs, undiluted Cocos Nucifera (Coconut) Oil was not a sensitizer in a Magnusson-Kligman maximization study.					33
Hydrogenated Coconut Oil					
Undiluted hydrogenated coconut oil was non-irritating to rabbit skin. In guinea pigs, undiluted hydrogenated coconut oil was not a sensitizer in a Buehler test.					33
Coconut Acid					
Undiluted coconut acid was minimally irritating to rabbit skin.					33
Sodium Cocoate					
In single-insult occlusive patch tests of a 5% aq. solution of a bar soap containing 13% sodium cocoate, scores of 1.6-4.0/8.0 were reported.					33
Elaeis Guineensis (Palm) Oil					
Undiluted Elaeis Guineensis (Palm) Oil was practically non- to minimally irritating to rabbit skin. Elaeis Guineensis (Palm) Oil, 5%, was non-allergenic in a maximization study.					26
Gossypium Herbaceum (Cotton) Seed Oil					
Cosmetic formulations containing 3.4-8.97% hydrogenated cottonseed oil were not irritating to rabbit skin.					27
Oryza Sativa (Rice) Bran Oil					
Undiluted Oryza Sativa (Rice) Bran Oil was not irritating to rabbits, and in a guinea pig maximization study, no reactions were observed when 5% was used at induction and 25% and 50% Oryza Sativa (Rice) Bran Oil were used at challenge. An Oryza Sativa (Rice) Bran Oil /Oryza Sativa (Rice) Germ Oil mixture, concentrations not stated, did not cause a contact allergy response. Undiluted hydrolyzed rice protein was also not irritating or sensitizing.					28
Oryza Sativa (Rice) Germ Oil					
Oryza Sativa (Rice) Germ Oil was not a primary dermal irritant.					28
Prunus Amygdalus Dulcis (Sweet Almond) Oil					
Undiluted Prunus Amygdalus Dulcis (Sweet Almond) Oil and two moisturizer formulations, each containing 25% Prunus Amygdalus Dulcis (Sweet Almond) Oil, were tested for skin irritancy in rabbits using occlusive patches. Undiluted Prunus Amygdalus Dulcis (Sweet Almond) Oil was nonirritating (PII = 0/4). The formulations containing 25% Prunus Amygdalus Dulcis (Sweet Almond) Oil were minimally irritating (PIIs = 0.28 and 0.72, respectively). In a 60-day cumulative irritation test, 10 and 100% Prunus Amygdalus Dulcis (Sweet Almond) Oil was applied to rabbits. When tested in 7 separate trials, 100% Prunus Amygdalus Dulcis (Sweet Almond) Oil produced mean maximum irritation indices (MMIIs) ranging from 0.34 to 1.34 (maximum score = 8). At a concentration of 10%, MMIIs for this ingredient ranged from 0-0.66. Results indicated that, when applied to the skin over a long period of time, Prunus Amygdalus Dulcis (Sweet Almond) Oil is slightly irritating; whereas, at 10% it is practically nonirritating. A maximization assay was used to determine the sensitizing potential of Prunus Amygdalus Dulcis (Sweet Almond) Oil. using guinea pigs. Intradermal induction used concentrations of 5% Amygdalus Dulcis (Sweet Almond) Oil, the dose-range phase of the experiment used a single dermal application of 5%, 10%, or 100% Prunus Amygdalus Dulcis (Sweet Almond) Oil, a booster induction injection of 100% Prunus Amygdalus Dulcis (Sweet Almond) Oil was applied occlusively for 48 h 1 wk later, challenge was with 5% Prunus Amygdalus Dulcis (Sweet Almond) Oil in petrolatum applied topically under occlusion for 24 h. Prunus Amygdalus Dulcis (Sweet Almond) Oil was non-sensitizing.					217

Table 7b. Dermal effects – Non-Human studies- – summarized from previous CIR reports

Ingredient	Concentration	Animals	Procedure	Results	Reference
Undiluted Prunus Amygdalus Dulcis (Sweet Almond) Oil was tested for irritancy in groups of 6 male albino rabbits. The test material was applied under occlusion to the clipped intact and abraded dorsal skin of each animal. Twenty-three hours later, patches were removed; sites were scored at 24 and 48 hours. The Primary Irritation Indices (PIIs) for seven test samples of Prunus Amygdalus Dulcis (Sweet Almond) Oil ranged from 0 to 0.18 (maximum score = 8), indicating that this ingredient is practically nonirritating to skin.					
Sesamum Indicum (Sesame) Seed Oil					
Undiluted Sesamum Indicum (Sesame) Seed Oil was non- or minimally irritating to rabbit skin.					55
Triticum Vulgare (Wheat) Germ Oil					
Triticum Vulgare (Wheat) Germ Oil, undiluted and at 2% in formulation, was non- to mildly irritating, and undiluted Triticum Vulgare (Wheat) Germ Oil was not sensitizing to guinea pigs.					30
PHOTOTOXICITY					
Elaeis Guineensis (Palm) Oil					
A facial lotion containing 1.5% Elaeis Guineensis (Palm) Oil was not phototoxic in the phototoxicity yeast assay.					26
Oryza Sativa (Rice) Bran Oil					
Oryza Sativa (Rice) Bran Oil, tested undiluted during induction at 10% at challenge, was not a photosensitizer in guinea pigs.					28
Oryza Sativa (Rice) Germ Oil					
Oryza Sativa (Rice) Germ Oil, ≤75%, was not phototoxic or photosensitizing.					28
COMEDOGENICITY					
Corylus Avellana (Hazel) Seed Oil					
A comedogenicity study was conducted in which 0.1 ml of Corylus Avellana (Hazel) Seed Oil (pH 6) was applied to the pinna of the ear of albino rabbits. No local irritation was noted at the application site. A “slight difference in the number and size of the pilosebaceous follicles” was noted via magnifying glass. A “slight excess of sebum and a dilation of the follicles” was noted upon microscopic examination of the treated areas					41

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
		Adansonia Digitata Seed Oil		
0.01% Adansonia Digitata Seed Oil in a lip product	106	HRIPT with 0.2 g test material, semi-occluded	not a dermal irritant or sensitizer	218
100% Adansonia Digitata Seed Oil	107	HRIPT with 0.02-0.05 ml test material, semi-occluded	not a dermal irritant or sensitizer	219
		Aleurites Moluccana Seed Oil		
0.005% Aleurites Moluccana Seed Oil in scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	not a dermal irritant or sensitizer	220
~3% in a skin cleanser	110	modified HRIPT; semi-occlusive; 10% dilution in distilled water	not a dermal irritant or sensitizer	221
		Arachis Hypogaea (Peanut) Oil		
dermatologic product containing 0.01% fluocinolone and refined Arachis Hypogaea (Peanut) Oil	peanut-sensitive subjects; 8 children, 6 adults	skin prick test with peanut extracts, a soln, of 50% glycerin (negative control), a solution of 1.8 mg/ml histamine phosphate in 50% glycerin (positive control), the complete test product, vehicle only (without fluocinolone), and refined Arachis Hypogaea (Peanut) Oil	1 child had a trace positive reaction	222
		patch test with product, vehicle only, and refined Arachis Hypogaea (Peanut) Oil	no reactions	
		Argania Spinosa Kernel Oil		
5% Argania Spinosa Kernel Oil in a face serum	108	primary cutaneous irritation	no primary irritation	223
5% Argania Spinosa Kernel Oil in a face serum	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	223
10% Argania Spinosa Kernel Oil in a skin salve	209	HRIPT; occlusive; applied neat	not a sensitizer	224
10% Argania Spinosa Kernel Oil in a skin salve	51	4-wk use test; applied to lips, hands/nails, elbows, knees, feet/heels	did not elicit significant dermal irritation or dryness; 2 subjects had level 1(mild, very slight erythema) on the lips, and 5 had level 1 erythema on the elbows, lips, or knees; 15 subjects reported subjective irritation	225
		Astrocaryum Murumuru		
1% Astrocaryum Murumuru Seed Butter in a lipstick	97	HRIPT with 150 mg test material, semi-occluded	not a dermal irritant or sensitizer	226
4% Astrocaryum Murumuru Seed Butter in a lipstick	108	HRIPT, occluded	not a dermal irritant or sensitizer	227
4% Astrocaryum Murumuru Seed Butter in a lipstick	108	HRIPT, occluded	not a dermal irritant or sensitizer	228
4% Astrocaryum Murumuru Seed Butter in a lipstick	108	HRIPT, occluded	not a dermal irritant or sensitizer	229
4% Astrocaryum Murumuru Seed Butter in a lipstick	106	HRIPT, occluded	not a dermal irritant or sensitizer	230
4% Astrocaryum Murumuru Seed Butter in a lipstick	106	HRIPT, occluded	not a dermal irritant or sensitizer	231
4% Astrocaryum Murumuru Seed Butter in a lipstick	108	HRIPT, occluded	not a dermal irritant or sensitizer	232

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
Avena Sativa (Oat) Kernel Oil				
3% Avena Sativa (Oat) Kernel Oil in a body and hand formulation	100	HRIPT with 0.2 ml, occluded	not a dermal irritant or sensitizer	233
Bassia Latifolia Seed Butter				
2% Bassia Latifolia Seed Butter in a body scrub	110	HRIPT with 1% aq. solution of the formulation, semi-occluded	not a dermal irritant or sensitizer	234
Borago Officinalis Seed Oil				
1% Borago Officinalis Seed Oil in a body and hand formulation	213	HRIPT with 0.2 g, occluded	not a dermal irritant or sensitizer	235
2% Borago Officinalis Seed Oil in a face serum	108	primary cutaneous irritation	no primary irritation	223
2% Borago Officinalis Seed Oil in a face serum	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	223
Brassica Campestris (Rapeseed) Oil				
5% Hydrogenated Rapeseed Oil in a baby oil	105	HRIPT with 0.2 ml, semi-occluded	not a dermal irritant or sensitizer	236
Brassica Oleracea Italica (Broccoli) Seed Oil				
0.5% Brassica Oleracea Italica (Broccoli) Seed Oil in a hair conditioner	102	HRIPT with 150 µl of test material, 10% dilution, semi-occluded	not a dermal irritant or sensitizer	237
Butyrospermum Parkii (Shea) Butter				
Butyrospermum Parkii (Shea) Butter and fractions of unsaponifiable lipids from Butyrospermum Parkii (Shea) Butter; the "liquid" sample was obtained from a supplier; the unsaponifiable fraction was obtained through low temperature crystallization of the supplied sample	21	single applications to normal skin and sodium lauryl sulfate (SLS)-irritated skin; right volar forearm was treated with 50 µl of each test material in 12 mm Finn chambers for 48 h; the left volar forearm was treated with 50 µl of 14% aq. SLS for 7 h, rinsed, dried, and then treated with 50 µl of each test material for 17 h; cutaneous blood flow (CBF) and transepidermal water loss (TEWL) were measured	normal skin: barely perceptible erythema observed in a "small" number of subjects at 24 h after treatment with shea butter; no irritation to the shea unsaponifiable fraction; no sig. difference in CBF or TEWL SLS-treated skin: 2 subjects had a slight- and moderate reaction to the unsaponifiable fraction; no sig. difference in CBF or TEWL	238
0.1% Butyrospermum Parkii (Shea) Butter in a scalp conditioner	114	primary cutaneous irritation; formulation diluted to 1%	no primary irritation	239
2% Butyrospermum Parkii (Shea) Butter in a cream	119	primary cutaneous irritation	no primary irritation	240
0.1% Butyrospermum Parkii (Shea) Butter in a scalp conditioner	110	HRIPT; occlusive; formulation diluted to 1%	not a dermal irritant or sensitizer	239
2% Butyrospermum Parkii (Shea) Butter in a cream	118 (irritation)/ 116 (sensitization)	HRIPT; occlusive	not a dermal irritant or sensitizer	240
4% Butyrospermum Parkii (Shea) Butter in a face cream	51	HRIPT with 20 µl test material, occluded	not a dermal irritant or sensitizer	241
4% Butyrospermum Parkii (Shea) Butter in an eye cream	108	HRIPT with 20 µl test material, occluded	not a dermal irritant or sensitizer	242
23.5% Butyrospermum Parkii (Shea) Butter in a lip gloss	104	HRIPT	not a dermal irritant or sensitizer	243
23.7% Butyrospermum Parkii (Shea) Butter in a lip gloss	104	HRIPT	irritation on induction days 5-9 in one subject; no sensitization	244

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
24.1% Butyrospermum Parkii (Shea) Butter in a lip wax	113	HRIPT	not a dermal irritant or sensitizer	245
24.1% Butyrospermum Parkii (Shea) Butter in a lip wax	2 runs	Episkin	average viability 67.3% - no irritation potential	246
24.7% Butyrospermum Parkii (Shea) Butter in a lip gloss	40	28-day use study, 2-6 times /day	1 subject with desquamation	247
45% Butyrospermum Parkii (Shea) Butter in a body/hand massage	109[a]	HRIPT	not a dermal irritant or sensitizer	248
45% Butyrospermum Parkii (Shea) Butter in a body/hand massage	109[a]	HRIPT	not a dermal irritant or sensitizer	249
45% Butyrospermum Parkii (Shea) Butter in a body/hand massage	109[a]	HRIPT	not a dermal irritant or sensitizer	250
45% Butyrospermum Parkii (Shea) Butter in a body/hand massage	109[a]	HRIPT	not a dermal irritant or sensitizer	251
45% Butyrospermum Parkii (Shea) Butter in a body/hand massage	31	2-week use study, 2 time per day	no erythema, edema, or dryness	252
60% Butyrospermum Parkii (Shea) Butter in a cuticle cream	111	HRIPT	not a dermal irritant or sensitizer	253
Camelina Sativa Seed Oil				
0.25% Camelina Sativa Seed Oil in a body powder	204	HRIPT with 0.1 g, semi-occluded	not a dermal sensitizer	254
7% Camelina Sativa Seed Oil in an oil treatment	103	HRIPT with 200 µl test material, semi-occluded	Grade 1 (mild erythema) reactions in 4 subjects for 1 or 2 patches in the induction phase, grade 1 (mild erythema in different subjects at the 48 h challenge reading. Study concluded test material was not a dermal irritant or sensitizer.	255
Camellia Sinensis Seed Oil				
0.0985% Camellia Sinensis Seed Oil in a lipstick	108	HRIPT with 0.2 g, occluded	not a dermal irritant or sensitizer	256
0.0985% Camellia Sinensis Seed Oil in a lipstick	108	HRIPT with 0.2 g, occluded	not a dermal irritant or sensitizer	257
Canola Oil				
74.7% Canola Oil in a body oil	101	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	258
Carthamus Tinctorius (Safflower) Oil				
5% Carthamus Tinctorius (Safflower) Seed Oil in a cleansing oil rinse-off	214	HRIPT with 0.2 ml of a 10% v/v aqueous solution, semi-occluded	3 subjects had a "?" reaction following a patch during the induction and 1 subject had definite erythema with no edema or damage to the epidermis (+D) following the 7th patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer.	259

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
30% Carthamus Tinctorius (Safflower) Seed Oil in a massage oil	107	HRIPT with 0.2 ml test material, semi-occluded	1 subject had slight erythema following the 7th patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer.	260
Caryocar Brasiliense Fruit Oil				
0.1% Caryocar Brasiliense Fruit Oil in a lipstick	100	HRIPT with 200 mg test material, semi-occluded	not a dermal irritant or sensitizer	261
Chenopodium Quinoa Seed Oil				
1% Chenopodium Quinoa Seed Oil in a UV SPF cream	105	HRIPT with 0.02 ml test material, occluded	"An acceptable level of irritation" was observed in the induction phase consisting of grade 1 (mild erythema) in 39 subjects, with one additional subject exhibiting a grade 2 (moderate erythema) reaction. No evidence of skin sensitization was observed.	262
1% Chenopodium Quinoa Seed Oil in a UV SPF cream	102	HRIPT with 0.02 ml test material, occluded	"An acceptable level of irritation" was observed in the induction phase, with 54% of the subjects exhibiting a grade 1 (mild erythema) reaction and 3% of the subjects exhibiting a grade 2 (moderate erythema) reaction. One subject had a strong reaction to the 3rd induction patch and discontinued the induction phase after the 6th application. At challenge, the subject had only papules at 96 h. Due to reactions to other materials tested at the same time, it could not be determined if the test material was the causative agent. No evidence of skin sensitization was observed in the remaining subjects.	263
Citrullus Lanatus (Watermelon) Seed Oil				
2% Citrullus Lanatus (Watermelon) Seed Oil in a facial oil	105	HRIPT, semi-occluded	not a dermal irritant or sensitizer	264
Cocos Nucifera (Coconut) Fruit Oil				
0.15% Cocos Nucifera (Coconut) Oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	not a dermal irritant or sensitizer	220
31% Cocos Nucifera (Coconut) Oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 subjects had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer.	265
Corylus Avellana (Hazel Seed) Oil				
1% Corylus Avellana (Hazel) Seed Oil in a moisturizing cream	25	Amended Draize patch test, 10% standard concentration	Non-irritating	266

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
1% Corylus Avellana (Hazel) Seed Oil in a moisturizing cream	32	60 day clinical study	"Fairly good acceptability"	267
5% Corylus Avellana (Hazel) Seed Oil in a massage oil	107	HRIPT with 0.2 ml test material, semi-occluded	1 subject had slight erythema following the 7th patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer.	260
		Crambe Abyssinica Seed Oil		
5% Crambe Abyssinica Seed Oil in a face and neck product	54	HRIPT; semi-occluded, undiluted	not a dermal irritant or sensitizer	268
100% Crambe Abyssinica Seed Oil in an unspecified product	107	HRIPT; undiluted	not a dermal irritant or sensitizer	213
		Elaeis Guineensis (Palm) Oil		
15.7% Sodium Palm Kernelate in a soap	42	28-day use test	good acceptability for use	269
61.6% Sodium Palmate in a soap	42	28-day use test	good acceptability for use	269
		Euterpe Oleracea Fruit Oil		
0.5% Euterpe Oleracea Fruit Oil in an eye treatment	104	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	270
		Glycine Soja (Soybean) Oil		
0.19% Glycine Soja (Soybean) Unsaponifiables in a face and neck product	50	HRIPT, occluded	not a dermal irritant or sensitizer	271
39% Hydrogenated Soybean Oil in a lipstick	108	HRIPT, occluded	not a dermal irritant or sensitizer	272
		Garcinia Indica Seed Butter		
0.3869% Garcinia Indica Seed Butter in a body and hand product	101	HRIPT, 0.2 g applied, occlusive	not a sensitizer; irritation was observed in one subject	273
		Gossypium Herbaceum (Cotton) Seed Oil		
3.6% Hydrogenated Cottonseed Oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 subjects had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer.	265
		Helianthus Annuus (Sunflower) Seed Oil		
6% Helianthus Annuus (Sunflower) Seed Oil in a skin cream	108	primary cutaneous irritation	no primary irritation	274
20% Helianthus Annuus (Sunflower) Seed Oil in a face serum	108	primary cutaneous irritation	no primary irritation	223
0.264% Helianthus Annuus (Sunflower) Seed Oil in a cream	57	HRIPT; Finn chambers, applied neat	not a dermal irritant or sensitizer	275
6% Helianthus Annuus (Sunflower) Seed Oil in a skin cream	106	HRIPT, occlusive	not a dermal irritant or sensitizer	274

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
20% Helianthus Annuus (Sunflower) Seed Oil in a face serum	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	223
1% Helianthus Annuus (Sunflower) Seed Oil in a soap	42	28-day use test	good acceptability for use	269
39.8% Helianthus Annuus (Sunflower) Seed Oil in a massage oil	107	HRIPT with 0.2 ml test material, semi-occluded	1 subject had slight erythema following the 7th patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer.	260
Helianthus Annuus (Sunflower) Seed Oil Unsaponifiables				
2% Helianthus Annuus (Sunflower) Seed Oil Unsaponifiables in a night product	100	HRIPT, semi-occluded	not a dermal irritant or sensitizer	271
2% Helianthus Annuus (Sunflower) Seed Oil Unsaponifiables in a face and neck product	100	HRIPT, semi-occluded	not a dermal irritant or sensitizer	271
Hippophae Rhamnoides Seed Oil				
5% Hippophae Rhamnoides Seed Oil	10	cutaneous local tolerance test, 0.02 ml single 48 h occlusive application	not an irritant; average irritation score of 0	276
Irvingia Gabonensis Kernel Butter				
0.31% Irvingia Gabonensis Kernel Butter in a face and neck product	52	HRIPT, occluded	not a dermal irritant or sensitizer	271
Limnanthes Alba (Meadowfoam) Seed Oil				
71.3% Limnanthes Alba (Meadowfoam) Seed Oil in a facial repair product	109	HRIPT, semi-occluded	7 subjects had ± on the first day of the induction only, no other reactions. Not a dermal irritant or sensitizer.	277
Linum Usitatissimum (Linseed) Seed Oil				
9.4% Linum Usitatissimum (Linseed) Seed Oil in mascara	105	HRIPT with 0.2 g test material, semi-occluded	not a dermal irritant or sensitizer	278
Luffa Cylindrica Seed Oil				
0.01% Luffa Cylindrica Seed Oil in a body wash	102	HRIPT; 0.2 ml of a 1% dilution using distilled water was applied to a 1" x 1" pad applied with a semi-occlusive patch	not a dermal irritant or sensitizer	279
Macadamia Ternifolia Seed Oil				
0.5% Macadamia Ternifolia Seed Oil in a cleansing oil rinse-off	214	HRIPT with 0.2 ml of a 10% v/v aqueous solution, semi-occluded	3 subjects had a "?" reaction following a patch during the induction and 1 subject had definite erythema with no edema or damage to the epidermis (+D) following the 7th patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer.	259
30% Macadamia Ternifolia Seed Oil in a body and hand product	55	HRIPT; semi-occluded, undiluted	not a dermal irritant or sensitizer	268

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
Mangifera Indica (Mango) Seed Oil				
2% Mangifera Indica (Mango) Seed Oil in a lipstick	100	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	280
3.87% Mangifera Indica (Mango) Seed Oil in an eyeliner	102	HRIPT with 0.2 g of test material, semi-occluded	not a dermal irritant or sensitizer	281
Mangifera Indica (Mango) Seed Butter				
1% Mangifera Indica (Mango) Seed Butter in a facial lotion	100	HRIPT with 200 µl test material, semi-occluded	not a dermal irritant or sensitizer	282
9% Mangifera Indica (Mango) Seed Butter in a body product	102	HRIPT with 0.2 g, semi-occluded	not a sensitizer	283
Moringa Oleifera Seed Oil				
0.01% Moringa Oleifera Seed Oil in a cleansing oil rinse-off	214	HRIPT with 0.2 ml of a 10% v/v aqueous solution, semi-occluded	3 subjects had a "?" reaction following a patch during the induction and 1 subject had definite erythema with no edema or damage to the epidermis (+D) following the 7th patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer.	259
Moringa Pterygosperma Seed Oil				
3% Moringa Pterygosperma Seed Oil in an eye treatment	104	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	284
Oenothera Biennis (Evening Primrose) Oil				
1.99% Oenothera Biennis (Evening Primrose) Oil in a foundation	600	HRIPT, occluded	not a dermal irritant or sensitizer	285
Olea Europaea (Olive) Fruit Oil				
0.7% Olea Europaea (Olive) Fruit Oil in a scalp conditioner	114	primary cutaneous irritation; formulation diluted to 1%	no primary irritation	239
0.1595% Olea Europaea (Olive) Fruit Oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	not a dermal irritant or sensitizer	220
0.7% Olea Europaea (Olive) Fruit Oil in a scalp conditioner	110	HRIPT; occlusive; formulation diluted to 1%	not a dermal irritant or sensitizer	239
1.6% Olea Europaea (Olive) Fruit Oil in a body lotion	110	HRIPT with 0.02 ml test material , occluded	1 subject had slight erythema following the 7th patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer.	286
10% Olea Europaea (Olive) Fruit Oil in a skin salve	209	HRIPT; occlusive applied neat	not a sensitizer	224
22% Olea Europaea (Olive) Fruit Oil in a body moisturizer	105	HRIPT, semi-occluded	not a dermal irritant or sensitizer	287
58.7% Olea Europaea (Olive) Fruit Oil in a conditioning hair oil	102	HRIPT with 0.2 ml, semi-occluded	not a dermal irritant or sensitizer	288

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
69.6% Olea Europaea (Olive) Fruit Oil in a foundation	209	HRIPT with 200 µl test material, occluded	not a dermal irritant or sensitizer	289
10% Olea Europaea (Olive) Oil in a skin salve	51	4-wk use test; applied to lips, hands/nails, elbows, knees, feet/heels	did not elicit significant dermal irritation or dryness; 2 subjects had level 1(mild, very slight erythema on the lips, and 5 had level 1 erythema on the elbows, lips, or knees; 15 subjects reported subjective irritation	225
Olea Europaea (Olive) Oil Unsaponfiables				
2.5% Olea Europaea (Olive) Oil Unsaponfiables in a bath body mist	107	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	290
Hydrogenated Olive Oil				
12% Hydrogenated Olive Oil in a lipstick	108	HRIPT, occluded	not a dermal irritant or sensitizer	272
Hydrogenated Olive Oil Unsaponifiables				
2% Hydrogenated Olive Oil Unsaponifiables in a face and neck product	50	HRIPT, occluded	not a dermal irritant or sensitizer	271
5% Hydrogenated Olive Oil Unsaponifiables in a skin cleansing product	57	HRIPT, semi-occluded, 10% dilution of product	not a dermal irritant or sensitizer	271
Sodium Olivate				
17.64% Sodium Olivate in a body bar soap	107	HRIPT, semi-occluded	not a dermal irritant or sensitizer	291
Orbignya Oleifera Seed Oil				
3.79% Orbignya Oleifera Seed Oil in a cream cleanser	104	HRIPT with 0.2 ml of a 10% dilution of formulation, semi-occluded	not a dermal irritant or sensitizer	292
Orbignya Speciosa Kernel Oil				
0.4125% Orbignya Speciosa Kernel Oil in a hair conditioner	104	modified HRIPT; semi-occlusive; 10% dilution in distilled water	not a dermal irritant or sensitizer	293
Persea Gratissima (Avocado) Oil				
0.2% Persea Gratissima (Avocado) Oil in a scalp conditioner	114	primary cutaneous irritation; formulation diluted to 1%	no primary irritation	239
0.2% Persea Gratissima (Avocado) Oil in a scalp conditioner	110	HRIPT; occlusive; formulation diluted to 1%	not a dermal irritant or sensitizer	239
10% Persea Gratissima (Avocado) Oil in a skin salve	51	4-wk use test; applied to lips, hands/nails, elbows, knees, feet/heels	did not elicit significant dermal irritation or dryness; 2 subjects had level 1(mild, very slight erythema on the lips, and 5 had level 1 erythema on the elbows, lips, or knees; 15 subjects reported subjective irritation	225

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
Plukenetia Volubilis Seed Oil				
0.51% Plukenetia Volubilis Seed Oil in a lipstick	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	294
Prunus Amygdalus Dulcis (Sweet Almond) Oil				
7% Prunus Amygdalus Dulcis (Sweet Almond) Oil in an oil treatment	103	HRIPT with 200 µl test material, semi-occluded	Grade 1 (mild erythema) reactions in 4 subjects for 1 or 2 patches in the induction phase, grade 1 (mild erythema in different subjects at the 48 h challenge reading. Study concluded test material was not a dermal irritant or sensitizer.	255
10% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a face serum	108	primary cutaneous irritation	no primary irritation	223
10% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a face serum	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	223
10% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a skin salve	209	HRIPT; occlusive applied neat	not a sensitizer	224
10% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a skin salve	51	4-wk use test; applied to lips, hands/nails, elbows, knees, feet/heels	did not elicit significant dermal irritation or dryness; 2 subjects had level 1(mild, very slight erythema on the lips, and 5 had level 1 erythema on the elbows, lips, or knees; 15 subjects reported subjective irritation	225
15% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a massage oil	107	HRIPT with 0.2 ml test material, semi-occluded	1 subject had slight erythema following the 7th patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer.	260
25% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 subjects had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer.	265
~31% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a facial oil	108	modified HRIPT; semi-occlusive; applied neat	not a dermal irritant or sensitizer	295
45.25% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a facial oil	109	HRIPT; semi-occlusive; applied neat	not a dermal irritant or sensitizer	296
46% Prunus Amygdalus Dulcis (Sweet Almond) Oil in a cuticle softener	106	modified Draize assay with an induction phase (3x/wk for 10 applications) and a challenge phase, applied neat, occlusive	not a dermal irritant or sensitizer	297
Prunus Armeniaca (Apricot) Kernel Oil				
2% Prunus Armeniaca (Apricot) Kernel Oil in a face cream	51	HRIPT with 20 µl test material, occluded	not a dermal irritant or sensitizer	241
2% Prunus Armeniaca (Apricot) Kernel Oil in an eye cream	108	HRIPT with 20 µl test material, occluded	not a dermal irritant or sensitizer	242
2.5% Prunus Armeniaca (Apricot) Kernel Oil in a cream	119	primary cutaneous irritation	no primary irritation	240

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
19.749% Prunus Armeniaca (Apricot) Kernel Oil in a face serum	108	primary cutaneous irritation	no primary irritation	223
0.005% Prunus Armeniaca (Apricot) Kernel Oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	not a dermal irritant or sensitizer	220
1% Prunus Armeniaca (Apricot) Kernel Oil in a cream	57	HRIPT; Finn chambers, applied neat	not a dermal irritant or sensitizer	275
2.5% Prunus Armeniaca (Apricot) Kernel Oil in a cream	118 (irritation)/ 116 (sensitization)	HRIPT; occlusive	not a dermal irritant or a sensitizer	240
19.749% Prunus Armeniaca (Apricot) Kernel Oil in a face serum	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	223
Prunus Domestica Seed Oil				
0.04% Prunus Domestica Seed Oil in a preshave lotion	105	HRIPT with 0.2 ml, occluded	not a sensitizer	298
Prunus Persica (Peach) Kernel Oil				
24% Prunus Persica (Peach) Kernel Oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 subjects had low-level, transient (+) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer.	265
Ribes Nigrum (Black Currant) Seed Oil				
0.1% Ribes Nigrum (Black Currant) Oil in a scalp conditioner	114	primary cutaneous irritation; diluted to 1%	no primary irritation	239
0.25% Ribes Nigrum (Black Currant) Oil in a cream	119	primary cutaneous irritation	no primary irritation	240
0.1% Ribes Nigrum (Black Currant) Oil in a scalp conditioner	110	HRIPT; occlusive; diluted to 1%	not a dermal irritant or sensitizer	239
0.2% Ribes Nigrum (Black Currant) Seed Oil is an eye mask	228	HRIPT, occluded	4 subjects had "?" or "+" reaction during induction that were not considered clinically relevant, no other reactions observed. Not sensitizing	299
0.2% Ribes Nigrum (Black Currant) Oil in a skin cream	106	HRIPT, occlusive	not a dermal irritant or sensitizer	274
0.25% Ribes Nigrum (Black Currant) Oil in a cream	118 (irritation)/ 116 (sensitization)	HRIPT; occlusive	not a dermal irritant or a sensitizer	240
0.2% Ribes Nigrum (Black Currant) Seed Oil is an eye mask	195	4-week safety in-use study	No adverse reactions reported. Product considered suitable for sensitive skin.	300
Rosa Canina Fruit Oil				
0.39% Rosa Canina Fruit Oil in a skin cream	108	primary cutaneous irritation	no primary irritation	274
0.39% Rosa Canina Fruit Oil in a skin cream	106	HRIPT, occlusive	not a dermal irritant or sensitizer	274
Rubus Chamaemorus Seed Oil				
2.5% Rubus Chamaemorus Seed Oil in product	10	Single occlusive patch test for 48 h with 25 µl	not an irritant	301

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
Rubus Idaeus (Raspberry) Seed Oil				
5% Rubus Idaeus (Raspberry) Seed Oil in a face and neck product	102	HRIPT, occluded	not a dermal irritant or sensitizer	271
Sesamum Indicum (Sesame) Seed Oil				
25% Sesamum Indicum (Sesame) Seed Oil in a face serum	108	primary cutaneous irritation	no primary irritation	223
8% Sesamum Indicum (Sesame) Seed Oil in a skin salve	209	HRIPT; occlusive applied neat	not a sensitizer	224
25% Sesamum Indicum (Sesame) Seed Oil in a face serum	108	HRIPT; occlusive; applied neat	not an irritant or a sensitizer	223
8% Sesamum Indicum (Sesame) Seed Oil in a skin salve	51	4-wk use test; applied to lips, hands/nails, elbows, knees, feet/heels	did not elicit significant dermal irritation or dryness; 2 subjects had level 1(mild, very slight erythema on the lips, and 5 had level 1 erythema on the elbows, lips, or knees; 15 subjects reported subjective irritation	225
Solanum Lycopersicum (Tomato) Seed Oil				
0.0023% Solanum Lycopersicum (Tomato) Seed Oil in a cream cleanser	104	HRIPT with 0.2 ml of a 10% dilution of the formulation, semi-occluded	not a dermal irritant or sensitizer	302
Theobroma Cacao (Cocoa) Seed Butter				
50.1% Theobroma Cacao (Cocoa) Seed Butter in a lip balm	106	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	303
Theobroma Grandiflorum Seed Butter[304]				
5% Theobroma Grandiflorum Seed Butter in a lip balm	106	HRIPT with 150 µl test material, semi-occluded	not a dermal irritant or sensitizer	305
Triticum Vulgare (Wheat) Germ Oil				
0.005% Triticum Vulgare (Wheat) Germ Oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	not a dermal irritant or sensitizer	220
Vaccinium Macrocarpon (Cranberry) Seed Oil				
0.04% Vaccinium Macrocarpon (Cranberry) Seed Oil in a face and neck product	53	HRIPT, occluded	not a dermal irritant or sensitizer	271
Vaccinium Myrtillus Seed Oil				
~1% Vaccinium Myrtillus Seed Oil in a facial oil	116	modified HRIPT; semi-occlusive; volatilized	not a dermal irritant or sensitizer	304
Vaccinium Vitis-Idaea Seed Oil				
5% Vaccinium Vitis-Idaea Seed Oil in product	10	Single occlusive patch test of 48 h with 0.02 ml	not an irritant	306
Vegetable Oil				
4% Vegetable Oil in a foundation	115	HRIPT, semi-occluded	1 subject had ± on the first day of the induction only, no other reactions. Not a dermal irritant or sensitizer.	307
4% Vegetable Oil in a lipstick	106	HRIPT with 0.2 g, occluded	not a dermal irritant or sensitizer	308
11% Vegetable Oil in an eye shadow	106	HRIPT, semi-occluded	not a dermal irritant or sensitizer	309

Table 8a. Dermal effects – Human studies

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
		Vitis Vinifera (Grape) Seed Oil		
39% Vitis Vinifera (Grape)Seed Oil in a preshave lotion	105	HRIPT with 0.2 ml, occluded	not a sensitizer	298
90% Vitis Vinifera (Grape) Seed Oil in a fragranced oil	105	HRIPT; semi-occluded; applied neat	not a dermal irritant or sensitizer	310
0.5% Hydrogenated Grapeseed Oil in a lip product	53	HRIPT; semi-occluded	not a dermal irritant or sensitizer	311
		Zea Mays (Corn) Germ Oil		
20% Zea Mays (Corn) Germ Oil in a cleansing oil rinse-off	214	HRIPT with 0.2 ml of a 10% v/v aqueous solution, semi-occluded	3 subjects had a "?" reaction following a patch during the induction and 1 subject had definite erythema with no edema or damage to the epidermis (+D) following the 7th patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer.	259
		COMEDOGENICITY		
		Ribes Nigrum (Black Currant) Seed Oil		
0.2% Ribes Nigrum (Black Currant) Seed Oil in an eye mask formulation	6	applied undiluted; occlusive	avg. score of 0.00 comedones/cm^2; non-comedogenic	312

[a] Same 109 panelists tested these 4 formulations hat differed only in color and fragrance.

Table 8b. Dermal effects – Human studies – summarized from previous CIR reports

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
Carthamus Tinctorius (Safflower) Oil				
Cosmetic formulations containing 3-5% Carthamus Tinctorius (Safflower) Seed Oil were not irritating to humans in occlusive patch tests and were not primary irritants or sensitizers in repeated insult patch tests.				32
Cocos Nucifera (Coconut) Fruit Oil				
An RIPT was performed using 103 subjects with a tanning butter containing 2.5% Cocos Nucifera (Coconut) Oil no erythematous reactions were seen at challenge; A bar soap containing 13% Cocos Nucifera (Coconut) Oil produced very mild irritation when tested as a 1% aq. solution on 106 subjects, and it was minimally to mildly irritating in a soap chamber test with a 8% aq. solution; the soap produced no unusual irritation response in a 2-wk normal use test; undiluted Cocos Nucifera (Coconut) Oil was not an allergen in 12 subjects.				*43*
Hydrogenated Coconut Oil				
Four lipstick formulations containing 10% hydrogenated coconut oil were tested with a single 48-h application on 204 females; there was no evidence of primary irritation and no indication of sensitization on retests performed 14 d later.				*43*
Potassium Cocoate				
In a test using 40 healthy subjects and 480 patients with active skin disease, 5% aq. potassium cocoate produced 5 positive responses.				*43*
Corylus Avellana (Hazel Seed) Oil				
A patch testing reference book by de Groot noted that the published literature does not contain recommended test concentrations concerning Hazel Seed Oil. To serve as a guide to the reader, de Groot reported that an unpublished (and at the time, ongoing) study found no irritant reaction in 1 to 20 patients suffering from or suspected to suffer from cosmetic product contact allergy who had been patch tested with 30% Hazel Seed Oil in petrolatum.				*41*
Elaeis Guineensis (Palm) Oil				
Elaeis Guineensis (Palm) Oil, 15% in petrolatum or cosmetic formulations containing 1.0-2.0%, was not an irritant or sensitizer in clinical studies. Bar soap flakes, tested at dilutions that contained ≤ 2.13% palm kernel oil, were not irritating or sensitizing.				26
Gossypium Herbaceum (Cotton) Seed Oil				
Patients that were hypersensitive to cottonseed proteins were not sensitive to cottonseed oil in a skin prick test				*27*
Hydrogenated Cottonseed Oil				
In a clinical patch test, the irritation potential of a cosmetic formulation containing 3.4% hydrogenated cottonseed oil was mildly low, and the severity of reaction to 10.4% hydrogenated cottonseed oil was acceptably low in a use study. Cosmetic formulations containing 10.6-20.86% hydrogenated cottonseed oil were not irritating or sensitizing.				*27*
Oryza Sativa (Rice) Bran Oil				
Rice is generally regarded as hypoallergenic, although some case studies of allergic reactions to raw rice have been reported. In clinical testing, formulations containing 1.04-8.0% Oryza Sativa (Rice) Bran Oil were not irritating or sensitizing. . Hydrolyzed rice protein was not irritating to human subjects.				*28*
Persea Gratissima (Avocado) Oil				
Persea Gratissima (Avocado) Oil was not an irritant or sensitizer when human subjects were patch tested with cosmetic formulations containing up to 10.7% Persea Gratissima (Avocado) Oil or in patch tests using 100% Persea Gratissima (Avocado) Oil.				31
Prunus Amygdalus Dulcis (Sweet Almond) Oil				
Undiluted Prunus Amygdalus Dulcis (Sweet Almond) Oil was non-irritating in a single insult patch test with 101 subjects, and it was non-irritating and non-sensitizing in an HRIPT using 52 subjects. Cosmetic formulations containing 0.1-25% were practically non-irritating and non-sensitizing in HRIPTs performed with 6906 subjects. In the Lanman-Maibach 21-day Cumulative Irritancy Assay, a moisturizer containing 25% Prunus Amygdalus Dulcis (Sweet Almond) Oil had a total irritancy score of 14/630.				217

Table 8b. Dermal effects – Human studies – summarized from previous CIR reports

Ingredient and Concentration	Subjects Completed	Method	Results	Reference
Sesamum Indicum (Sesame) Seed Oil				
In clinical testing, undiluted Sesamum Indicum (Sesame) Seed Oil was not irritating. Cosmetic formulations containing 8-14.3% Sesamum Indicum (Sesame) Seed Oil were non- to essentially non-irritating. Prophetic patch testing with formulations containing 10-11% Sesamum Indicum (Sesame) Seed Oil were not irritating with or without UV light. Patients with contact allergy to Sesamum Indicum (Sesame) Seed Oil were patch tested, and most had positive reactions to sesamol, sesamin, and sesamolin.				55
Triticum Vulgare (Wheat) Germ Oil				
In clinical testing, Triticum Vulgare (Wheat) Germ Oil was not an irritant or a sensitizer.				30
PHOTOTOXICITY/PHOTOSENSITIZATION				
Cocos Nucifera (Coconut) Oil				
Bar soaps made with 13% Cocos Nucifera (Coconut) Oil, tested as a 3% aqueous solution, tested using 10 subjects, and a similar soap, prepared as 1 or 3% aqueous solutions, tested on 52 panelists, did not produce any evidence of photosensitization.				33
Sodium Cocoate				
Bar soaps 13% sodium cocoate, prepared as a 3% aqueous solution, tested using 10 subjects did not produce any evidence of photosensitization.				33
Prunus Amygdalus Dulcis (Sweet Almond) Oil				
Formulations containing 0.1% - 2.0% Prunus Amygdalus Dulcis (Sweet Almond) Oil, tested for photosensitization in a total of 764 subjects, did not manifest photosensitivity in any of the test subjects.				217
Oryza Sativa (Rice) Bran Oil				
Formulations containing 1.04% Oryza Sativa (Rice) Bran Oil were not photosensitizing.				*28*

Table 9a. Ocular irritation – Non-Human and Human

Ingredient	Concentration	Test Group	Procedure	Results	Reference
NON-HUMAN					
Adansonia Digitata Seed Oil					
baobab oil	100%		MatTek EpiOcular MTT viability assay; 100 µl of test material for 16-256 min	non-irritating	210
Aleurites Moluccana Seed Oil					
Aleurites Moluccana oil			Draize test	not an ocular irritant	313
Aleurites Moluccana oil			in vitro conjunctival cell assay	not cytotoxic	313
Aleurites Moluccana oil			ocular burn treatment efficacy test	no adverse effects	314
Butyrospermum Parkii (Shea) Butter					
Butyrospermum Parkii (Shea) Butter	undiluted	3 male Kleinrussen Chbb:HM rabbits	0.1 ml instilled into the conjunctival sac of one eye for 24 h	not irritating; mild conjunctival reactions	315
Crambe Abyssinica Seed Oil					
Crambe Abyssinica Seed Oil	undiluted		details not provided	an ocular irritant, but not corrosive	213
Fragaria Ananassa (Strawberry) Seed Oil					
Fragaria Ananassa (Strawberry) Seed Oil	5-50% in a lipophilic solvent		neutral red release test	IC_{50} >50%; negligible cytotoxicity	316
Hippophae Rhamnoides Seed Oil					
Hippophae Rhamnoides Seed Oil	5-50% in a lipophilic solvent		neutral red release test	IC_{50} >50%; negligible cytotoxicity	317
Linum Usitatissimum (Linseed) Seed Oil					
mascara containing 9.4% Linum Usitatissimum (Linseed) Oil	diluted at 0-50% in mineral oil		neutral red release test	NR_{50}>50%; slightly cytotoxic	318
mascara containing 9.4% Linum Usitatissimum (Linseed) Oil	67.1% solution in mineral oil		hen's egg test-chorioallantoic membrane assay (HET-CAM)	moderately irritating	318
mascara containing 9.4% Linum Usitatissimum (Linseed) Oil	66.9% solution in mineral oil		reconstituted epithelial culture assay	slightly cytotoxic	318
Olea Europaea (Olive) Fruit Oil					
Olea Europaea (Olive) Fruit Oil, "high purity"	undiluted	rabbits; number not specified	Draize test	not irritating	313
Olea Europaea (Olive) Fruit Oil, "high purity"			in vitro study using human conjunc-tival epithelial cells	did not induce cellular necrosis or apoptosis	313
Ribes Nigrum (Black Currant) Seed Oil					
eye mask containing 0.2% Black Ribes (Black Currant) Seed Oil	50% dilution		HET-CAM assay	practically no irritation	319
Rubus Chamaemorus Seed Oil					
product containing 2.5% Rubus Chamaemorus Seed Oil			neutral red release assay	negligible cytotoxicity; product was considered well tolerated	320
Vaccinium Vitis-Idaea Seed Oil					
Vaccinium Vitis-Idaea Seed Oil	5-50% in a lipophilic solvent		neutral red release test	IC_{50}> 50%; negligible cytotoxicity	321

Table 9a. Ocular irritation – Non-Human and Human

Ingredient	Concentration	Test Group	Procedure	Results	Reference
			Zea Mays (Corn) Oil		
Zea Mays (Corn) Oil, "high purity"	undiluted	rabbits, number not specified	Draize test	not irritating	313
Zea Mays (Corn) Oil, "high purity"			in vitro study using human conjunctival epithelial cells	did not induce necrosis or apoptosis	313
			HUMAN STUDIES		
			Linum Usitatissimum (Linseed) Seed Oil		
9.4% Linum Usitatissimum (Linseed) Seed Oil in a mascara		33 female subjects	4 wk study; 16 wore contact lenses, 17 had "sensitive" eyes	no subjective irritation and no adverse reports; clinically safe for use by contact lens-wearers	322
			Ribes Nigrum (Black Currant) Seed Oil		
0.2% Ribes Nigrum (Black Currant) Seed Oil in an eye mask	undiluted	52 subjects	4-wk in-use study	no adverse reactions; safe for contact-lens wearers	323

Table 9b. Ocular irritation – Non-Human - summarized from previous CIR reports

Ingredient	Concentration	Test Group	Procedure	Results	Reference
Cocos Nucifera (Coconut) Oil					
Undiluted Cocos Nucifera (Coconut) Oil, instilled into rabbit eyes without rinsing, produced minimal eye irritation.					33
Hydrogenated Coconut Oil					
Undiluted hydrogenated coconut oil produced mild irritation in one study, minimal irritation in another, negligible or minimal irritation in eight additional tests. Two lipstick formulations containing 10% hydrogenated coconut oil both produced slight conjunctivitis.					33
Coconut Acid					
Undiluted coconut acid produced mild irritation in rabbit eyes in two studies and minimal irritation in a third.					33
Elaeis Guineensis (Palm) Oil					
Undiluted Elaeis Guineensis (Palm) Oil and cosmetic lotions and creams containing 1.5-2.0% Elaeis Guineensis (Palm) Oil were minimally irritating to the eyes of rabbits, while one lotion containing 1.5% Elaeis Guineensis (Palm) Oil was moderately irritating.					26
Hydrogenated Palm Oil					
Hydrogenated palm oil suppositories were mildly irritating to rabbit eyes.					26
Hydrogenated Cottonseed Oil					
Cosmetic formulations containing 3.4-12.3% hydrogenated cottonseed oil were mildly irritating to the eyes of rabbits.					27
Oryza Sativa (Rice) Bran Oil					
A mixture of Oryza Sativa (Rice) Bran Oil and Oryza Sativa (Rice) Germ Oil, concentrations not stated, were not irritating to rabbit eyes. Undiluted Oryza Sativa (Rice) Bran Oil was considered minimally irritating.					28
Oryza Sativa (Rice) Germ Oil					
Oryza Sativa (Rice) Germ Oil, concentration not stated, was not a primary irritant.					28
Prunus Amygdalus Dulcis (Sweet Almond) Oil					
The ocular irritation potential of undiluted Prunus Amygdalus Dulcis (Sweet Almond) Oil and cosmetic formulations containing up to 25% Prunus Amygdalus Dulcis (Sweet Almond) Oil were evaluated using rabbits. Undiluted Prunus Amygdalus Dulcis (Sweet Almond) Oil was practically nonirritating or minimally irritating, and formulations containing up to 25% Prunus Amygdalus Dulcis (Sweet Almond) Oil were nonirritating to minimally irritating. In most instances, reactions that occurred were limited to conjunctival irritation, which cleared by the third day of observation.					217
Sesame Indicum (Sesame) Seed Oil					
Undiluted Sesamum Indicum (Sesame) Seed Oil was non- to minimally irritating to rabbit eyes, and a lipstick containing 10-11% Sesamum Indicum (Sesame) Seed Oil was not an ocular irritant.					55
Triticum Vulgare (Wheat) Germ Oil					
Undiluted Triticum Vulgare (Wheat) Germ Oil was, at most, a minimal ocular irritant, and 2% in a water emulsion was not irritating.					30

Table 10. **Clinical Trials/Case Studies**

Ingredient	Patients/Condition	Effect/Observation	Reference
Aleurites Moluccana Seed Oil			
Aleurites Moluccana oil	15; mild, stable plaque psoriasis	efficacy study "just enough (oil) to moisten the plaque" was applied 3 x daily for 12 wks; no side effects or adverse events were reported.	324
Anacardium Occidentale (Cashew) Seed Oil			
Anacardium Occidentale (Cashew) Seed Oil	37-year-old male resin researcher	presentation of bullae on his right leg after dropping pure oil from a bottle on his right thigh; skin was thoroughly washed immediately; erythema developed 10 days after exposure Patch testing was performed with cashew nut oil 3% alcohol, cashew nut oil 0.3% alcohol, cashew nut oil 0.03% alcohol, and urushiol 0.01% petrolatum.; a "+" reaction was reported on day 2 and "++" reactions on days 3 and 4 to the 3% dilution,; a "+" reactions to the 0.3% dilution and urushiol was reported on days 2-4; a "?+" reaction was observed on days 2 and 3 and a "+" reaction was observed on day 4 to the 0.03% dilution	325
Cocos Nucifera (Coconut) Oil			
Cocos Nucifera (Coconut) Oil		*did not produce adverse effects in several therapeutic studies*	33
Glycine Soja (Soybean) Oil			
Glycine Soja (Soybean) Oil	7; history of immediate hypersensitivity reaction after the ingestion of soybeans	a double-blind crossover study; the patients were first skin tested by the puncture method with a crude whole soybean extract, a partially hydrogenated oil, a non-hydrogenated oil, and a cold-pressed soybean oil; olive oil from a retailer was used as a negative control. Since all 7 patients had negative skin tests to the oils and positive reactions to the crude soybean extract, they were challenged orally with capsules of each of the oils in random order on 4 separate days. None of the patients reacted to the oral challenges; the researchers remarked that while a reaction to the cold-pressed soybean oil did not occur in this study, cold-pressed oils may contain soybean protein and should be avoided	63
soy oil proteins	4; known allergy to soybean	Sera was used to examine the allergenicity; neither the IgE nor the IgG_4 in the sera reacted to protein in the soy oil	23
Helianthus Annuus (Sunflower) Oil			
refined and cold-pressed sunflower oils	patients had anaphylactic reactions following ingestion of sunflower seeds	no reactions were seen upon oral or open challenge with refined or cold-pressed sunflower oils, both of which were shown to contain detectable amounts of protein.	18
	1 woman; desensitized to mugwort (of the Compositae family) pollen for a yr, then had an anaphylactic reaction to sunflower (also of the Compositae family) seeds	a delayed positive reaction to sunflower oil in a skin prick test was discovered; prick test results with 10 control subjects were negative. In an oral challenge test, a delayed reaction was again observed, with symptoms occurring 2.25-8 h after administration.	326
Macadamia Seed Oil			
Macadamia Seed Oil in a lipstick (species description or concentration were not reported)	28-year-old woman; chelitis	Chelitis case reported after lipstick use; patient was patch tested with ingredients contained in the lipstick, Positive reactions to diisostearyl malate and Macadamia Seed Oil were reported; the condition . improved after discontinuing use of lipsticks containing these 2 ingredients	327
Olea Europaea (Olive) Fruit Oil			
Olea Europaea (Olive) Fruit Oil		Throughout the literature, it is stated that sensitization to Olea Europaea (Olive) Fruit Oil is considered rare. Case reports have been described, however, and generally involved patients with venous eczema, some type of dermatitis or lesion, or an occupational exposure. Patch testing with Olea Europaea (Olive) Fruit Oil produced positive reactions in most of these cases, and these results were usually regarded as allergenic. The concentrations of Olea Europaea (Olive) Fruit Oil tested were not always given, but when stated, test concentrations giving positive results, ranged from 30-100%. When the constituents of olive oil were tested as well, the results of that testing were negative.	328-335
Olea Europaea (Olive) Fruit Oil		Whether the reactions to olive oil were contact sensitization or irritation were investigated using open and occlusive testing. It was concluded that olive oil presented as a weak irritant rather than a contact sensitizer in the few case studies observed.	336

Table 10. **Clinical Trials/Case Studies**

Ingredient	Patients/Condition	Effect/Observation	Reference
Persea Gratissima (Avocado) Oil			
Persea Gratissima (Avocado) Oil	1 female; dermatitis around the eyes and earlobes	Patch testing with her sunscreen resulted in positive results. In subsequent patch testing of the individual ingredients, a positive reaction to undiluted oil, but not to the active ingredient, was observed; 20 controls subjects were used, and reactions to Persea Gratissima (Avocado) Oil were not seen	[337]
Sesamum Indicum (Sesame) Seed Oil			
Sesamum Indicum (Sesame) Seed Oil in an ointment	female	Pruritic erythema, papules, and vesicles appeared after application of the ointment; patch testing was performed with the ointment and with the individual ingredients, including undiluted Sesamum Indicum (Sesame) Seed Oil Both the ointment and Sesamum Indicum (Sesame) Seed Oil produced positive reactions on days 2, 3, 4, and 1; the other components did not cause a reaction Results were negative in patch testing of Sesamum Indicum (Sesame) Seed Oil using 20 healthy subjects.	[338]

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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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'Reviewed by the Cosmetic Ingredient Review Expert Panel. Wilbur Johnson, Senior Scientific Analyst and Writer, prepared this report. Address correspondence to him at Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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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).

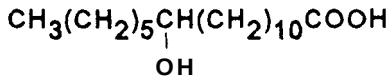

FIGURE 1
Chemical formula for Hydroxystearic Acid (Wenninger and McEwen 1995a).

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"	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

'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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7

Final Report on the Safety Assessment of Hydrolyzed Collagen

Hydrolyzed Collagen is a hydrolysate derived from animal byproducts. It is used in cosmetic products as a conditioner or moisturizer at concentrations less than 5 percent.

Hydrolyzed Collagen was practically nontoxic when administered orally or dermally in acute animal toxicity studies. This ingredient was minimally irritating to rabbit eyes when tested full-strength. Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested full-strength. Subchronic dermal studies on 2 cosmetic formulations containing 2 percent Hydrolyzed Collagen were negative for systemic toxicity. Hydrolyzed Collagen was nonsensitizing in guinea pigs.

In clinical studies, Hydrolyzed Collagen produced no skin irritation, sensitization, or indication of phototoxicity.

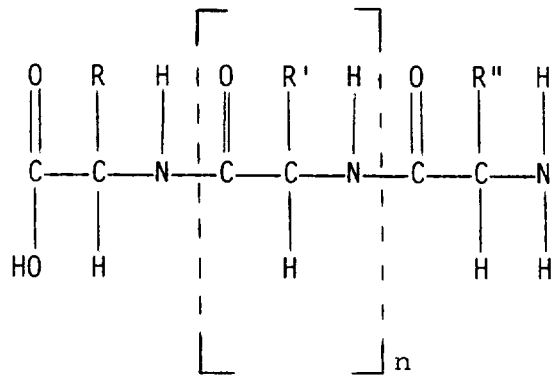
On the basis of the available animal and clinical data, it is concluded that Hydrolyzed Collagen is safe as a cosmetic ingredient in the present practices of use and concentration.

INTRODUCTION

This report presents the available information published between 1940 and the present and the unpublished cosmetic industry data on Hydrolyzed Collagen (formerly Hydrolyzed Animal Protein).

CHEMICAL AND PHYSICAL PROPERTIES

Hydrolyzed Collagen is defined as a collagen hydrolysate derived by acid, enzyme, or other method of hydrolysis.[1] It is a polypeptide of molecular weight 1000 to 10,000 with the following generalized structure:[2]



The typical amino acid composition is given in Table 1 along with the corresponding structures of R, R′, and R″.

Hydrolyzed Collagen is an off-white to white hygroscopic powder. It is also available as a viscous, amber aqueous solution.[2] The physicochemical properties of Hydrolyzed Collagen and its solution are given in Table 2.

Hydrolyzed Collagen can be prepared by any 1 of 3 methods: alkaline hydrolysis of bovine skin products followed by enzymatic hydrolysis to the desired molecular weight, enzymatic hydrolysis of fresh animal byproducts or bovine-derived leather, or acid or alkaline hydrolysis of chrome leather fold splinters with inorganic acids or lyes to a defined molecular weight. The hydrolysate produced by the latter method is purified in an aqueous solution and then by precipitation and filtration to effectively remove the heavy ions.[2] Acid hydrolysis tends to split the polypeptide bond between proline (or hydroxyproline) and other amino acids, with very little specificity for which amino acid donates its amino group to the peptide bond.[3]

The spectrum of amino acids resulting from the hydrolysis of collagen differs substantially from that of other proteins by its high content of glycine and proline and low content of histidine, tryptophan, and cystine (Table 1). Collagen also contains 2 amino acids, hydroxyproline and hydroxylysine, not found in other proteins.[4]

Hydrolyzed Collagen is analyzed primarily by column chromatography. The literature cites numerous chromatographic methods.[5-9] Its solution can be positively identified by comparison to a standard infrared spectrum.[10] The 2 amino acids found only in collagen, hydroxyproline and hydroxylysine, allow for differentiation between collagen hydrolysates and other protein hydrolysates.[4]

Hydrolyzed Collagen is stable under the conditions of normal cosmetic use. However, the addition of perfumes high in aldehyde content may cause color reactions and odor changes due to the reaction of active carbonyl groups with the amino group of the hydrolysate.[2,11] Burnett[11] has found that cosmetics containing proteins are better preserved at successively lower pH values, whereas Frech et al.[12] found that sodium acetate is an effective preservative in protein hydrolysate solutions. Hydrolyzed Collagen has shown a 15 to 45 percent reduction in viscosity after storage for 1 month at room temperature.[13]

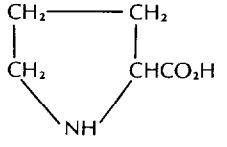
TABLE 1. Typical Amino Acid Composition of Hydrolyzed Collagen[2]

Amino Acid	*R, R', or R''*	*Typical Composition* (%)*
Glycine	$-H$	20.0–30.5
Alanine	$-CH_3$	8.0–11.0
Serine	$-CH_2OH$	2.9–4.1
Threonine	$-CHOHCH_3$	1.8–2.6
Proline	CYCLIC†	13.7–18.0
Hydroxyproline	CYCLIC†	12.1–14.5
Valine	$-CHCH_3CH_3$	2.1–3.4
Isoleucine	$-CHCH_3CH_2CH_3$	1.3–1.8
Leucine	$-CH_2CHCH_3CH_3$	2.8–3.5
Phenylalanine	$-CH_2C_6H_5$	1.1–2.6
Tyrosine	$-CH_2C_6H_4OH$	0.2–1.0
Cystine/cysteine	$-$†$/-CH_2-SH$	0.0–0.9
Methionine	$-CH_2CH_2SCH_3$	0.7–0.9
Aspartic acid	$-CH_2CO_2H$	5.7–9.0
Glutamic acid	$-CH_2CH_2CO_2H$	10.0–11.7
Arginine	$-CH_2CH_2CH_2C(NH)_2NH_2$	7.8–9.0
Histidine	$NHCHNCHCCH_2-$	0.7–1.0
Lysine	$-CH_2CH_2CH_2CH_2NH_2$	3.9–5.2
Hydroxylysine	$-(CH_2)_2CHOHCH_2NH_2$	0.7–1.2

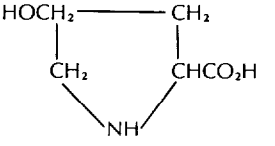
*Prepared by alkaline hydrolysis of bovine skin products to form a food-grade gelatin, followed by enzyme hydrolysis to the desired molecular weight.

†These do not conform to the generalized formula.

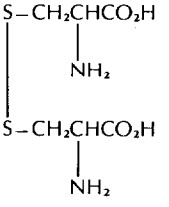
Proline:



Hydroxyproline:



Cystine:



USE

Cosmetic Use

Hydrolyzed Collagen is used in cosmetics, primarily hair and skin care products, because of its conditioning and moisturizing properties. It is generally used

TABLE 2. Physicochemical Properties of Hydrolyzed Collagen[2]

Property	Value	
	Powder	*Solution*
Molecular weight	1000 to 10,000	–
Solids content (105°C/16 hours)	–	35% min.
Moisture content (vacuum oven 90°C/6 hours)	8.0% max.	–
pH	–	4.0–6.5 (10% aqueous solution)
Nitrogen	12.0% min.	8.0% min.
Ash content	12.0% max.	5.0% max.
Iron	–	3 ppm max.
Heavy metals	–	25 ppm max.

at concentrations <5 percent in the following product categories: baby shampoos, bath, eye makeup, hair, hair coloring, makeup, manicuring, personal cleanliness, shaving, skin care, and tanning preparations.[2,13–16]

Table 3 presents the FDA product formulation data for Hydrolyzed Collagen.[15] The cosmetic product formulation computer printout that is made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations. Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at <100 percent concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

In 1981, approximately 95 percent of the 923 formulations containing Hydrolyzed Collagen incorporated the hydrolysate at concentrations of <5 percent: 23 percent of these at concentrations ≤0.1 percent, 50 percent at concentrations >0.1 to 1 percent, and 22 percent at concentrations >1 to 5 percent. Hair preparations accounted for 66 percent of the total product listings of Hydrolyzed Collagen, with the second highest listing (16 percent) found in skin care products.[15]

The formulation data presented in Table 3 indicate that cosmetic products containing Hydrolyzed Collagen may contact all external body surfaces and hair, as well as the eyes. These products may be used daily or occasionally over a period of up to several years. The frequency and length of application could result in continuous exposure.

Noncosmetic Use

Hydrolyzed Collagen is used in soaps to impart a soft texture to skin.[11] It is also used in a treatment for duodenal and gastric ulcers and gastritis.[17] A protective film-forming composition containing Hydrolyzed Collagen is also applied to cow teats.[18]

GENERAL BIOLOGY

Substantivity

The literature contains many studies documenting the substantivity of Hydrolyzed Collagen to human hair and skin. Hydrolyzed Collagen generally is adsorbed on hair; the amount of protein adsorbed is measured by hydroxyproline analysis.[13,19] Kim et al. found that adsorbed Hydrolyzed Collagen increased the tensile strength and elongation of hair. Increasing concentrations of Hydrolyzed Collagen increased adsorption.[19,20] Hydrolyzed Collagens of average molecular weight 1000 and 2000 have greater substantivity to hair and skin, respectively, than those of average molecular weight 10,000.[13,21] Brooks[22] stated that Hydrolyzed Collagen substantivity is due to the distribution of terminal amino groups along (primarily at the lysine and arginine residues) and at the ends of the polypeptide.

Cooperman and Johnsen[23] studied the penetration of Hydrolyzed Collagen into both unbleached and bleached hair strands. In the hair strands treated with the lower molecular weight Hydrolyzed Collagens, the cuticle contained the highest percentage of protein. However, the cortex, by virtue of its greater bulk, contained the greater amount of protein. Hair strands treated with the higher molecular weight Hydrolyzed Collagens had equal quantities of protein in the cuticle and cortex. Protein penetration increased with increasing damage to hair.

As a hygroscopic compound, Hydrolyzed Collagen helps bind water to the hair and skin surfaces.[11,13,24,25] Brooks[22] found that Hydrolyzed Collagen binds water better at higher relative humidities and at pH 5 rather than pH 7 or 9. Hydrolyzed Collagen potentiates epidermal metabolism by providing a suitable, moist environment on the surface of the epidermis for healthy skin and hair.[11]

The amphoteric nature of Hydrolyzed Collagen makes it an acceptable buffering agent for alkali in permanent waving products. It is incorporated in waving formulations to avoid or minimize damage to hair. A protective application of Hydrolyzed Collagen is sometimes made prior to waving. Hydrolyzed Collagen is also used in hair dyes to insure uniformity in dyeing.[11]

General Effects

Various enzymes will hydrolyze collagen: trypsin,[26] intracellular proteolytic enzymes of *Oidiodendron kalari*,[27] *Streptomyces griseus* protease,[28,29] collagenases of genera *Bacteroides, Clostridium,* and *Peptostreptococcus*,[30,31] rat hepatic lysosomal extracts,[32] collagenases from rabbit synovial fibroblasts,[33] and cathepsin B and collagenolytic cathepsin from human placenta.[34]

TABLE 3. Product Formulation Data[15]

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)						
			>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Hydrolyzed Collagen									
Baby shampoos	35	1	–	–	–	–	–	–	1
Bath oils, tablets, and salts	237	2	–	–	–	–	2	–	–
Bubble baths	475	2	–	–	–	–	–	2	–
Other bath preparations	132	2	–	–	–	–	–	2	–
Eyeliner	396	1	–	–	–	–	–	–	1
Eye shadow	2582	6	–	–	–	–	–	5	1
Mascara	397	28	–	–	–	–	–	15	13
Other eye makeup preparations	230	5	–	–	–	–	1	1	2
Hair conditioners	478	174	2	1	2	13	60	79	17
Hair sprays (aerosol fixatives)	265	7	–	–	–	–	–	1	6
Hair straighteners	64	7	–	–	–	–	–	7	–
Permanent waves	474	70	–	–	1	8	24	21	16
Hair rinses (noncoloring)	158	34	–	–	–	1	10	17	6
Hair shampoos (noncoloring)	909	224	–	–	–	3	36	133	52
Tonics, dressings, and other hair grooming aids	290	35	1	–	1	–	11	17	5
Wave sets	180	39	–	–	2	–	4	19	14
Other hair preparations (noncoloring)	177	18	–	–	–	1	6	7	4
Hair tints	15	14	–	–	–	–	13	1	–
Hair rinses (coloring)	76	24	–	–	–	–	–	–	24

Hair bleaches	111	7	–	–	–	–	3	3	1
Other hair coloring preparations	49	1	–	–	–	–	–	1	–
Blushers (all types)	819	5	–	–	–	–	–	5	–
Face powders	555	5	–	–	–	–	–	2	3
Makeup foundations	740	10	–	–	–	–	–	8	2
Lipstick	3319	15	–	–	–	–	–	9	6
Makeup bases	831	15	–	–	–	–	–	10	5
Cuticle softeners	32	3	–	–	–	–	–	1	2
Nail creams and lotions	25	6	–	1	–	1	2	1	1
Nail polish and enamel	767	1	–	–	–	–	1	–	–
Nail polish and enamel remover	41	2	–	–	–	–	–	–	2
Other manicuring preparations	50	6	–	–	–	–	3	1	2
Bath soaps and detergents	148	3	–	–	–	–	1	2	–
Aftershave lotions	282	3	–	–	–	–	–	3	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	27	–	–	–	–	4	14	9
Face, body, and hand skin care preparations (excluding shaving preparations)	832	46	–	–	–	1	4	37	4
Moisturizing skin care preparations	747	43	–	–	1	1	11	23	7
Night skin care preparations	219	11	–	–	–	1	5	5	–
Paste masks (mudpacks)	171	6	–	–	–	–	3	1	2
Skin fresheners	260	7	–	–	–	–	1	5	1
Wrinkle smoothers (removers)	38	1	–	–	–	–	1	–	–
Other skin care preparations	349	7	–	1	–	–	2	2	2
1981 TOTALS		923	3	3	7	30	208	461	211

In other studies, a proteinase from *Pseudomonas aeruginosa* catalyzed the hydrolysis of collagen,[35] whereas anthocyanosides isolated from *Vaccinium myrtillis* decreased collagen hydrolysis.[36] Hydrolyzed Collagen induced the activity of an extracellular collagenase produced by a marine *Vibrio*,[37] and a secondary vitamin K deficiency in rats increased the hydrolysis of collagen.[38] The epimerization of trans-4-hydroxy-L-proline to cis-4-hydroxy-D-proline during acid hydrolysis of collagen has also been documented.[39]

Collagen hydrolysates have been studied for their stimulatory effect on the healing of open wounds.[40] The ears of 1 rabbit were incised: 1 was kept as a control and 1 was treated with Hydrolyzed Collagen. The same rate of healing was observed for the first few days; thereafter, the treated ear healed more rapidly.[16]

Animal Toxicology

Acute Toxicity

Oral

Hydrolyzed Collagen (100 percent) was analyzed for acute oral toxicity in rats in 2 tests. LD_{50}s were >10 g/kg and >15 g/kg, respectively. In each study, investigators concluded that Hydrolyzed Collagen was nontoxic by ingestion[41,42] (Table 4).

Two shampoo formulations, each containing 2 percent Hydrolyzed Collagen, were tested for acute oral toxicity in mice and rats. LD_{50}s were >15 ml/kg in both mice and rats. The investigators concluded that each formulation was practically nontoxic[43,44] (Table 4).

A hair conditioner containing 0.5 percent Hydrolyzed Collagen had an oral LD_{50} of >40.0 ml/kg in rats, estimated by interpolation from the probit response curve. The investigators' observations included decreased activity, rales, diarrhea, salivation, and an increase in body weight[46] (Table 4).

Ocular Irritation

Four lots of Hydrolyzed Collagen (100 percent) were evaluated for ocular irritation by a modified Draize eye test. A 0.1 ml sample of Hydrolyzed Collagen

TABLE 4. Acute Oral Toxicity of Hydrolyzed Collagen

Compound	*Species*	*LD_{50}*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100%	Rat	>10 g/kg	Nontoxic	41
Hydrolyzed Collagen, 100%	Rat	>15 g/kg	Nontoxic	42
Hydrolyzed Collagen, 2% in a shampoo	Mouse	>15 g/kg	Practically nontoxic	43
Hydrolyzed Collagen, 2% in a shampoo	Rat	>15 g/kg	Practically nontoxic	44
Hydrolyzed Collagen 0.5% in a hair conditioner	Rat	>40.0 ml/kg	–	45

was instilled into the conjunctival sac of 1 eye of each of 6 albino rabbits. Eyes were scored by Draize classification (max, 110) at 1, 2, 3, 4, and 7 days or until negative. The 4 lots had average irritation scores of 5, 4, 3, 0; 6, 3, 1, 0; 5, 3, 3, 0; and 6, 3, 3, 0 on Days 1, 2, 3, and 4, respectively. As judged by the Draize classification of eye irritation, Hydrolyzed Collagen was mildly irritating[47] (Table 5).

Hydrolyzed Collagen was evaluated as a 50 percent aqueous solution in a modified Draize eye irritation test. A 0.1 ml sample of the solution was instilled into the conjunctival sac of 1 eye of each of 6 albino rabbits; the other eye served as a control. The eyes were examined for 7 days or until negative. Average irritation scores were 1 and 0 (max, 110) on Days 1 and 2, respectively. As judged by the Draize classification of eye irritation, the test solution was minimally irritating[48] (Table 5).

A shampoo formulation containing 2 percent Hydrolyzed Collagen was tested for ocular irritation in 9 albino rabbits. The shampoo was diluted to a concentration of 20 percent (w/v) with distilled water, and a 0.1 ml sample was instilled into the conjunctival sac of the left eye. Each right eye served as a control.

TABLE 5. Ocular Irritation of Hydrolyzed Collagen

Compound	*Number of Rabbits/Test Groups*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100% (4 lots)	6	Average irritation scores (max, 110) on Days 1, 2, 3, and 4, respectively were: Lot 1–5, 4, 3, 0 Lot 2–6, 3, 1, 0 Lot 3–5, 3, 3, 0 Lot 4–6, 3, 3, 0; Hydrolyzed Collagen was concluded to be mildly irritating	47
Hydrolyzed Collagen, 50% aqueous solution	6	Average irritation scores (max, 110) were 1 and 0 on Days 1 and 2, respectively; test solution concluded to be minimally irritating	48
Hydrolyzed Collagen, 0.2% in a shampoo, tested as a 20% (w/v) aqueous dilution	9/ 3 rinsed 6 unrinsed	No irritation observed in rinsed eyes; unrinsed eyes had mean total scores (max, 130) of 3.33, 0.67, 0.0, and 0.0 at 24, 48, and 72 hours and 7 days, respectively; concluded that shampoo was a nonirritant but could cause minimal irritation under test conditions	49
Hydrolyzed Collagen, 0.5% in a hair conditioner	9/ 3 unrinsed 3 rinsed 3 received 1:9 dilution with water	Conjunctival scores of 2 or less (max, 110) seen in all unrinsed eyes, 1 rinsed, and 1 dilution eye; effects cleared by 72, 72, and 48 hours, respectively; concluded that hair conditioner was a mild, transient irritant under all test conditions	50

The eyes of 3 rabbits were rinsed with water 2 seconds after application; the other 6 were not rinsed. Eyes were scored at 24, 48, and 72 hours and 7 days. The unrinsed treated eyes had conjunctival irritation at 24 hours consisting of slight erythema (6 rabbits), very slight edema (3 rabbits), and slight discharge (1 rabbit). Irritation decreased progressively, and all unrinsed eyes were normal at 72 hours. No irritation was observed in the rinsed eyes throughout the 7-day period. Mean total scores for the unrinsed eyes were 3.33, 0.67, 0.0, and 0.0 (max, 130) at 24, 48, and 72 hours and 7 days, respectively. The shampoo was found to be a nonirritant. However, under test conditions, it could cause minimal eye irritation[49] (Table 5).

A hair conditioner containing 0.5 percent Hydrolyzed Collagen was tested for ocular irritation in 9 albino rabbits. A 0.1 ml sample was applied to the right eye of each rabbit. The rabbits were divided into groups of 3: Group I received the product full-strength, Group II received a full-strength application followed by rinsing 4 seconds later, and Group III received a 1:9 dilution of the conditioner with water. Ocular reactions were recorded 24, 48, and 72 hours and 7 days after instillation of the test material. Conjunctival effects (score of 2 or less, max, 110) were seen in all 3 rabbits of Group I and in 1 rabbit of Groups II and III. These effects had disappeared by 72 hours in Groups I and II and by 48 hours in Group III. The investigators concluded that the hair conditioner was a mild, transient irritant when applied full-strength with or without rinsing, or diluted 1:9 with water[50] (Table 5).

Dermal/Primary Skin Irritation

Hydrolyzed Collagen (100 percent) was tested for primary skin irritation using 6 rabbits. The backs of the rabbits were clipped and divided into 2 sections, of which 1 was abraded. Hydrolyzed Collagen was applied to both sections under gauze pads for 24 hours. Sites were scored upon patch removal and 48 hours later. No reactions were noted. The investigators concluded that Hydrolyzed Collagen was nonirritating[41] (Table 6).

Hydrolyzed Collagen was applied full-strength to the shaved backs of 9 rabbits in a modified Draize primary skin irritation test. A 0.1 ml sample was applied to each rabbit by occlusive filter disc for 24 hours. Four lots of Hydrolyzed Collagen were evaluated. Sites were graded upon disc removal and 48 hours later. Average irritation socres (max, 4) for the 4 lots were 0.25, 0.20, 0.16, and 0.26. The investigators concluded the Hydrolyzed Collagen was minimally irritating[51] (Table 6).

Hydrolyzed Collagen was evaluated as a 50 percent aqueous solution for primary skin irritation using 9 albino rabbits. Samples of 0.1 ml were applied by occlusive filter disc to the shaved skin of the rabbits for 24 hours. Applications were made for 3 consecutive days. Sites were scored for irritation 24 hours after each application. The maximum average irritation response was 1.33 (max, 4); the test solution was considered mildly irritating[52] (Table 6).

Various concentrated solutions of a soap containing 26 to 28 percent Hydrolyzed Collagen were brushed onto the skin of guinea pigs (number unspecified). Skin changes were monitored clinically and microscopically. Body weights were recorded and necropsies performed on a number of the animals. No internal in-

juries were noted. The soap was considered significantly less irritating than a lauryl sulfate solution of the equivalent concentration[53] (Table 6).

A shampoo formulation containing 2 percent Hydrolyzed Collagen was evaluated for skin irritation and acute dermal toxicity. Three groups of 4 rabbits each received doses of 1.5, 3, and 6 ml/kg of the test shampoo, and a fourth group received 6 ml/kg of a regular shampoo as a comparative control. Backs of all rabbits were clipped; 2 in each group also were abraded. The shampoos were applied full-strength under an occlusive wrap for 24 hours. Sites were evaluated according to Draize at 24, 48, and 72 hours and 7 and 14 days. At 24 and 48 hours, moderate erythema (score of 2 on 0 to 4 scale) was seen in all control rabbits and 1 high-dose nonabraded rabbit. Slight erythema (score of 1) was noted in all others, and no edema was noted. The skin appeared darkened at 48 hours in 1 control rabbit and in the high-dose rabbit with moderate erythema. At 72 hours, no erythema or edema was noted. However, the same 2 rabbits still had darkened skin, whereas peeling and scaling of the skin was observed in the others. At 7 and 14 days, treated skin sites appeared normal except for areas of pustular dermatitis due to a secondary infection; 1 high-dose rabbit had darkened, thickened skin (Day 7), and 1 had dry, cracked, and peeling skin (Day 14). The investigators reported that application of the shampoo caused erythema and burning of the skin of the rabbit. Its application, under exaggerated conditions, was responsible for the degeneration and necrosis of the skin of some rabbits and was followed by secondary bacterial infection in others as manifested by a pustular dermatitis. There were no significant differences in hematological and urinalysis values. A dose-related decrease in feed consumption and body weight gain was observed. However, there were no other signs of systemic toxicity. Necropsy of the 3 rabbits that died during the study (2 control, 1 high dose) indicated that acute pneumonia was the prime contributing factor[54] (Table 6).

Two other shampoo formulations, each containing 2 percent Hydrolyzed Collagen, were evaluated for skin irritation using identical tests. The first shampoo was administered full-strength, whereas the second was diluted to 20 percent (w/v) in distilled water. Samples of 0.5 ml of the shampoo or dilution were administered to the intact and abraded skin of 6 rabbits and occluded for 24 hours. Sites were scored according to Draize at 24, 48 (shampoo only), and 72 hours. Moderate to severe irritation was observed in the rabbits treated with the full-strength shampoo; very slight to mild irritation was seen in the rabbits receiving the dilution treatments (Table 6). The shampoo and dilution had primary irritation scores (PIS) of 4.67 and 1.46, respectively, where a score of 5 (max, 8) is considered to be a primary skin irritant. The investigators concluded that the shampoo was a dermal irritant to the intact and abraded skin of rabbits, whereas the dilution was classified as a nonirritant. However, it was noted that mild irritation can occur under these test conditions.[55,56]

A hair conditioner containing 0.5 percent Hydrolyzed Collagen was evaluated for dermal irritation in a combined irritation and phototoxicity test. Occlusive patches containing 0.20 ml of the conditioner were applied to the backs of 6 clipped rabbits for 48 hours. Sites were scored for erythema and edema at 49, 72, and 96 hours; all rabbits had a score of 0 (max, 8). It was concluded that the hair conditioner was not a primary dermal irritant[57] (Table 6).

TABLE 6. Primary Skin Irritation/Dermal Toxicity of Hydrolyzed Collagen

Compound	*Test Method*	*Number and Species of Animals*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100%	Applied under gauze pads for 24 hours. I, A*	6 rabbits	No reactions PII† of 0; nonirritating	41
Hydrolyzed Collagen, 100% (4 lots)	Occlusive disc for 24 hours–I	9 rabbits	Average irritation scores (max, 4) for 4 lots were 0.25, 0.20, 0.16, and 0.26; concluded to be minimally irritating	51
Hydrolyzed Collagen, 50% aqueous solution	Occlusive disc for 24 hours, 3 consecutive applications–I	9 rabbits	Maximum average irritation response was 1.33 (max, 4); test solution considered mildly irritating	52
Hydrolyzed Collagen, 26–28% in a soap	Various concentrations brushed on–I	Guinea pigs, unspecified number	Considered significantly less irritating than a lauryl sulfate solution of the equivalent concentration; no internal injuries noted	53
Hydrolyzed Collagen, 2% in a shampoo	Occlusive patch for 24 hours–I, A–doses of 1.5, 3, and 6 ml/kg	12 rabbits	Moderate erythema observed in all control rabbits and 1 high-dose rabbit (score of 2, max, 4), slight erythema (score of 1) in all others, clearing by 72 hours; no edema noted; several rabbits had darkened or cracked and peeling skin, pustular dermatitis due to secondary infection in others; dose-related decrease in feed con-	54

			sumption and body weight gain; no other signs of systemic toxicity; conclusion: under exaggerated conditions, caused erythema and burning of rabbit skin and was responsible for the degeneration and necrosis of skin, allowing secondary infection	
Hydrolyzed Collagen, 2% in a shampoo	Occlusive patch for 24 hours–I, A	6 rabbits	Moderate to severe and well-defined erythema was observed in 5 and 1 rabbits, respectively, at 24 hours, with similar results at 72 hours; very slight, slight, and moderate edema was observed in 1, 3, and 2 rabbits, respectively, at 24 hours, decreasing minimally by 72 hours; PIS‡ of 4.67 (max, 8); concluded to be dermal irritant on I and A skin	55
Hydrolyzed Collagen, 2% in a shampoo–diluted to 20% (w/v) in distilled water	Occlusive patch for 24 hours–I, A	6 rabbits	Well-defined and very slight erythema was observed in 3 and 3 rabbits, respectively, at 24 hours, diminishing to only very slight scores in 5 rabbits at 72 hours; slight and very slight edema was observed in 1 and 3 rabbits, respectively, at 24 hours, clearing totally by 72 hours; PIS of 1.46 (max, 8); concluded to be a nonirritant on I and A skin but can cause mild irritation under study conditions	56
Hydrolyzed Collagen, 0.5% in a hair conditioner	Occlusive patch for 48 hours–I	6 rabbits	No erythema or edema; all rabbits had individual score of 0 (max, 8); concluded to be not a primary dermal irritant	57

*I, intact; A, abraded.
†PII, primary irritation index.
‡PIS, primary irritation scores.

Subchronic Toxicity

Dermal

A hair preparation containing 2 percent Hydrolyzed Collagen was tested for subchronic dermal toxicity. Three groups of 2 male and 2 female rabbits received 100, 1000, or 3200 mg/kg of the test shampoo. Control groups received 2000 mg/kg of a marketed antidandruff shampoo, 3220 mg/kg of the test shampoo without the active drug ingredient, and 1 ml water/kg. All test and control applications were made daily for 30 days, were left on the skin for 15 minutes, and were then removed with water. The skin of 1 male and 1 female in each group was abraded weekly. The only treatment-related finding was local skin irritation. No deaths, abnormal behavior, gross or microscopic lesions were associated with treatment.[58]

Another shampoo containing 2 percent Hydrolyzed Collagen was tested for dermal toxicity using Yorkshire pigs (white). Three groups of 2 male and 2 female pigs (1 of each sex abraded twice weekly) received applications of 0.5, 1.0, and 2.0 ml/kg. Control groups received 2.0 ml/kg saline and 2.0 ml/kg of another marketed shampoo. Applications were made to the clipped back of each pig twice daily for 4 weeks. Treated sites were rinsed with warm water 1 hour after each application. All pigs were given a general physical examination before and at 4 weeks observation. Body weights were recorded weekly, and blood samples were obtained for routine hematological and serum chemistry evaluations. All animals were necropsied. The abraded skin of 1 high-dose female was slightly irritated; the skin of all others appeared unremarkable. No dose-related effects were determined by physical examination, hematological and serum chemistry evaluations, necropsy examination, and histopathological evaluation. A statistically significant dose-regression relationship for male gonad weights was not considered indicative of systemic toxicity due to the immaturity and variation in size of the testes of these young pigs. Minimal focal inflammatory cellular infiltration was noted in the treated dermis of 1 middle-dose and 1 high-dose pig. However, this same condition was observed in the untreated skin of 1 saline control and 1 high-dose pig.[59]

Sensitization

Hydrolyzed Collagen was tested for sensitization using 2 male white guinea pigs. A 0.1 percent solution of Hydrolyzed Collagen in physiological saline was injected intracutaneously into the clipped back or flank of each guinea pig every other day or 3 times weekly for a total of 10 injections. The first injection consisted of 0.05 ml of the test solution; each succeeding injection consisted of 0.1 ml. Sites were scored for diameter, height, and color 24 hours after each injection. After a 2-week rest period, a challenge injection of 0.05 ml was administered into a different site. Induction injections gave average diameter scores of 7.6 and 8.9 mm, average heights of 0.3 and 0.2 mm, and an average color of pink for the 2 guinea pigs. On challenge, 1 animal had no reaction, whereas the second had a reaction of diameter 5 mm, no height, and a pink color. Both animals had a sensitization score of 0 (9 or above is severely sensitizing), classifying Hydrolyzed Collagen as a nonsensitizer.[41]

A shampoo formulation containing 2 percent Hydrolyzed Collagen was

tested for sensitization using a modification of the Buehler and Griffith[60] method. A total of 3 inductive applications, 1 per week, were made to the clipped back of 10 guinea pigs. The first inductive application consisted of 0.5 ml of a 1, 5, and 10 percent dilution (distilled water v/v) occlusively patched on 3 separate sites on the right side of the animal for 24 hours. The second and third inductions consisted of 0.5 ml of a 10 percent aqueous dilution occlusively patched on the left side of the animal for 6 hours. A 24-hour challenge patch was applied 2 weeks later to an untreated site on the animal's right side. Sites were scored for erythema 24 hours after each application. Dinitrochlorobenzene (DNCB) was tested as a positive control. The first and third inductive patches produced no erythema, and the second produced very slight erythema in 2 guinea pigs. No erythema was observed at challenge. The investigators concluded that the shampoo formulation did not cause sensitization.[45]

Phototoxicity

A shampoo formulation containing 2 percent Hydrolyzed Collagen was tested for phototoxicity using 2 guinea pigs. The shampoo was tested as a 20 percent (w/v) mixture in distilled water, and 8-methoxypsoralen was tested undiluted as a positive control. The back of each animal was clipped and divided into 4 sites; 2 received 0.1 ml of the shampoo and 2 received 0.05 ml of 8-methoxypsoralen. Fifteen to twenty minutes later, the right side of each animal was shielded with cardboard while the animals were irradiated for 1 hour with UVA light (320 to 400 nm) using a No. F40 BL 40W Westinghouse Blacklight. Sites were graded for erythema (max, 4) 24 hours after exposure. All exposed and unexposed sites treated with the shampoo dilution had scores of 0. 8-Methoxypsoralen gave a mean score of 3.5 for the irradiated sites and a score of 0 for the nonirradiated sites. The investigators concluded that the shampoo formulation was not phototoxic in guinea pigs.[61]

A hair conditioner containing 0.5 percent Hydrolyzed Collagen was also analyzed for phototoxicity using 6 rabbits. One rabbit received 8-methoxypsoralen as a positive control. A 0.20 ml sample of the conditioner was applied to a gauze patch, evaporated for 5 minutes, and then placed on the clipped back of the rabbit and occluded. Two patches were applied to each animal. Two hours later, 1 patch was removed and the other protected with aluminum foil while the animals were irradiated for 15 minutes with Sylvania lights No. F-40-BLB. The patches were then replaced until 48 hours posttreatment, at which time all patches were removed. Sites were scored for erythema and edema (max, 8) 1 hour later and at 72 and 96 hours. Each rabbit had an individual score of 0 for both irradiated and nonirradiated sites. The hair conditioner was neither a primary dermal irritant nor a phototoxic irritant to rabbit skin.[57]

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

Hydrolyzed Collagen (100 percent) was tested for skin irritation on 20 humans. A single patch containing 0.1 ml of Hydrolyzed Collagen was applied to the volar forearm or the inner aspect of the arm of each subject. A standard con-

trol was also tested. Reactions were recorded 2 and 24 hours after patch removal. The irritation score for Hydrolyzed Collagen and the controls in all 20 subjects was 0 (max, 4). No significant difference in irritancy potential existed between Hydrolyzed Collagen and the control[62] (Table 7).

Patch tests were performed on 33 subjects (18 men and 15 women) using Hydrolyzed Collagen at concentrations of 2 and 20 percent. Occlusive patches containing Hydrolyzed Collagen at each concentration were applied to the breast or arm for 24 hours. Sites were scored at 24, 48, and 72 hours; no reactions were observed[63] (Table 7).

A 21-day cumulative irritation test was conducted on a hair conditioner containing 0.5 percent Hydrolyzed Collagen. Semiocclusive patches with 0.5 ml of the conditioner were applied to the upper part of the back of 20 subjects for 24 hours. Patches were then removed, evaluated 30 minutes later, and a new patch was applied. These procedures were repeated for 15 applications, allowing for 21-day continuous exposure. Mineral oil was used as the standard control. Of the 17 subjects who completed the study, only 1 had any reaction, giving a cumulative irritation score of 0.5 (max, 84). The mean cumulative irritation score of 0.03 was exactly comparable to that of mineral oil (0.03). The investigators concluded that the product as used by label directions would not present any medical hazard to the consumer[64] (Table 7).

Various compositions of a soap containing 26 to 28 percent Hydrolyzed Collagen were applied daily to the skin as a 5 percent solution for 10 to 48 days. A large number of healthy subjects and people with dermatitis were used. A low degree of irritation was seen even at high concentrations of the least irritating composition. No sensitization was observed. The treated skin area was examined microscopically; those with acute dermatitis had moderate irritation[53] (Table 7).

Five cosmetic formulations were evaluated for irritation and sensitization in repeated insult patch tests (RIPT). Three of these, a morning cream, a suntan lotion, and a night cream, containing 3.0, 2.2, and 3.0 percent Hydrolyzed Collagen, respectively, were tested in the same manner. A series of 10 48-hour occlusive patches containing the undiluted formulation was applied to the back of each subject. Sites were graded after each removal and 24 hours after removal of the tenth patch (morning cream and suntan lotion only). After approximately an 11-day rest period, challenge patches were applied, occluded for 48 hours, and scored upon removal and 24 hours later. Scattered irritant responses after the third application were seen in the 103 subjects who completed the induction phase for the morning cream and suntan lotion. The maximum number of responses seen at any 1 reading for the suntan lotion was 11 with erythema and 8 with very mild erythema. Maximum response to the morning cream was 5 with erythema and 10 with very mild erythema. Of the 96 subjects who completed the challenge phase, 1 had erythema and 1 had very mild erythema to the suntan lotion at 24 hours. However, the panelist with erythema had a negative reaction on rechallenge. No reactions were observed on challenge with the morning cream. Two of the 113 panelists completing the induction phase testing of the night cream had irritant responses: 1 had very mild erythema and 1 had erythema. One of the 103 subjects completing the challenge phase had erythema. The investigators concluded that the morning cream, suntan lotion, and night cream were mildly irritating, definitely irritating, and nonirritating, respectively, whereas none of the formulations gave significant evidence of sensitization[65,66] (Table 7).

TABLE 7. Clinical Irritation and Sensitization

Ingredient	*Type of Test*	*Number of Humans*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100%	Single patch-type and duration not specified	20	Average irritation score of 0 for all 20 subjects (max, 4); no significant difference between test material and control	62
Hydrolyzed Collagen, 2 and 20%	Single, 24-hour occlusive patch	33	No reactions were observed	63
Hydrolyzed Collagen, 0.5% in a hair conditioner	21-day cumulative irritation test	17	One subject had a reaction, giving a cumulative irritation score of 0.5 (max, 84); mean cumulative irritation score of 0.03 was exactly comparable to control; product should not present any medical hazard; nonirritant	64
Hydrolyzed Collagen, 26–28% in a soap—tested as 5% solution of the soap	Applied daily to skin for 10–48 days	Large number (unspecified), both healthy subjects and some with dermatitis	Low degree of irritation, no sensitization was observed; those with acute dermatitis showed moderate irritation	53
Hydrolyzed Collagen, 3.0% in a morning cream	RIPT*	103–I† 96–C	Scattered irritant responses, maximum response at any one reading was 10 very mild erythemas and 5 erythemas; no reactions on challenge; mildly irritating and nonsensitizing	65
Hydrolyzed Collagen, 2.2% in a suntan lotion	RIPT	103–I 96–C	Scattered irritant responses, maximum response at any one reading was 8 very mild erythemas and 11 erythemas; 2 reactions to the challenge, 1 very mild erythema and 1 erythema; negative reaction on rechallenge; definitely irritating and nonsensitizing	65
Hydrolyzed Collagen, 3.0% in a night cream	RIPT	113–I 103–C	Two irritant responses: 1 very mild erythema and 1 erythema; 1 erythema reaction on challenge; nonirritating and nonsensitizing	66
Hydrolyzed Collagen, 0.5% in a mascara	RIPT	205	One subject exhibited faint erythema during the induction phase; no reactions at challenge; nonirritating and nonsensitizing	67

TABLE 7. (Continued)

Ingredient	*Type of Test*	*Number of Humans*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 0.5% in a hair conditioner tested as a 0.1% dilution	RIPT	207–I 201–C	Mean cumulative irritation scores (max, 50) as follows: 167 subjects had score of 0 22 subjects had score of 1 12 subjects had score of 2 1 subject had score of 3 1 subject had score of 4 3 subjects had score of 5 1 subject had score of 6; one subject exhibited erythema at challenge; however, this panelist reacted to 10 of the other 13 products and had no reaction on rechallenge	68
Hydrolyzed Collagen, 0.5% in a mascara	Controlled use test, 4 weeks of daily use	27	No reactions were observed	69

*Repeated insult patch test.
†I, induction; C, challenge.

The fourth formulation tested by RIPT was a mascara containing 0.5 percent Hydrolyzed Collagen. Occlusive patches containing the undiluted mascara were applied to the upper backs of 205 subjects on Monday, Wednesday, and Friday for 3 consecutive weeks. Patches were removed and sites graded just prior to the next scheduled patch application. Following a 2-week rest, 2 consecutive 48-hour challenge patches were applied to adjacent sites on the back. Sites were graded at 48 and 96 hours. One subject had faint erythema during the induction phase; no reactions were observed at challenge. The mascara was found to be neither an irritant nor a sensitizer[67] (Table 7).

The fifth formulation evaluated by RIPT was a hair conditioner containing 0.5 percent Hydrolyzed Collagen. A 0.1 percent dilution of the conditioner was applied using semioccluded patches to the upper backs of the subjects for 48 hours. Patches were then removed, sites evaluated, and new patches applied for a total of 10 applications. Following a 2-week rest period, challenge patches were applied to the subjects' thighs. Mean cumulative irritation scores (max, 50) were as follows: 167 subjects had a score of 0, 22 subjects had a score of 1, 12 subjects had a score of 2, 3 subjects had scores of 3, 4, and 6 (each), and 3 subjects had a score of 5. Of the 201 subjects completing the challenge phase of the study, 1 had erythema. However, this panelist also reacted to 10 of the other 13 substances being tested. A rechallenge on the other thigh produced no reaction[68] (Table 7).

A mascara formulation containing 0.5 percent Hydrolyzed Collagen was evaluated by a 4-week controlled use test. Twenty-seven women used the product daily as per normal instructions. No reactions were observed[69] (Table 7).

A prospective study of cosmetic-induced dermatitis by 11 dermatologists of the North American Contact Dermatitis Group (NACDG) identified 1 case of dermatitis associated with use of Hydrolyzed Collagen from among a total of 487 cases.[70]

Phototoxicity/Photosensitization

A mascara formulation containing 0.5 percent Hydrolyzed Collagen was tested for phototoxicity/photosensitization on a panel of 23 humans. Occlusive patches containing 0.1 g/cm^2 of the mascara were applied to the backs of the subjects for 24 hours. Patches were then removed, evaluated, and irradiated with 3 times the individual's MED using a xenon arc solar simulator (150 W) filtered to produce a continuous UVA-UVB emission spectrum (290 to 400 nm). Sites were evaluated 48 hours later, and the procedures of application, patching, and irradiation were repeated for a total of 7 applications. No reactions were observed. The investigators concluded that the mascara was neither phototoxic nor a photosensitizer.[71]

Domsch et al.[72] have found that UV-induced erythema was decreased by rubbing Hydrolyzed Collagen (mean molecular weight of 1500) into the skin. A 10 percent solution of Hydrolyzed Collagen applied immediately and 24 hours after irradiation decreased erythema by 20 percent at 24 hours and 25 percent at 48 hours.

SUMMARY

Hydrolyzed Collagen is a collagen hydrolysate derived by acid, enzyme, or other method of hydrolysis. It is a white to off-white hygroscopic powder of molecular weight 1000 to 10,000 and is also available as a viscous, amber aqueous solution.

Hydrolyzed Collagen can be prepared by any 1 of 3 methods: alkaline hydrolysis of bovine skin products followed by enzymatic hydrolysis to the desired molecular weight, enzymatic hydrolysis of fresh animal byproducts or bovine-derived leather, or acid or alkaline hydrolysis of chrome leather fold splinters with inorganic acids or lyes to a defined molecular weight. The hydrolysis of collagen yields a high content of glycine and proline compared to other proteins, as well as 2 unique amino acids, hydroxyproline and hydroxylysine. Hydrolyzed Collagen is usually analyzed by column chromatography.

Hydrolyzed Collagen is used in cosmetics, primarily hair and skin care products, because of its conditioning and moisturizing properties. It is usually incorporated at concentrations <5 percent and was in 923 formulations reported in 1981. Cosmetic products containing Hydrolyzed Collagen may contact all external body surfaces and hair, as well as the eyes. Frequency and length of application could result in continuous exposure.

Many studies have documented the substantivity of Hydrolyzed Collagen to human hair and skin. Hydrolyzed Collagen generally is adsorbed on hair, although it has been shown to penetrate the cuticle and cortex. Increasing concentrations of Hydrolyzed Collagen increased adsorption, as did increasing damage to hair. Hydrolyzed Collagen also binds water to the hair and skin surfaces and is used as a buffering agent for alkali in permanent waving preparations.

Acute toxicity studies found Hydrolyzed Collagen and formulations containing Hydrolyzed Collagen practically nontoxic when administered orally to mice and rats. Dermal studies gave no indication of systemic toxicity when formulations containing Hydrolyzed Collagen were applied to rabbits and guinea pigs. However, a shampoo formulation (2 percent Hydrolyzed Collagen) administered to rabbits under exaggerated conditions did cause erythema and burning, leading to degeneration and necrosis of the skin.

Hydrolyzed Collagen was minimally irritating to rabbit eyes when tested full-strength and in formulation. Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested full-strength, whereas a 50 percent aqueous solution of Hydrolyzed Collagen was mildly irritating. Shampoo formulations containing Hydrolyzed Collagen (2 percent) were generally nonirritating when tested as dilutions. However, these were irritating under the exaggerated conditions of a full-strength application. A soap (26 to 28 percent Hydrolyzed Collagen) and hair conditioner (0.5 percent Hydrolyzed Collagen) produced no dermal irritation in guinea pigs and rabbits, respectively.

Subchronic dermal studies on 2 cosmetic formulations containing 2 percent Hydrolyzed Collagen were negative for systemic toxicity in rabbits and Yorkshire pigs.

Hydrolyzed Collagen was nonsensitizing in guinea pigs. Cosmetic formulations containing Hydrolyzed Collagen (2, 2, and 0.5 percent) were also nonsensitizing and nonphototoxic in guinea pigs and rabbits.

In clinical studies, Hydrolyzed Collagen produced no skin irritation. Formulations containing Hydrolyzed Collagen at concentrations ranging from 0.5 to 28 percent produced some irritation. However, no significant evidence of sensitization was observed in any study. No phototoxicity or photosensitization was evident in a study of a mascara containing 0.5 percent Hydrolyzed Collagen. It has been reported that UV-induced erythema was decreased by rubbing Hydrolyzed Collagen into the skin after irradiation.

CONCLUSION

On the basis of the available animal and clinical data presented in this report, the Panel concludes that Hydrolyzed Collagen is safe as a cosmetic ingredient in the present practices of use and concentration.

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4

Final Report on the Safety Assessment of Isostearic Acid

Isostearic Acid is a mixture of fatty esters consisting mainly of methyl branched isomers of octadecanoic acid and is used at concentrations up to 10% in a wide variety of cosmetic products. In rats, the acute oral LD50 is estimated to be greater than 32 ml/kg. The raw ingredient produced no significant skin or eye irritation in Draize rabbit irritation tests.

In clinical studies, 100 subjects showed no signs of irritation after a 24 h single insult skin patch with undiluted Isostearic Acid. Thirty-four percent Isostearic Acid was neither an irritant nor a sensitizer in 168 subjects, and gave no indication of phototoxicity in a subset of this population.

It is concluded that Isostearic Acid is safe as a cosmetic ingredient in the present practices of use. Consideration for the compound's potential for production of human comedogenicity is noted.

CHEMISTRY

Composition

Isostearic Acid is the Cosmetic, Toiletry and Fragrance Association (CTFA) adopted name for a complex blend of branched-chain saturated isomers of octadecanoic acid. The chemical literature sometimes uses the term Isostearic Acid to refer specifically to the isomer 16-methylheptadecanoic acid (CAS Number 2724-58-5). However, the ingredient which is used in cosmetics is a mixture of the 18 carbon isomers generally branching with the methyl group.[1,2] According to CTFA Specifications, Isostearic Acid consists of approximately 80% branched chain C_{16} and C_{18} acids and 20% straight-chain C_{14}, C_{16}, and C_{18} acids.[3] Approximate values for the distribution of the different types of fatty acids present in Isostearic Acid are listed in Table 1.

Isostearic Acid is prepared by dimerizing the fatty acids of Tall Oil, Soybean Oil, or Tallow in the presence of a catalyst. The reaction mixture is then separated into monomer and dimer fractions by distillation. The monomer fraction which is rearranged during the reaction is further refined by hydrogenation, solvent separation, and an additional distillation.[4,5]

Methods for the laboratory synthesis of 16-methylheptadecanoic acid have also been described.[6-10]

TABLE 1. Fatty Acid Components of Isostearic Acid.

Component	*Level (%)*
Methyl-branched isomers of octadecanoic acid	approx. 80
C14 linear saturated fatty acid (Myristic)	1–10
C18 linear saturated fatty acid (Stearic)	1–10
C16 linear saturated fatty acid (Palmitic)	4–8
C18 Oleic acid	0–2

Data from Ref. 4.

Physical Properties

Isostearic Acid is a clear, oily liquid with little odor. It is insoluble in water but easily soluble in such organic solvents as ethanol, acetone, ethyl ether, carbon tetrachloride, and others. Its alkaline salts are readily soluble in water.[2]

The different isomers are mutually soluble and show virtually identical properties. Since it is a mixture, the melting point of Isostearic Acid is much lower than one would expect for a saturated fatty acid of similar molecular weight.[2] Whereas the melting point of 16-methylheptadecanoic acid has been reported as 69.5°–69.7°C,[7] Isostearic Acid is a liquid at room temperature.

Table 2 presents CTFA specifications for Isostearic Acid[3] as well as measured values for the chemical and physical properties of Isostearic Acid obtained from three different commercial sources.[2]

Studies on the molecular and crystalline structures of 16-methylheptadecanoic acid have been conducted,[11,12] and infrared data are available.[13] The surface chemistry of Isostearic Acid as a cosmetic ingredient has also been studied.[14]

Reactivity

Isostearic Acid should participate in chemical reactions common to long chain, saturated fatty acids.

TABLE 2. Chemical and Physical Properties of Isostearic Acid.

Mol. wt.	*Solid pt.*	*Viscosity*	*Sp. gr.*	*Iodine value*	*Acid value*	*Sapon. value*
284	10 °C max.	50 cps 25 °C	0.89 25 °C	3.0 max.[a]	191.0–201.0[a]	197.0–204.0[a]
			0.906 25 °C	3.0	191.0–201.0	197.0–204.0
				8[b]	180–200	185–205
				8	177	189

[a]CTFA Specification.
[b]Resulting from chain branching, not from double bonds.
Data from Refs. 2,3.

Analytical Methods

Gas chromatography,[15,16] mass spectrometry,[17] infrared spectrometry,[13] and x-ray crystallography[11] have been used in the study of Isostearic Acid or its component isomers.

Impurities

Isostearic Acid typically contains unsaponifiable matter and moisture at levels of 3.0% and 1.0%, respectively.[4] Analysis of one sample of Isostearic Acid revealed unsaponifiables at 4% and moisture at 0.01%.[2]

USE

Purpose in Cosmetics

Isostearic Acid is an emollient[18] which shows some of the same chemical properties as stearic acid and has physical properties similar to those of oleic acid. It is used as a replacement for stearic acid when "smoother and more easily spreading" products are desired without the use of oleic acid. Emulsions using Isostearic Acid have desirable organoleptic properties and resist degradation of color and odor. This ingredient is also employed in synthesizing a wide variety of esters that are used in cosmetic formulations.[2]

Scope and Extent of Use in Cosmetics

Table 3 lists product types and the number of product formulations containing Isostearic Acid as reported by the Food and Drug Administration (FDA) in 1981. It is contained in a wide variety of cosmetic products at concentrations generally less than 5%; one fragrance preparation and one suntan product were reported to contain Isostearic Acid in the 5%–10% range.[19] Unpublished safety data (reviewed elsewhere in this report) on a skin cleansing product containing 35% Isostearic Acid suggest possible use at higher concentrations.[20,21]

The cosmetic product formulation computer printout which is made available by the FDA is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations. Ingredients are listed in prescribed concentration ranges under specific product type categories. Certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration. The value reported by the cosmetic formulator in such a case may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. The fact that data are submitted only within the framework of preset concentration ranges also provides the opportunity for a two- to 10-fold overestimation of the actual concentration of an ingredient in a particular product.

Potential Interactions with Other Ingredients

Chemical interactions of Isostearic Acid with the other ingredients in cosmetic formulations have not been reported.

TABLE 3. Product Formulation Data on Isostearic Acid.

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)			
			>5-10	>1-5	>0.1-1	≤0.1
Isostearic Acid						
Eyeliner	396	2	–	1	1	–
Eye shadow	2582	17	–	2	14	1
Mascara	397	9	–	9	–	–
Blushers (all types)	819	20	–	10	9	1
Face powders	555	13	–	1	2	10
Makeup foundations	740	12	–	11	1	–
Lipstick	3319	8	1	–	6	–
Makeup bases	831	17	–	11	6	–
Rouges	211	1	–	1	–	–
Bath soaps and detergents	148	3	–	3	–	–
Other personal cleanliness products	227	2	–	–	2	–
Shaving cream (aerosol brushless, and lather)	114	2	–	2	–	–
Other shaving preparation products	29	1	–	–	1	–
Skin cleansing preparations (cold creams, lotions liquids and pads)	680	5	–	3	2	–
Face, body, and hand skin care preparations (excluding shaving preparations)	832	6	–	3	3	–
Moisturizing skin care preparations	747	19	–	8	11	–
Night skin care preparations	219	2	–	1	1	–
Skin lighteners	44	1	–	1	–	–
Suntan gels, creams, and liquids	164	1	1	–	–	–
Other suntan preparations	28	1	–	1	–	–
1981 TOTALS		142	2	68	59	12

Data from Ref. 19.

Surfaces to which Commonly Applied

Products containing Isostearic Acid are applied to all areas of the skin, hair, nails, and mucous membranes (Table 3). They may be applied as many as several times a day and remain in contact with the skin for various periods of time following each application. Daily or occasional use may extend over many years.

BIOLOGICAL PROPERTIES

Although branched chain fatty acids are not usually found in animal tissues,[22] the 16-methylheptadecanoic acid component of Isostearic Acid has been isolated from a number of animal sources. Hydrogenated mutton fat,[23]

wool,[7] and milk fat[15,16,24] have been found to contain trace amounts of 16-methylheptadecanoic acid. Likewise, it appeared in relatively small amounts in the mitochondrial and microsomal fractions of rat pituitary homogenate.[22] It was also detected in bovine muscle, where its relative concentration was significantly correlated with subjective evaluations of tenderness and flavor.[25]

Isostearate and other branched chain fatty acids supported the growth of a sterol requiring Mycoplasma (strain Y) which was unable to synthesize or alter the chain length of either saturated or unsaturated fatty acids.[26]

The incorporation of free fatty acids into myxoviruses was shown through the use of branched chain fatty acids as molecular markers. Gas–liquid chromatography revealed the presence of incorporated 16-methylheptadecanoic acid.[27]

Metabolism

Acyl coenzyme A synthetase of rat liver homogenate was found to activate Isostearic Acid.[28] Iso-fatty acids are metabolized in a way similar to that of straight-chain fatty acids by the mitochondrial and microsomal fractions of rat liver homogenate. In contrast, however, with the straight-chain fatty acids which are successively oxidized at the β carbon to yield two carbon fractions, the iso-fatty acids are also oxidized to a large extent at the ω carbon to ultimately form three carbon dicarboxylic acids. The enzymes catalyzing the ω-hydroxylation are present in the mitochondrial and microsomal fractions of liver homogenate, whereas the enzymes catalyzing the further oxidation into carboxylic acids have been demonstrated in the soluble fraction.[17]

Animal Toxicology

Acute Studies

Oral toxicity

The acute oral toxicity of Isostearic Acid was evaluated in three studies on the undiluted ingredient[29-31] and two studies on product formulations containing the ingredient.[32,33] In each study, young adult albino rats were fasted overnight and administered a single dose of the undiluted ingredient or product formulation by gastric intubation. They were then allowed free access to food and water for two weeks. The results and other details of these studies are summarized in Table 4. From these data, the acute oral LD50 of Isostearic Acid in rats is between 32 and 64 ml/kg.

Primary skin irritation and phototoxicity

The potentials for primary skin irritation caused by undiluted Isostearic Acid,[34] 15% Isostearic Acid in corn oil[30] and three product formulations containing Isostearic Acid[20,32,35] were evaluated using the Draize rabbit skin patch test technique. In each study, 0.5 ml samples were applied and occluded for 24 h, after which time the patch sites were graded for erythema and edema on the Draize scale. The results and other details of these studies are summarized in Table 5. The undiluted ingredient produced minimal irritation of the rabbit skin, whereas no irritation was noted when it was diluted to 15% in corn oil. Product

TABLE 4. Acute Oral Toxicity Tests on Isostearic Acid.

Concentration (%)	*Dose*	*Dose of Isostearic Acid (adjusted for dilution)*	*Animals*	*Results*	*Comments*	*Ref.*
100	2.0–64.0 ml/kg	2.0–64.0 ml/kg	5 rats at each of 6 dose levels	no deaths at doses up to 32 ml/kg; 3 died at 64.0 ml/kg	Slight nasal hemorrhage at 32.0 ml/kg; moderate to severe nasal hemorrhage at 64.0 ml/kg with erratic locomotion prior to death. Two survivors at 64.0 ml/kg were severely debilitated. LD50 between 32.0 and 64.0 ml/kg	29
100	5 g/kg	5 g/kg	10	no deaths		31
100	15.9 g/kg	15.9 g/kg	5 rats	no deaths		30
4.0 (in product formulation)	15.0 g/kg	0.6 g/kg	5 rats	no deaths		32
2.0 (in product formulation)	15.9 g/kg	0.32 g/kg	5 rats	no deaths		33

TABLE 5. Draize Primary Skin Irritation Tests on Isostearic Acid.

Concentration (%)	*Number of rabbits*	*Primary irritation index (max = 8)*	*Comments*	*Refs.*
100	6	0.63	Minimal irritation	34
100	6	0.3	Minimal transient irritation	37
15 (in corn oil)	6	0.0	No signs of irritation	30
35 (in product formulation)	9	1.89	Moderate irritation by product formulation	20
4 (in product formulation)	9	0.39	Minimal irritation by product formulation	32
4 (in product formulation)	9	0.06	Minimal irritation by product formulation	35
1.25 (aqueous solution of product formulation)	9	0.00	No signs of irritation by aqueous solution of product formulation	20

formulations containing Isostearic Acid produced minimal to moderate skin irritation, most probably by virtue of the other ingredients present in the formulations.

In a primary skin irritation and phototoxicity test, 200 mg of 100% Isostearic Acid was applied to the dorsal surface of New Zealand rabbits. The test material was applied for 2 h under gauze patches to 1-in^2 skin areas on both the left- and right-hand sides. The patch on the right-hand side was removed and exposed to 5×10^7 ergs/cm^2 black light (320–450 nm). The nonirradiated areas were shielded with aluminum foil during the light exposure. A positive Oxsoralen control was treated in a similar manner. The investigators concluded that the test material was mildly irritating without light exposure and only moderately irritating following light exposure. The investigator reported that a statistically significant difference was not detected between the nonirradiated and radiated sites.[36]

Eye irritation

The Draize rabbit eye irritation procedure or a modification of the test was used to evaluate undiluted Isostearic Acid[30,37] and four product formulations containing Isostearic Acid.[20,32,33,35] In each study, a 0.1 ml sample was instilled into the conjunctival sac of one eye of each rabbit with no washing; the untreated eye served as a control. Treated eyes were examined and graded on the Draize eye irritation scale at 1, 2, 3, 4, and 7 days. The results and other details of these studies are summarized in Table 6. The undiluted ingredient produced only minimal eye irritation which cleared by 24 h. Some of the product formulations produced moderate eye irritation, which is greater than that produced by the ingredient alone.

Comedogenicity

Comedogenicity* studies were conducted on two sunscreen formulations, one containing 2.5% Isostearic Acid and the other without Isostearic Acid.[38-40]

*Comedones are also known as blackheads.

TABLE 6. Draize Eye Irritation Tests on Isostearic Acid.

Type of product formulation	*Isostearic Acid concentration (%)*	*Number of rabbits*	*Ocular irritation index (max = 110)*					*Comments*	*Ref.*
			24 h	*48 h*	*72 h*	*4 days*	*7 days*		
None	100	3	0	0	0	0	0	Transient conjunctival irritation at 1 h; all eyes normal by 24 h.	30
None	100	6	0.3	0	0	0	0	Eyes unwashed; minimal transient irritation.	37
		3	0	0	0	0	0	Eyes washed with tepid water; no irritation.	
Skin cleanser	35 (in product formulation)	6	34	14	6	4	0	Moderate reversible eye irritation which gradually cleared; all eyes normal by Day 7.	20
Face color	4 (in product formulation)	6	1	0	0	0	0	Transient conjunctival irritation at 24 h; all eyes normal by 48 h.	32
Mascara	4 (in product formulation)	6	8	6	4	1	0	Minimal eye irritation which gradually cleared; all eyes normal by Day 7	35
		retest of same animals	2	1	0	0	0	after initial application and by 72 h after repeat application.	
Face makeup foundation	2 (in product formulation)	3	–	0	0	0	0	Transient conjunctival irritation at 24 h; all eyes normal by 48 h.	33

– No data.

The formulation containing Isostearic Acid was tested in two separate assays;[38,39] 1 ml of the product was applied to the glabrous inner portion of the right ear of each of nine rabbits. The left ear was untreated and served as a control. The test material was applied five days per week for a total of 20 applications. Observations of grossly appearing enlarged pores and hyperkeratosis were made daily, and terminal biopsies were made with histologic comparison of treated and control skin. The product containing Isostearic Acid was significantly comedogenic and irritating to rabbit ears under the conditions of this test. An identical assay on the product without Isostearic Acid[40] showed the formulation to be irritating but not comedogenic to the ears of six rabbits.

Clinical Assessment of Safety

Primary Skin Irritation

A 24 h occlusive patch test procedure was used to evaluate the primary skin irritation caused by undiluted Isostearic Acid[30] and by four product formulations containing Isostearic Acid.[21,33,41,42] The results and other details of these studies are summarized in Table 7. The undiluted ingredient tested "negative" in the single insult patch test; product formulations containing Isostearic Acid produced up to minimal irritation, most probably by virtue of the other ingredients present in the formulations.

A sunscreen formulation containing 2.5% Isostearic Acid was applied to the backs of 10 subjects. Approximately 50–200 mg of the test formulation containing 1.2–5.0 mg Isostearic Acid was used in the test. The test sites were occluded for 48 h before removal. No irritation was reported.[43]

In another study,[44] 19 women participated in a controlled-use test on the skin cleanser formulation containing 35% Isostearic Acid. The product was ap-

TABLE 7. Clinical 24-Hour Single Insult Patch Tests with Isostearic Acid.

Product type	*Isostearic Acid concentration (%)*	*Number of subjects*	*Results*	*Ref.*
None	100	100	"negative"	30
Face color	4 (in product formulation)	19	No signs of irritation	41
Mascara	4 (in product formulation)	18	No signs of irritation	42
Skin cleanser	0.44 (1.25% aqueous solution of product formulation containing 35% Isostearic Acid)	80 (20 each for four versions of the product formulation)	PIIs = 0.13 to 0.18; (max = 4.0) minimal irritation	21
Face makeup foundation	0.2 (10% in peach kernel oil of product formulation containing 2% Isostearic Acid)	104	"negative"	33

plied once on one cheek the first day and twice on the same cheek on Days 2–4 of the study. The other cheek, cleansed with soap, served as a control. None of the 19 participants noted discomfort. Although three reported mild to moderate dryness on the area treated with the cleanser, the product compared favorably to the control soap.

A sunscreen containing 2.5% Isostearic Acid was tested in a 21-day repeated insult patch test on 19 subjects. The test material, 0.2 g of formulation, was placed on nonwoven fabric patches and semioccluded on the backs of the subjects for 24 h. A total of 15 applications of the material were applied over a 21-day test period. A Cumulative Irritation Index (CII) of 0.87 out of a maximum score of 84 was reported. The investigator did not consider this value of CII to be clinically significant.[45]

Irritation/Sensitization

One hundred three subjects completed a repeated insult patch test of 10% Isostearic Acid dissolved in mineral oil. Each subject received a patch to the intact skin of the upper back under semiocclusion. The patches remained in place for 48 h (72 h on weekends) at which time they were removed, the sites were examined for irritation and new patches were applied. These procedures were repeated 10 times, followed by a two-week nontreatment period and rechallenge. The test ingredient had a mean cumulative irritation score of 0.243 ± 0.068. Mineral oil was included in the study as a nonirritating control and had a mean cumulative irritation score of 0.177 ± 0.042. Propylene glycol, a positive control as a known mild irritant, had a mean cumulative score of 0.388 ± 0.071. The investigators reported there were no skin reactions consistent with ingredient-induced sensitization.[46]

A repeated insult patch test was performed on 168 subjects (115F, 53M) using 0.1 ml of a 35% mineral oil solution of Isostearic Acid. The test material was applied at 48 h intervals, three times per week for three weeks on the back of the subjects. The test area was occluded for 24 h before removal, and washed with distilled water. The test sites were read at 48 h, after which fresh test material and the occlusive patch were reapplied. After a three-week nontreatment period, the test area, as well as a previously untreated site, were challenged using the same procedure as previously noted. The sites were scored for sensitization at 24, 48, and 72 h. The investigator noted that only transient reactions were observed during the test and that Isostearic Acid was neither an irritant nor a sensitizer.[47]

A sunscreen containing 2.5% Isostearic Acid was tested in a 21-day repeated insult patch test. Approximately 200 mg of the test formulation, which is equivalent to 5 mg of Isostearic Acid, was applied at 48 h intervals for 10 applications to the backs of 235 Caucasian females. Following a two-week nontreatment period, the subjects were re-exposed for 48 h. There were no reactions during the induction phase of the study, and the investigator concluded that the formulation's potential for sensitization was extremely low, or nonexistent.[48]

A mascara formulation containing 2.85% Isostearic Acid was tested in a repeated insult patch test on 98 subjects.[49] The induction phase of the procedure consisted of 10 consecutive occlusive patch applications to the same site over a period of two weeks. A single occlusive challenge patch was applied to

TABLE 8. Clinical Repeated Insult Patch Tests with Isostearic Acid.

Product Type	*Concentration (%)*	*Number of subjects*	*Results*	*Ref.*
None	35 (mineral oil dil.)	168	No irritation; no sensitization	47
None	10 (mineral oil dil.)	103	None to mild irritation; no sensitization	46
Mascara	2.85	98	1/98 show some irritation; no sensitization	49
Sunscreen	2.5	235	No irritation potential; as sensitizer, extremely low or nonexistent	48

the original contact site and/or a virgin site after a 10- to 14-day nontreatment period. During the induction phase of the experiment, one subject exhibited some skin irritation. There were no reactions at challenge and thus no indications of skin sensitization. The results of all repeated insult patch tests are summarized in Table 8.

Phototoxicity and Photosensitization

Twenty-eight of the 168 subjects tested for irritation and sensitization discussed above were randomly selected to test the ability of 35% Isostearic Acid in mineral oil to induce a phototoxic or photosensitive reaction following ultraviolet exposure. The test protocols were the same except that the forearm was used as a test site. The 28 subjects were divided into two groups; 19 received only UVA and 9 received both UVA and UVB. The UVA (320–400 nm) light was applied for 15 min to the 19 subjects (4.4 $\mu W/cm^2$ at the skin surface measured at a 360 nm wavelength peak). The UVB was applied at two times Mean Erythema Dose (MED) to nine subjects from a 150 watt Xenon Arc Solar Simulator emitting at 280–320 nm. The subjects receiving the UVB exposure were also exposed for 5 min to UVA as previously described. The investigator noted that only transient reactions were observed, and that Isostearic Acid was not a photosensitizer.[47]

SUMMARY

Isostearic Acid is a mixture of fatty esters consisting mainly of methyl branched isomers of octadecanoic acid. It is reported by the FDA to be used at concentrations up to 10% in a wide variety of cosmetic products which may be applied to all areas of the body; data have also been received on a product containing 35% Isostearic Acid.

Studies with rat liver homogenate suggest Isostearic Acid is readily metabolized following ingestion. In rats, the acute oral LD50 is estimated to be greater than 32 ml/kg. The raw ingredient produced no significant skin or eye irritation in Draize rabbit irritation tests, whereas variable degrees of irritation were produced by product formulations containing Isostearic Acid. A product for-

mulation both with and without 2.5% Isostearic Acid was tested in a rabbit ear comedogenicity assay. The formulation without Isostearic Acid was irritating but did not produce comedones; however, the formulation with Isostearic Acid was both irritating and comedogenic.

In clinical studies, 100 subjects showed no signs of irritation after a 24 h single insult skin patch with undiluted Isostearic Acid, and product formulations containing up to 4% Isostearic Acid produced, at most, minimal irritation when similarly tested on a total of 221 subjects. In another study, 35% Isostearic Acid in mineral oil was neither an irritant nor a sensitizer in 168 subjects. A subset population of 25 individuals from this study group, when tested in a similar manner but exposed to UVA + UVB, gave no indication that Isostearic Acid is a photosensitizer. Isostearic Acid at 10% in mineral oil was similarly not irritating nor sensitizing to 103 subjects. Product formulations containing 2.5%–2.85% Isostearic Acid produced no evidence of contact sensitization when tested in repeated insult patch tests on a total of 333 subjects.

DISCUSSION

The Panel expresses concern regarding the production of comedones in the rabbit ear assay by a product formulation containing commercially available Isostearic Acid. The Panel recognizes that currently available tests are inadequate to predict the potential for human comedogenicity of an ingredient as used in a product formulation. However, it is a potential health effect that should be considered when Isostearic Acid is used in cosmetic formulations.

CONCLUSION

On the basis of the available information presented in this report, the Panel concludes that Isostearic Acid is safe as a cosmetic ingredient in the present practices of use.

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3

Final Report on the Safety Assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are fatty acids with hydrocarbon chains ranging in length from 12 to 18 carbons with a terminal carboxyl group. These fatty acids are absorbed, digested, and transported in animals and humans. Little acute toxicity was observed when Oleic, Lauric, Palmitic, Myristic, or Stearic Acid or cosmetic formulations containing these fatty acids were given to rats orally at doses of 15–19 g/kg body weight. Feeding of 15% dietary Oleic Acid to rats in a chronic study resulted in normal growth and health, but reproductive capacity of female rats was impaired. Results from topical application of Oleic, Palmitic, and Stearic Acid to the skin of mice, rabbits, and guinea pigs produced little or no apparent toxicity. Studies using product formulations containing Oleic and Stearic acids indicate that neither is a sensitizer or photosensitizing agent. Animal studies also indicate that these fatty acids are not eye irritants. Lauric, Stearic, and Oleic Acids were noncarcinogenic in separate animal tests. In primary and cumulative irritation clinical studies, Oleic, Myristic, and Stearic Acids at high concentrations were nonirritating. Cosmetic product formulations containing Oleic, Lauric, Palmitic, and Stearic Acids at concentrations ranging up to 13% were not primary or cumulative irritants, nor sensitizers. On the basis of available data from studies using animals and humans, it is concluded that Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are safe in present practices of use and concentration in cosmetics.

INTRODUCTION

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are long hydrocarbon chain carboxylic acids, known as fatty acids. They are usually produced by hydrolysis of common animal and vegetable fats and oils. Fatty acids are generally used as intermediates in the manufacture of their alkali salts, which

are in turn used as emulsifiers, emollients, and lubricants in a variety of cosmetic creams, cakes, soaps, and pastes.

CHEMISTRY

Structure and Nomenclature

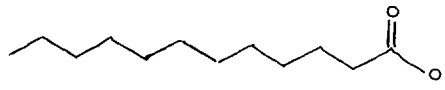
Lauric, Myristic, Palmitic, and Stearic Acids are saturated fatty acids of 12-, 14-, 16-, and 18-carbon lengths. Oleic Acid is an 18-carbon *cis*-mono unsaturated fatty acid. These fatty acids consist of long hydrocarbon chains with a terminal carboxyl group. Synonyms for the fatty acids (Table 1) were obtained from the following sources: Windholz et al.,[1] Estrin et al.,[2] Morrison and Boyd,[3] Lehninger,[4] and Osol.[5] Structural formulae are presented in Figure 1. A summary of some physicochemical properties appears in Table 2. Since the saturated fatty acids bear the carboxyl functional group and basically

TABLE 1. Synonyms for the Fatty Acids

Fatty acid	*Synonyms*
Oleic Acid	*cis*-9-Octadecenoic acid *cis*-Δ^9-Octadecenoic acid 9-Octadecenoic acid Oleinic acid Elaic acid Red oil 18:1Δ^9
Lauric Acid	n-Dodecanoic acid Dodecanoic acid Laurostearic acid Dodecoic acid 12:0
Palmitic Acid	n-Hexadecanoic acid Hexadecanoic acid Hexadecoic acid Hexadecylic acid Cetylic acid 16:0
Myristic Acid	n-Tetradecanoic acid Tetradecanoic acid Tetradecoic acid 14:0
Stearic Acid	n-Octadecanoic acid Octadecanoic acid Cetylacetic acid Stearophanic acid 18:0

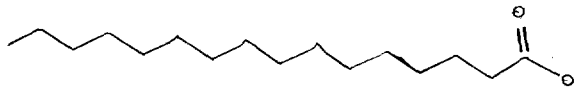
OLEIC ACID

$C_{18}H_{34}O_2$



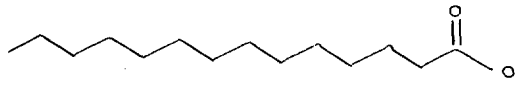
LAURIC ACID

$C_{12}H_{24}O_2$



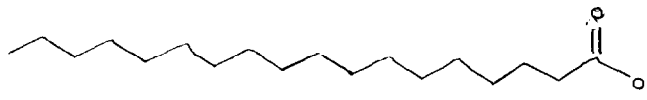
PALMITIC ACID

$C_{16}H_{32}O_2$



MYRISTIC ACID

$C_{14}H_{28}O_2$



STEARIC ACID

$C_{18}H_{36}O_2$

FIG. 1. Structural formulae of fatty acids.

differ from each other by 2–6 methylene groups, their properties are similar. The *cis* double bond of Oleic Acid alters several physical properties relative to those of Stearic Acid.[4]

Description and Source

Fatty acids have been found in marine and freshwater organisms,[6] bacteria,[4] and vegetable oils and animal fats.[3] Although mammalian tissues

TABLE 2. Physicochemical Properties of the Fatty Acids

Property	*Lauric Acid*	*Myristic Acid*	*Palmitic Acid*	*Stearic Acid*	*Oleic Acid*
CAS Registry No.	143-07-7	544-63-8	57-10-3	57-11-4	112-80-1
Empirical formula[a]	$C_{12}H_{24}O_2$	$C_{14}H_{28}O_2$	$C_{16}H_{32}O_2$	$C_{18}H_{36}O_2$	$C_{18}H_{34}O_2$
Molecular weight	200.31[a], 200.33[b]	228.36[a], 228.38[b]	256.42[a], 256.43[b]	284.47[a], 284.50[b]	282.45[a], 282.47[b]
Density (g/ml, °C)	0.8679_4^{50b}	0.8528_4^{70a}	0.8527_4^{62b}	0.847^{70a}	0.895_{25}^{25a}
Melting point (°C)	44, 48[a]	58.5[a], 58[b], 54.4[c]	63–64[a]	69–70[a, c], 71.2[b]	16.3[b]
Boiling point (°C, P in atm)[a]	225_{100}	250.5_{100}	215_{15}	383_1 (decomposes at 360_1)	286_{100}
Solubility[a, b, d]					
Water	Insol.	Insol.	Insol.	Insol.	Insol.
Alcohol	v. sol.—ethanol propanol—1 g/ml	sol.—abs. ethanol v. sol.—methanol	v. sol.—ethanol + heat v. sol.—propanol	sl. sol.—1 g/21 ml ethanol	v. sol.—ethanol
Chloroform	sol.	sol.	v. sol.	sol.—1 g/2 ml	v. sol.
Benzene	v. sol.	v. sol.	sol.	sl. sol.—1 g/5 ml	v. sol.
Ether	v. sol.	sl. sol.	v. sol.	v. sol.	v. sol.
Viscosity (cp, °C)[c]	7.3^{50}	5.06^{75}	7.1^{75}	9.04^{75}	23.01^{30}
Iodine number[a]	—	—	—	—	89.9
Acid value	280.1[c]	245.7[c]	218.0[c]	197.2[c]	198.6[a]

[a]Ref. 1.
[b]Ref. 7.
[c]Ref. 6.
[d]Ref. 8.
Insol., insoluble; sl. sol., slightly soluble; sol., soluble; v. sol., very or freely soluble.

normally contain trace amounts of free fatty acids, conjugated forms can be found in several tissues.[4] Free fatty acids have been found in human sebum and epidermal tissue.[9,10]

Oleic Acid, in esterified form, is found in many vegetable oils and animal fats, frequently constituting greater than 50% of the total fatty acid concentration. Oils rich in Oleic Acid include olive (80%), peanut (60%), teaseed (85%), and pecan (85%) oils; very few fats contain less than 10% Oleic Acid.[6]

Pure Oleic Acid is a colorless to pale yellow, oily liquid at temperatures above 5–7°C. At 4°C, it solidifies to a crystalline mass. Upon exposure to oxygen, it darkens gradually, and it decomposes when heated to 80–100°C at atmospheric pressure.[1,8,11] Oleic Acid has a characteristic lardlike odor and taste.[1,8]

Lauric Acid is one of the three most widely distributed naturally occurring saturated fatty acids; the others are Palmitic and Stearic Acids. Its common name is derived from the laurel family, Lauraceae. The fatty acid content of the seeds is greater than 90% Lauric Acid. Sources of Lauric Acid include coconut and palm kernel oils, babassu butter (approximately 40%) and other vegetable oils, and milk fats (2–8%). Camphor seed oil has a high Lauric Acid content.[1,6,8]

Lauric Acid occurs as a white or slightly yellow, somewhat glossy crystalline solid or powder[1,8] or as a colorless solid[11] with a slight odor of bay oil.[1]

The glyceryl ester of Palmitic Acid is widely distributed, being found in practically all vegetable oils and animal (including marine animal) fats at concentrations of at least 5%. Palmitic Acid is the major component of lard and tallow (25–30%), palm oil (30–50%), cocoa butter (25%), and other vegetable butters. Chinese vegetable tallow is reported to contain 60–70% Palmitic Acid.[1,6]

Palmitic Acid occurs as a mixture of solid organic acids obtained from fats that are primarily composed of Palmitic Acid with varying quantities of Stearic Acid. Its appearance ranges from a hard, white or faintly yellow, slightly glossy crystalline solid to a white or yellow-white powder,[8] white crystalline scales,[1] or colorless crystals.[11]

Myristic Acid is a solid organic acid usually obtained from coconut oil, nutmeg butter (*Myristica fragrans* Houtt), palm seed oils, and milk fats.[1,6] Seed oils of the plant family, Myristaceae, contain the largest amounts of Myristic Acid (up to 80%), but small amounts have been measured in most animal fats and vegetable oils.

Myristic Acid occurs as a hard, white or faintly yellow, glossy crystalline solid, as a white or yellow-white powder,[8] or as colorless leaflets.[11]

Stearic Acid is found primarily as a glyceride in animal fats and oils; lard and tallow contain approximately 10 and 20% Stearic Acid, respectively.[1,6] Most vegetable oils contain 1–5% Stearic Acid; cocoa butter contains about 35%.

Stearic Acid occurs as hard, white or faintly yellow, somewhat glossy crystals or leaflets or as an amorphous white or yellow-white powder.[1,5,8,12] It has a slight odor and taste resembling tallow.[1,8]

Method of Manufacture and Impurities

The fatty acids are usually produced by the hydrolysis of common animal and vegetable fats and oils followed by fractionation of the resulting fatty acids. Fatty acids that are used in foods, drugs, and cosmetics normally exist as mixtures of several fatty acids depending on the source and manufacturing process.

Processing operations in the manufacture of fatty acids from fats are known to alter their chemical compositions. The processes (e.g., distillation, high temperature and pressure hydrolysis, and bleaching) may result in *cis-trans* isomerization, conjugation of polyunsaturates, polymerization, and dehydration.[6]

Cosmetic-grade Oleic, Lauric, Palmitic, Myristic, and Stearic Acids occur as mixtures of fatty acids depending on their method of manufacture and source. The individual fatty acids predominate in the mixture ranging from 74% (Oleic Acid) to 95% (Myristic Acid). All contain varying amounts of unsaponifiable matter, and some grades also contain glyceryl monoesters of fatty acids. Butylated hydroxytoluene may be added to all five fatty acid preparations as an antioxidant.[13–17] In cosmetics containing unsaturated materials, the concentration range for butylated hydroxytoluene should be 0.01 to 0.1%.[18] Butylated hydroxytoluene has been used in some lanolin products containing unsaturated fatty acids, alcohols, esters, sterols, and terpenols, at concentrations ranging from 200 to 500 ppm.[19] Data on the components, impurities, and additives of these cosmetic grade fatty acids are presented in Table 3. Comparisons of specifications for cosmetic, food, and drug grade fatty acids are presented in Tables 4, 5, 6, 7, and 8. Cosmetic grade specifications for fatty acid composition are presented in Table 9.

Fourteen FAPC (Fatty Acid Producers Council of the Soap and Detergent Association) categories of fatty acids are contrasted by titer and iodine value. Typical fatty acid compositions are reported.[6] FDA files contain some composition data on Oleic and Stearic Acids, which were submitted with Food Additive Petitions (Notes from the composition data in CIR files).

Oleic Acid is produced by the hydrolysis and fractionation (e.g., saponification and distillation) of animal or vegetable fats and oils.[1,5,11,16] Preparation of Oleic Acid from animal tallow and olive has been reported.[1,5] It is also obtained as a byproduct in the manufacture of solid Stearic and Palmitic Acids. Crude (unpurified, unbleached) Oleic Acid of commerce, or red oil, contains Stearic and Palmitic Acids in varying quantities.[5,20]

Several commercial grades of Oleic Acid are available, distinguished by varying proportions of saturated fatty acids. The commercial grade contains 7–12% saturated acids and some unsaturated acids and is usually derived from edible sources (internally administered Oleic Acid must be derived from edible sources[5]). Oleic Acid derived from tallow contains varying amounts of linolenic and Stearic Acids and small but significant quantities of elaidic (*trans*-9-octadecenoic) acid, some of which is generated from certain processing operations (e.g., distillation and high-temperature bleaching with clays).[1,5,6]

Hawley[20] reported several technical grades of Oleic Acid: chick edema factor-free grade, U.S. Pharmacopeia (USP) grade, Food Chemicals Codex (FCC) grade, and purified technical grade Oleic Acid. The latter technical

TABLE 3. Components, Impurities, Additives in Cosmetic-Grade Fatty Acids[13–17]

Cosmetic-grade fatty acid	*Components in Mixture (%)*	*Minor Impurities (%)*	*Additives*
Oleic Acid	9-Octadecenoic acid (68–74)[a] 9,12-Octadecadienoic acid (4–12) 9-Hexadecenoic acid (7–11) Hexadecanoic acid (4) Tetradecanoic acid (3) 9-Tetradecenoic acid (1–3) Heptadecanoic acid (1–2) Pentadecanoic acid (0.5–2) Octadecanoic acid (1) Octadecatrienoic acid (1) Decanoic acid Dodecanoic acid	Unsaponifiable material (1.5 max)	Butylated hydroxytoluene[b] (BHT)
Lauric Acid	Dodecanoic acid (90 min) Tetradecanoic acid (6 max) Decanoic acid (5 max) Hexadecanoic acid (2 max)	Unsaponifiable material (0.3 max) (mostly hydrocarbon) Glyceryl monolaurate[b] (0.07 max)	BHT[b]
Palmitic Acid	Hexadecanoic acid (80 min) Octadecanoic acid (11 max) Tetradecanoic acid (7 max) Heptadecanoic acid (4.5 max) Pentadecanoic acid (1 max)	Unsaponifiable material (0.3 max) (mostly hydrocarbon) Glyceryl monopalmitate[b] (0.07 max)	BHT[b]
Myristic Acid	Tetradecanoic acid (95 min) Hexadecanoic acid (4 max) Dodecanoic acid (3 max)	Unsaponifiable material (0.2 max) (mostly hydrocarbon) Glyceryl monomyristate[b] (0.07 max)	BHT[b]
Stearic Acid	Octadecanoic acid (39–95)[a] Hexadecanoic acid (5–50) Tetradecanoic acid (0–3) 9-Octadecenoic acid (0–5) Heptadecanoic acid (0–2.5) Eicosanoic acid (0–2) Pentadecanoic acid (0–1)	9-Hexadecenoic acid 9,12-Octadecadienoic acid Unsaponifiable material (0.3 max) Glyceryl monostearate (0.07 max)	BHT[b]

[a] These are concentration ranges of a typical analysis.
[b] Present in some grades.

grade Oleic Acid contains ≥ 90% Oleic Acid and has a 4% maximum linoleic acid content and a 6% maximum saturated fatty acid content.

Lauric Acid is produced by the hydrolysis, usually via saponification, of animal or vegetable fats and oils followed by fractional distillation.[11,22] Lauric Acid is commonly isolated from coconut oil,[1,11] and several patents describe its chemical synthesis.[1]

Palmitic Acid is produced by the hydrolysis and fractionation of palm oil, tallow oil, coconut oil, Japan Wax, Chinese vegetable tallow, and spermaceti. Fractionation is usually by distillation or crystallization.[1,11,20] Palmitic Acid can also be obtained in the manufacturing process for Stearic Acid.

TABLE 4. Comparison of Specifications: Cosmetic and Food Grades

Oleic Acid	*Cosmetics*[21]	*Foods*[8]
Iodine value	83.0–99.0	83–103
Acid value	190.0–207.0	196–204
Saponification value	198.0–207.0	196–206
Unsaponifiable matter	1.0% max	2% max
Arsenic		3 ppm max
Heavy metals (e.g., Pb)		10 ppm max
Residue on ignition		0.01% max
Titer (solidification point)	2–6°C	< 10°C
Water content		0.4% max

TABLE 5. Comparison of Specifications: Cosmetic and Food Grades

Lauric Acid	*Cosmetics*[13, 14]	*Foods*[8]
Iodine value	0.5 max	3.0 max
Acid value	273–283	252–287
Saponification value	276–284	253–287
Unsaponifiable matter	0.3% max	0.3% max
Arsenic		3 ppm max
Heavy metals (e.g., Pb)		10 ppm max
Residue on ignition		0.1%
Titer (solidification point)	38–44°C	26–44°C
Water content		0.2% max

TABLE 6. Comparison of Specifications: Cosmetic and Food Grades

Palmitic Acid	*Cosmetics*[21]	*Foods*[8]
Iodine value	1.0 max	2.0 max
Acid value	213–221	204–220
Ester value	3.0 max	
Saponification value	216.5–220.5	205–221
Unsaponifiable matter	0.25% max	1.5% max
Arsenic		3 ppm max
Heavy metals (e.g., Pb)		10 ppm max
Residue on ignition		0.1%
Titer (solidification point)	59.4–60.4°C	53.3–62°C
Water content		0.2% max

The following methods have been used in the preparation of Myristic Acid: isolation from tall-oil fatty acids from 9-ketotetradecanoic acid, by electrolysis of a mixture of methyl hydrogen adipate and decanoic acid, by Maurer oxidation of myristanol, and from cetanol.[1] The most common means of preparation is by fractional distillation of hydrolyzed coconut oil, palm kernel oil,[20] or coconut acids.[11]

Commercial Stearic Acid has several crystalline forms and contains varying relative concentrations of other fatty acids depending on the sources and processing methods used.[9] Commercial Stearic Acid is primarily a mixture of

TABLE 7. Comparison of Specifications: Cosmetic and Food Grades

Myristic Acid	*Cosmetics*[13,14]	*Foods*[8]
Iodine value	0.5 max	1.0 max
Acid value	243–249	242–249
Saponification value	243–249	242–251
Unsaponifiable matter	0.2% max	1% max
Arsenic		3 ppm max
Heavy metals (e.g., Pb)		10 ppm max
Residue on ignition		0.1% max
Titer (solidification point)	52–54°C	48–55.5°C
Water content		0.2% max

TABLE 8. Comparison of Specifications: Cosmetic and Food Grades

Stearic Acid	*Cosmetics "95.0%"*[21]	*Foods*[8]
Iodine value	1.0 max	7 max
Acid value		196–211
Ester value	3.0 max	
Saponification value	196.4–200.4	197–212
Unsaponifiable matter	0.25% max	1.5% max
Arsenic		3 ppm max
Heavy metals (e.g., Pb)		10 ppm max
Residue on ignition		0.1% max
Titer (solidification point)	67.2–68.2°C	54.5–69°C
Water content		0.2% max

varying amounts of Stearic and Palmitic Acids. Palmitic Acid/Stearic Acid ratios in commercial preparations depend on several factors, such as source, geographical and climatic influences, genetic uniformity, and fat location site (in animals).[6]

Methods of processing for Stearic Acid include hydrolysis of tallow or hydrogenation of unsaturated fatty acids (e.g., Oleic Acid) in cottonseed and other vegetable oils, followed by methods of isolation, such as fractional distillation or crystallization.[1,5,6,9,11,17] A successive series of pressing operations has been used to separate the liquid unsaturated fatty acids from the solid saturated fatty acids.[6] The Palmitic Acid/Stearic Acid ratio obtained from tallow hydrolysis and triple-pressing or solvent crystallization is 55%/45%. Concentrations of Stearic Acid as high as 95–99%[6,9] have been reported from the hydrogenation of unsaturated fatty acids.

Both double-pressed (two successive pressings to expel unsaturated fatty acids) and triple-pressed Stearic Acid are used by the cosmetic industry.[6,9] Triple-pressed Stearic Acid is a product containing 1.5% 14C (14-carbon), 0.5% 15C, 50% 16C, 1% 17C, and 47% 18C fatty acids, with less than 0.2% Oleic Acid. Double-pressed Stearic Acid typically contains about 2.5% 14C, 50% 16C, 1% 17C, 40% 18C fatty acids, and 6% Oleic Acid.[6]

TABLE 9. Cosmetic-grade Specifications for Fatty Acid Composition (Reported as maximal or minimal acceptable percentage in composition)[21]

Fatty acid chain length[a]	*Oleic Acid*	*Lauric Acid*	*Palmitic Acid*	*Myristic Acid*	*Stearic Acid 37.5%*	*Stearic Acid 42.5%*	*Stearic Acid 95.0%*
8:0–12:0	1.0 max						
10:0		5 max					
12:0		90 min	1.3 max	3 max	0.1 max	0.1 max	Trace (< 0.05)
14:0	5.0 max	6 max	2.5 max	95 min	4.3 max	4.1 max	1.6 max
14:1			Trace (< 0.05)		0.1 max	0.1 max	Trace (< 0.05)
15:0	2.5 max		0.6 max		0.6 max	0.7 max	0.8 max
16:0	7.5 max	2 max	92.5–97.5	4 max	49.0–54.0	49.0–54.0	5.0 max
16:1	4.5–7.5		0.4 max		0.3 max	0.1 max	Trace (< 0.05)
17:0	1.5 max		2.3 max		2.5 max	2.7 max	2.0 max
18:0	3.5 max		5.0 max		35.0–40.0	40.0–45.0	92.5–97.5
18:1	70.0 min		0.4 max		5.5 max	0.6 max	0.6 max
18:2	2.0–12.0 max						
18:3	2.2 max						
16:0 + 18:0					89.0 min	94.0 min	97.5 min
16:0 + 18:0 + 14:0			97.5 min				
20:0			Trace (< 0.05)		0.1 max	0.1 max	Trace (< 0.05)

[a]A form of shorthand notation was used to denote the length of the fatty acid carbon chain and the number of double bonds in the chain (e.g., Myristic Acid—14:0; Oleic Acid—18:1). Information on the position and configuration of double bonds in unsaturated fatty acids was not included (e.g., elaidic acid, the *trans* isomer of Oleic Acid, would also be denoted as 18:1).

Three types of Stearic Acid distinguished by average Stearic Acid concentration, their specifications, and infrared spectra are included in *CTFA's Compendium of Cosmetic Ingredient Composition*.[21] These Stearic Acids, 37.5%, 42.5%, and 95.0%, have minimum Stearic plus Palmitic Acid concentrations of 89.0%, 94.0%, and 97.5%, respectively. Regular pharmaceutical grade Stearic Acid specifies a 40.0% minimum of either Stearic or Palmitic Acid and a 90.0% minimum for their sum.[23] Purified pharmaceutical grade Stearic Acid specifies a 90.0% minimum Stearic Acid content and a 96.0% minimum for the sum.[73] A comparison of these Stearic Acids is presented in Table 9.

Reactivity and Stability

Chemical reactions of the fatty acids are typical of reactions of carboxylic acids and alkanes (or alkenes, in the case of Oleic Acid). Typical reactions of carboxylic acids include reduction to form aldehydes and alcohols, esterification, formation of metal salts, high-pressure hydrogenation, formation of amides and acid halides, alkoxylation, and pyrolysis. Reactions of alkanes and alkenes are dehydrogenation and hydrogenation, halogenation and hydration.[3,6] Halogenation across carbon–carbon double bonds is a useful method for the quantitative titration for relative unsaturation.[4]

Insoluble stearates and oleates are formed in reactions of Stearic Acid and Oleic Acid with heavy metals and calcium. Oxidizing agents, such as nitric acid and potassium permanganate, added to Oleic Acid are known to produce various derivatives of this acid.[5] Other oxidation routes for fatty acids include oxidation via bacterial action, enzyme-catalyzed hydrolysis and oxidation, and autooxidation from atmospheric oxygen.[6]

A significant increase in lipid peroxide concentration has been observed after 18-h UVA-irradiation of Oleic Acid.[24]

Analytical Methods

Two basic methods for the analysis of the fatty acids have been reported by the cosmetic industry. Primarily, gas chromatography (GC) of fatty acid methyl esters, prepared by the boron trifluoride-methanol method, is used for the separation and relative identification of fatty acids in a mixture.[21,25] Infrared spectra of the fatty acids are used for fingerprinting, functional group identification, and impurity screening.[6,13–17,26] Determination of physicochemical properties also aids in positive identification of a specific fatty acid.[6,25]

Basic analysis of the fatty acids by GC[4,25] has evolved by technical advances in methylation procedures[23,27] and development of new derivatization reactants and techniques that allow easier detection of smaller quantities of fatty acids.[28] A method for the GC of nonmethylated fatty acids has been reported.[29]

Flame ionization detection (FID) is usually coupled with the GC of fatty acid methyl esters. Mass spectrometry (MS) has also been used with GC for compound identification.[30]

Thin-layer chromatography[30,31] and high-performance liquid chromatography (HPLC) are also used in fatty acid identification and quantitation. Precolumn chemical derivatization (e.g., forming benzyl, dansyl, phenacyl, and naphthacyl derivatives) of fatty acids is followed by reversed-phase HPLC. Methods of detection include ultraviolet and fluorescence spectroscopic and refractive index detection. The analysis of fatty acids by HPLC has been reviewed.[32,33]

Mass spectrometry with temperature profiling of the chemical ionization source has been reported as a method for initial compound separation. Its coupling with a second MS allows direct analysis of complex lipid sources.[34]

Other separation methods include centrifugal liquid and adsorption chromatography.[35] Identification procedures range from methods, such as gravimetry[25] and histochemical staining,[36] to ultraviolet, infrared, and nuclear magnetic resonance spectroscopy.[6,37,38]

USE

Cosmetic Use

The fatty acids, Oleic, Lauric, Palmitic, Myristic, and Stearic Acids, are primarily used as intermediates in the manufacture of corresponding alkali salts, which are, in turn, used as emulsifiers, emollients, and lubricants in a variety of cosmetic creams, cakes, soaps, and pastes.[5,9,39-41] They may also be used as base components (of the oil phase) of many cosmetic formulations.[38]

Emollient creams containing fatty acids are slightly alkaline, ranging in pH from 7.5 to 9.5. Other ingredients in these creams include sodium, potassium, and ammonium hydroxide, diethanolamine, triethanolamine, isopropanolamines, amino glycol, and borax.[9]

Stearic Acid is contained in 2465 cosmetic products listed by the Food and Drug Administration (FDA) in the 1981 product formulation data table.[41] Oleic Acid is contained in 424, Myristic Acid in 36, Palmitic Acid in 29, and Lauric Acid in 22 cosmetic formulations in several product categories[41] (Table 10).

The reported concentrations of the fatty acids in cosmetic products primarily range from 0.1 to 25%. Stearic Acid is found in cosmetics in all product categories of the FDA table; most products appear in skin care, makeup, and shaving preparation categories. Oleic Acid is found primarily in hair coloring and eye makeup preparation product categories. Lauric, Palmitic, and Myristic Acids are contained in skin care, shaving, and noncoloring hair preparations and personal cleanliness products.

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the tabular format listing preset ingredient concentration ranges and product categories in accordance with Title 21 section 720.4 of the Code of Federal Regulations.[42]

Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. Data

TABLE 10. Product Formulation Data[41]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)					
			> 25–50	> 10–25	> 5–10	> 1–5	> 0.1–1	≤ 0.1
Oleic Acid								
Baby shampoos	35	1	—	1	—	—	—	—
Baby lotions, oils, powders, and creams	56	1	—	—	—	1	—	—
Other baby products	15	2	—	1	—	1	—	—
Bath oils, tablets, and salts	237	1	—	—	1	—	—	—
Eyeliner	396	16	—	1	—	7	8	—
Eye shadow	2582	5	—	—	—	2	3	—
Eye makeup remover	81	2	—	—	—	2	—	—
Mascara	397	41	—	—	23	11	7	—
Other eye makeup preparations	230	1	—	—		1	—	—
Sachets	119	4	—	—	—	—	4	—
Other fragrance preparations	191	8	—	—	—	2	6	—
Hair conditioners	478	1	1	—	—	—	—	—
Permanent waves	474	1	—	—	—	—	—	1
Hair shampoos (noncoloring)	909	9	—	2	—	7	—	—
Tonics, dressings, and other hair grooming aids	290	1	—	—	—	—	1	—
Hair dyes and colors (all types requiring caution statement and patch test)	811	205	—	150	—	49	5	1
Hair tints	15	14	—	13	—	1	—	—
Hair shampoos (coloring)	16	7	—	—	—	6	1	—
Hair lighteners with color	2	1	—	—	—	1	—	—
Hair bleaches	111	8	3	3	1	1	—	—
Blushers (all types)	819	10	—	—	—	10	—	—
Face powders	555	1	—	—	—	—	1	—
Makeup foundations	740	20	—	—	—	15	5	—
Lipstick	3319	1	—	—	1	—	—	—
Makeup bases	831	5	—	—	—	2	2	1
Other makeup preparations (not eye)	530	4	—	3	—	—	1	—

TABLE 10. (Continued)

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*					
			> 25–50	*> 10–25*	*> 5–10*	*> 1–5*	*> 0.1–1*	*≤ 0.1*
Nail basecoats and undercoats	44	1	—	1	—	—	—	—
Bath soaps and detergents	148	5	—	—	—	4	1	—
Other personal cleanliness products	227	3	—	—	1	2	—	—
Aftershave lotions	282	3	—	—	—	—	2	1
Shaving cream (aerosol, brushless, and lather)	114	2	—	—	—	2	—	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	10	—	—	—	5	5	—
Face, body, and hand skin care preparations (excluding shaving preparations)	832	11	—	1	1	2	7	—
Hormone skin care preparations	10	1	—	—	—	1	—	—
Moisturizing skin care preparations	747	14	—	—	—	4	10	—
Other skin care preparations	349	2	—	—	—	1	1	—
Suntan gels, creams, and liquids	164	2	—	—	—	2	—	—
1981 TOTALS		424	4	176	28	142	70	4
Lauric Acid								
Hair shampoos (noncoloring)	909	3		1		2	—	—
Tonics, dressings, and other hair grooming aids	290	3	—	—	—	—	3	—
Deodorants (underarm)	239	5	—	—	—	—	4	1
Other personal cleanliness products	227	4	—	—	1	—	2	1
Shaving cream (aerosol, brushless, and lather)	114	3	—	—	1	2	—	—

Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	3	—	—	—	3	—	—
Moisturizing skin care preparations	747	1	—	—	—	—	1	—
1981 TOTALS		22	—	1	2	7	10	2
Palmitic Acid								
Eye shadow	2582	1	—	—	1	—	—	—
Hair shampoos (noncoloring)	909	2	—	—	—	2	—	—
Makeup foundations	740	2	—	—	—	1	1	—
Bath soaps and detergents	148	1	—	—	1	—	—	—
Shaving cream (aerosol, brushless, and lather)	114	4	—	—	3	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	8	—	1	1	6	—	—
Face, body, and hand skin care preparations (excluding shaving preparations)	832	3	—	—	—	1	2	—
Moisturizing skin care preparations	747	3	—	—	—	1	2	—
Night skin care preparations	219	3	—	2	—	1	—	—
Other skin care preparations	349	1	—	—	—	1	—	—
Suntan gels, creams, and liquids	164	1	—	1	—	—	—	—
1981 TOTALS		29	—	4	6	13	6	—

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*						
			> 50	*> 25–50*	*> 10–25*	*> 5–10*	*> 1–5*	*> 0.1–1*	*≤ 0.1*
Myristic Acid									
Mascara	397	2	—	—	—	—	—	2	—
Hair shampoos (noncoloring)	909	2	—	—	—	—	2	—	—
Bath soaps and detergents	148	3	—	—	1	2	—	—	—
Other personal cleanliness products	227	2	—	—	2	—	—	—	—

TABLE 10. (Continued)

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)						
			> 50	> 25–50	> 10–25	> 5–10	> 1–5	> 0.1–1	≤ 0.1
Beard softeners	4	2	—	2	—	—	—	—	—
Shaving cream (aerosol, brushless, and lather)	114	16	—	—	—	1	15	—	—
Other shaving preparation products	29	1	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	5	—	—	1	3	1	—	—
Face, body, and hand skin care preparations (excluding shaving preparations)	832	2	—	—	—	—	1	1	—
Moisturizing skin care preparations	747	1	—	—	—	—	—	1	—
1981 TOTALS		36	—	2	4	6	19	5	—
Stearic Acid									
Baby lotions, oils, powders, and creams	56	9	—	—	—	2	5	2	—
Other baby products	15	1	—	—	1	—	—	—	—
Other bath preparations	132	3	—	—	—	—	2	1	—
Eyebrow pencil	145	9	—	—	4	5	—	—	—
Eyeliner	396	55	—	5	6	4	29	11	—
Eye shadow	2582	128	—	—	—	—	111	17	—
Eye lotion	13	1	—	—	—	—	1	—	—
Eye makeup remover	81	1	—	—	—	—	—	1	—
Mascara	397	139	—	5	5	20	83	26	—
Other eye makeup preparations	230	26	—	—	—	2	20	4	—
Colognes and toilet waters	1120	3	—	—	—	—	3	—	—
Perfumes	657	3	—	—	—	—	3	—	—
Sachets	119	32	—	—	—	8	23	1	—
Other fragrance preparations	191	34	—	—	—	3	27	4	—

Hair conditioners	478	18	—	—	—	—	9	7	2
Hair sprays (aerosol fixatives)	265	1	—	—	—	—	1	—	—
Hair straighteners	64	6	—	—	—	2	—	4	—
Hair shampoos (noncoloring)	909	17	—	—	1	9	4	3	—
Tonics, dressings, and other hair grooming aids	290	18	1	—	1	4	7	4	1
Hair dyes and colors (all types requiring caution statement and patch test)	811	76	—	—	—	—	76	—	—
Hair bleaches	111	4	—	—	—	—	1	3	—
Other hair coloring preparations	49	8	—	—	8	—	—	—	—
Blushers (all types)	819	47	—	—	—	2	44	1	—
Face powders	555	2	—	—	—	—	—	2	—
Makeup foundations	740	190	—	—	2	3	179	6	—
Lipstick	3319	27	—	—	6	—	14	7	—
Makeup bases	831	263	—	—	1	1	256	5	—
Rouges	211	9	—	—	—	1	7	1	—
Makeup fixatives	22	1	—	—	—	—	1	—	—
Other makeup preparations (not eye)	530	20	—	—	1	—	18	1	—
Cuticle softeners	32	10	—	—	1	1	5	3	—
Nail creams and lotions	25	6	—	—	—	—	6	—	—
Other manicuring preparations	50	2	—	—	—	1	1	—	—
Bath soaps and detergents	148	13	—	—	9	1	3	—	—
Deodorants (underarm)	239	8	—	—	1	1	6	—	—
Other personal cleanliness products	227	8	—	—	1	—	7	—	—
Aftershave lotions	282	5	—	—	—	—	3	2	—
Shaving cream (aerosol, brushless, and lather)	114	100	—	7	11	63	16	3	—
Shaving soap (cakes, sticks, etc.)	7	1	—	1	—	—	—	—	—
Other shaving preparation products	29	6	—	—	2	—	4	—	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	173	—	—	18	12	118	24	1

TABLE 10. (Continued)

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*						
			> 50	*> 25–50*	*> 10–25*	*> 5–10*	*> 1–5*	*> 0.1–1*	*≤ 0.1*
Face, body, and hand skin care preparations (excluding shaving preparations)	832	432	—	2	32	39	325	34	—
Hormone skin care preparations	10	3	—	—	1	1	1	—	—
Moisturizing skin care preparations	747	327	—	2	11	21	259	33	1
Night skin care preparations	219	67	—	—	3	9	48	6	1
Paste masks (mud packs)	171	15	—	—	1	5	9	—	—
Skin lighteners	44	11	—	—	3	—	8	—	—
Skin fresheners	260	4	—	—	4	—	—	—	—
Wrinkle smoothers (removers)	38	4	—	—	—	—	4	—	—
Other skin care preparations	349	55	—	—	13	8	31	3	—
Suntan gels, creams, and liquids	164	48	—	—	1	3	36	8	—
Indoor tanning preparations	15	3	—	—	—	—	—	3	—
Other suntan preparations	28	13	—	—	—	—	12	1	—
1981 TOTALS		2465	1	22	148	231	1826	231	6

submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

Products containing these fatty acid ingredients may contact the skin, hair, and eyes. Use of Oleic and Stearic Acids in lipstick and manicuring preparations may lead to ingestion of small quantities of these ingredients. Frequency of application of the fatty acids may range from once per week to several times per day, from less than 1 h to several hours, due to the variety of cosmetic products in which they are contained.

Noncosmetic Use

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are used in foods as plasticizing, lubricating, binding, and defoaming agents and as reagents in the manufacture of other food-grade additives.[8,20,43] Myristic Acid is used as a flavoring agent in foods.[11]

Straight-chain monobasic carboxylic acids from fats and oils derived from edible sources, such as the fatty acids, Oleic, Lauric, Palmitic, Myristic, and Stearic Acids, are accepted as safe for use in food and in the manufacture of food-grade additives providing they meet particular conditions and specifications.[42] The unsaponifiable matter in the fatty acid or fatty acid-derived food additive must not exceed 2%, the food additive must be free of chick-edema factor, and it must be produced and labeled in accordance with good manufacturing practice.[42]

The fatty acids as a group are permitted as direct food additives.[42] Oleic Acid derived from tall oil and Oleic Acid meeting the specifications in Section 172.860 are permitted as direct food additives.[42] Oleic Acid is also allowed as a food additive in preparations of Polysorbate 80 for which it was used as a reagent.[42] Stearic Acid is permitted as a direct food additive in chewing gum base.[42]

Particular salts of fatty acids are allowed as direct food additives.[42] These salts are not reviewed in this report.

There are no limitations other than the observance of current good manufacturing practice[42] on the use of Oleic and Stearic Acids as indirect food additives.[42] These two fatty acids are also listed as substances that are GRAS.[42]

Regulation of Oleic and Stearic Acids as GRAS substances is based on reviews and evaluation by the Select Committee on GRAS Substances (SCOGS).[44,45] Monographs prepared for these evaluations also are available.[46,47] Several additional reports on fatty acid salts and various ester derivatives have been developed by SCOGS.[48]

FDA files contain both published and unpublished data on the Oleic Acid Group fatty acids (and some of their salts) in the form of Flavor and Extract Manufacturers' Association Monographs, Food Additive Safety Profiles, GRAS Monographs, GRAS Petitions, Food Additive Petitions, and Color Additive

Petitions.* The agency's food safety evaluation of these fatty acids and their salts as direct and indirect food additives and as GRAS substances was based on reviews of these data (document dates range from 1928 to 1977).

Unpublished data from industry submissions to FDA include a two-generation feeding and reproduction study in the rat using Oleic Acid derived from tall oil,[49] a 90-day subchronic oral toxicity study of food-grade Oleic Acid in rats,[50] a 52-day subchronic feeding study of rats using Stearic Acid mixed with lactate salts,[51] a 1-month feeding study of control rats using Stearic Acid as a diet supplement,[52] and a 209-day chronic oral toxicity study of control rats fed a diet supplement of Stearic Acid.[53]

Fatty acids have pharmaceutical uses as lubricants in tablet formulations, in the manufacture of their salts for ointment base emulsifiers,[5] and as calorie sources in parenteral and enteral nutrition therapy.[54] Stearic Acid is widely used in the pharmaceutical coating of enteric pills and bitter remedies and in the preparation of suppositories and ointments.[1,5]

None of the five Oleic Acid Group fatty acids are currently on the Over-The-Counter (OTC) Ingredient list of substances currently being reviewed by OTC scientific panels.[55] Several OTC advisory review panels have determined the level of efficacy of Stearic Acid in the (1) miscellaneous external drug product, (2) topical analgesic including antirheumatic, otic, burn, sunburn treatment, and prevention products, (3) antimicrobial II, and (4) contraceptive and other vaginal drug products categories. However, no determination of its safety was made.[56] Sodium Oleate is under review as a stimulant laxative by the OTC Panel for review of laxatives.[55] The ingredients, "fatty acid," "Oleic Acid," and "Stearic Acid" are listed as "inactive ingredients for approved prescription drug products" that are not required in labeling of these products.[57] The "Inactive Ingredient" list also contains common sources for the fatty acids, such as olive, peanut, cottonseed, nutmeg, tall, and coconut oils.

Fatty acids are used in the manufacture of soaps, detergents, metal salts, driers, and rubber; they are used as solvents for water-insoluble compounds, in polishing compounds, lubricating oils, waterproofing, in candles, phonograph records, insulators, modeling compounds, and as intermediates in chemical synthesis.[1,11,20,43]

Recent clinical uses for fatty acids are their conjugation with antibodies to aid incorporation of the proteins into membranes[58] and their conjugation with antigens for immune potentiation.[59] A derivative of Stearic Acid is commonly used as a paramagnetic probe in the measurement of membrane fluidity by electron spin resonance spectroscopy,[60] and radioactive Palmitic Acid is a diagnostic radiotracer in positron emission tomography.[61]

BIOLOGY

Absorption, Distribution, Metabolism, Excretion

The digestion of dietary fatty acids, their absorption in micellar aggregates, and their transport esterified to glycerol in chylomicrons and very low density

*A listing of these documents was obtained through the Freedom of Information Act. Copies of and notes taken from originals have been placed in Cosmetic Ingredient Review (CIR) files.

lipoproteins has been reviewed.[62-65] Oleic, Palmitic, Myristic, and Stearic Acids are primarily transported via the lymphatic system, and Lauric Acid is transported by the lymphatic and (as a free fatty acid) portal systems.[64] Fatty acids originating from adipose tissue stores are either bound to serum albumin or remain unesterified in the blood.[66,67]

Absorption and distribution studies of some fatty acids were reported in GRAS evaluations and scientific literature reviews of Stearic[45,46] and Oleic Acids[44,47] and the sodium salts of oleate and palmitate.[68] Metabolizable energy values and digestibility coefficients were calculated for Oleic and Stearic Acids in rats, pigs, and chickens. Distribution of radioactivity into various lipid classes in lymph from the thoracic duct of rats was followed for Oleic and Palmitic Acids.

Another monograph on Stearic Acid reviewed its digestion, absorption, and metabolism.[69] It was noted that several investigators found that increasing fatty acid chain length slightly decreased their digestibility; Stearic Acid was the most poorly absorbed of the common fatty acids.[70,71]

Oleic Acid has been reported to penetrate the skin of rats.[72] On histological examination, fluorescence from absorbed Oleic Acid was found in epidermal cell layers of skin removed from treated rats within 10 min of its application. The path of penetration was suggested to be via the hair follicles.[73] Only minute amounts of Oleic Acid were visualized in the blood vessels throughout the experiment. Skin permeability was shown to increase with the lipophilic nature of a compound.[74]

Radioactivity has been traced to the heart, liver, lung, spleen, kidney, muscle, intestine, adrenal, blood, and lymph, and adipose, mucosal, and dental tissues after administration of radioactive Oleic, Palmitic, and Stearic Acids.[69,75,76] The sites of the radioactive atoms (^{3}H, ^{14}C, ^{131}I) were not stated in these studies. Radioactive fatty acids were administered orally, intravenously, intraperitoneally, and intraduodenally into rats, dogs, sheep, chicks, frogs, and humans in various physiological states. Uptake and transport of fatty acids into the brain have been observed.[77]

Proposed mechanisms for fatty acid uptake by different tissues range from passive diffusion to facilitated diffusion or a combination of both.[78,79] Fatty acids taken up by the tissues can either be stored in the form of triglycerides (98% of which occurs in adipose tissue depots) or they can be oxidized for energy via the β-oxidation and tricarboxylic acid cycle pathways of catabolism.[80]

The β-oxidation of fatty acids occurs in most vertebrae tissues (except the brain) using an enzyme complex for the series of oxidation and hydration reactions resulting in the cleavage of acetate groups as acetyl-CoA (coenzyme A). An additional isomerization reaction is required for the complete catabolism of Oleic Acid.[63] Alternate oxidation pathways can be found in the liver (ω-oxidation) and in the brain (α-oxidation).[81-83]

Fatty acid biosynthesis from acetyl-CoA takes place primarily in the liver, adipose tissue, and mammary glands of higher animals. Successive reduction and dehydration reactions yield saturated fatty acids up to a 16-carbon chain length. Stearic Acid is synthesized by the condensation of palmitoyl-CoA and acetyl-CoA in the mitochondria, and Oleic Acid is formed via a monooxygenase system in the endoplasmic reticulum.[4,82]

Fatty acid metabolism has been extensively studied under various physiological conditions,[84-86] in mammalian development,[87,88] in various organisms,[89] as affected by xenobiotics, such as ethanol[90,91] and drugs.[92] The regulation of fatty acid metabolism has been reviewed.[93-96]

Simultaneous ingestion of trace amounts of ^{14}C-triolein (10 μCi) and ^{3}H-Oleic Acid (20 μCi) in 42 g of carrier fat by patients with normal fecal fat excretion resulted in estimated fecal excretion of less than 10% of both substances.[97] Gastrointestinal transit times for ^{14}C-triolein, ^{3}H-Oleic Acid, and a nonabsorbable marker, $^{51}CrCl_3$, did not differ significantly.

Fatty acid metabolism has been studied in several tissues. Interest in the correlation between fatty acids, cholesterol, and coronary heart disease has spurred extensive research on myocardial fatty acid metabolism.[98-101] Fatty acid metabolism has also been studied in the liver,[102-104] the intestine and intestinal microflora,[105,106] the lungs,[107] the kidneys,[108-110] skeletal muscle,[111] bone and cartilage,[112] and oral mucosal epithelium.[113]

Maternal–Fetal Transfer

Free fatty acids readily cross the placental barrier in rabbits, guinea pigs, rats, and humans.[114-118] A bolus of 1-^{14}C-Palmitic Acid was injected over 10 sec into the carotid artery of 4 pregnant guinea pigs ranging in gestational age from 48 to 65 days.[119] The fetal side of the placenta was perfused in situ. A rapid decline in maternal plasma radioactivity and a rapid appearance of radioactivity in the perfusate were observed. The disappearance profile of fetal radioactivity essentially paralleled that of maternal radioactivity after a lag time of 1.6 min. Other studies of maternal–fetal transfer of fatty acids were performed primarily with albumin-bound or lipoprotein-emulsified 1-^{14}C-Palmitic Acid.[119,120]

Dietary Fat and Coronary Heart Disease

The Select Committee on GRAS Substances stated its "concern over the role of saturated versus polyunsaturated fatty acids in the etiology of arteriosclerosis and associated vascular diseases" in their review of Stearic Acid.[45] The Committee noted a joint statement by the Food and Nutrition Board of the National Research Council and the Council on Foods and Nutrition of the American Medical Association that acknowledged the importance of reducing the intake of saturated fatty acids and cholesterol.[121] Cholesterol has been reviewed by Cosmetic Ingredient Review.[122]

Current studies and reviews confirm the correlation between dietary saturated fatty acid intake and the incidence of atherosclerosis and thrombosis found in earlier studies and reports.[123,124] Research is now focused on the mechanism(s) of induction and the elucidation of the multifactorial influence of diet on coronary heart disease.[100,101]

TABLE 11. Antimicrobial Activity of Fatty Acids[125, 126]

Organism	*Oleic Acid*	*Lauric Acid*	*Palmitic Acid*	*Myristic Acid*	*Stearic Acid*
	Minimal Inhibitory Concentration (mM)				
Aspergillus niger	—	> 4	—	—	—
Bacillus cereus	—	> 2	—	—	—
Bacillus subtilis	—	> 2, 0.5[b]	—	—	—
Candida albicans	NI[a]	2.49	NI	4.37	NI
Candida utilis	—	4, 1[b]	—	—	—
Micrococcus lysodeikticus	—	> 2	—	—	—
Penicillium citrinum	—	4	—	—	—
Pseudomonas aeruginosa	NI	NI	—	—	—
Streptococcus pneumoniae	NI	0.062	0.48	0.218	NI
Saccharomyces cerevisiae	—	> 4	—	—	—
Staphylococcus aureus	NI	2.49	NI	4.37	NI
Streptococcus Group A	1.77	0.124	3.9	0.547	NI
Streptococcus β-hemolytic type	—	0.249	3.9	2.18	NI

[a]NI, not inhibitory at concentrations tested (1.0 mg/ml or 3–6.0 m*M*).
[b]1st value obtained by agar dilution method, 2nd value obtained by broth dilution method.

Antimicrobial Activity

The antibacterial activities of Oleic, Lauric, Palmitic, Myristic, and Stearic Acids were studied by placing them in liquid broths containing different microorganisms.[125] Minimal inhibitory concentrations at 37°C were determined. Results of this study and of other studies on bacteria and fungi[126] are presented in Table 11.

The effects of Oleic, Lauric, Palmitic, Myristic, and Stearic Acids on aflatoxin B_1 production and growth of the fungus *Aspergillus parasiticus* were studied.[127] Concentrations of 5 m*M* fatty acid were added to liquid medium containing "three drops of the emulsifier, Tween-80." Myristic, Palmitic, and Stearic Acids stimulated and Oleic Acid inhibited toxin synthesis. Lauric Acid inhibited fungal growth.

The antiviral activity of Oleic Acid and other unsaturated fatty acids was studied.[128] These fatty acids inactivated enveloped viruses, such as herpes, influenza, Sendai, and Sindbis viruses at concentrations from 5 to 50 μg/ml. "Naked" viruses, such as polio, SV40, and encephalomyocarditis viruses, were not affected, indicating a direct memebrane effect. Stearic Acid did not inactivate any of the viruses at the concentrations tested.

TOXICOLOGY

Reviews of the literature from 1933 to 1976 were prepared for the safety evaluations of Oleic and Stearic Acids as GRAS substances by FDA[44–47] and of Stearic Acid as a fragrance raw material by Research Institute for Fragrance

Materials (RIFM).[69] RIFM Reviews of Oleic and Myristic Acids have been prepared and are pending publication. A subchronic oral toxicity study of Palmitic Acid was presented in a GRAS monograph on sodium oleate and sodium palmitate.[68]

Oral Toxicity Studies

Acute Oral Toxicity

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids were tested for acute oral toxicity to rats (Table 12).

Administration of doses up to 21.5 ml/kg of Oleic Acid and up to 10.0 g/kg of Palmitic and Myristic Acids (commercial grades) by gavage to albino rats resulted in no deaths and no significant gross lesions at necropsy.[129,130] Doses of 10.0 g/kg of commercial grade Lauric Acid and of 25% (w/v) Stearic Acid in corn oil produced the deaths of 1 rat in each group. At necropsy of these rats, congested lungs and kidneys and advanced autolytic changes were observed. No significant gross lesions were found at necropsy of 2 rats of the 0.464 and 4.64 g/kg triple-pressed Stearic Acid dose groups. Transient signs of toxicity were observed in rats of the higher dose groups of 10.0 and 21.5 ml/kg Oleic Acid, 10.0 g/kg 25% Stearic Acid in corn oil, and the 4.64 and 10.0 g/kg Lauric, Palmitic, Myristic, and triple-pressed Stearic Acids. Signs of toxicity included slight depression, depressed righting and placement reflexes, oily and unkempt fur, mucoid diarrhea, excessive salivation, and sero-sanguineous discharge from the muzzle and eyes.

A cream formulation containing 5% Oleic Acid administered to rats at a dose of 5 ml/kg produced no mortalities. Signs of toxicity included transient weakness in the legs and colored urine and feces.[131]

Oral administration of a 5.0 g/kg dose of a product formulation containing 8.7% Lauric Acid to rats produced slight toxicity and no deaths.[132]

A shave cream formulation containing 2.2% Palmitic Acid administered to rats at a dose of 5 g/kg produced no deaths and was classified as "non-toxic."[133]

White rats were fed a diet containing 50% Stearic Acid.[144] Treated male rats died after an average of 8.2 days and female rats died after 10.2 days. Spasms and paralysis of the extremities of some rats and cardiac irregularities were observed immediately preceding death. With a lower concentration of 15% Stearic Acid in the diet, the rats lived for a much longer period.

In three studies, groups of 5 male albino rats received oral doses of 0.464–10.0 g/kg "eutectic, triple-pressed" Stearic Acid and 25% (w/v) Stearic Acid in corn oil,[130] or approximately 16% Stearic Acid in ethylene oxide and water (65% solution in ethylene oxide diluted 1:3 in water).[134] There were 2 deaths in the 4.64 g/kg dose group of the first study and 1 death in the 10.0 g/kg dose groups of the second and third studies.

A dose of 5 g/kg of a face cream formulation containing 13% Stearic Acid produced no deaths when administered to albino rats by gavage.[135] Skin lotion formulations containing 2.8% Stearic Acid administered at doses of 15 g/kg by gavage to groups of 10 albino rats resulted in 1 death in 1 group.[136,137]

At necropsy of the rat that died, fibrous tissue around the heart and reddish fluid throughout the thoracic cavity were observed. Normal behavior and appearance were observed, and there were no gross alterations in surviving rats. Slight dehydration and depression were observed in 1 rat.

In other studies, testing for acute oral toxicity of skin lotion formulations containing 2.8% Stearic Acid by administration of 5 ml/kg[140-143] and 5 g/kg[138,139] doses of the formulations resulted in few, if any, deaths. At necropsy of the rats that died, fibrous tissue encasing the heart and lungs was observed.

Subchronic and Chronic Oral Toxicity

Feeding of 5% Oleic Acid or 50% Stearic Acid diets to chicks for 4 weeks had no adverse effects (Table 13).[145,146] Decreased clotting time, moderate hyperlipemia, and severe phlebothrombosis following initiation with an intravenous injection of lipopolysaccharide from *Salmonella typhosa* were observed in rats fed high-fat diets containing 5% Stearic Acid.[147,148] Rats fed diets containing 4.6 g/kg/day Palmitic Acid for 6 weeks developed hyperlipemia.[148] A diet containing 50% Stearic Acid fed to rats for 8 weeks resulted in a microscopic "foreign body-type reaction" in adipose tissue.[149] Rats fed high-fat diets containing 6% Stearic Acid for 9 weeks developed severe aortic atherosclerosis and thrombosis induced by *S. typhosa* lipopolysaccharide; high mortality was also observed.[147]

Feeding 15% Oleic Acid diets to rats for 10–16 weeks had no adverse effects on growth or general health.[150] Of 4 female weanling rats fed the diet for 16 weeks, "all 4 were able to become pregnant; however 2 died at parturition, a litter was eaten at birth, and the remaining litter died within 3 days of birth." Mating of 7 adult female rats fed the diet for 16 weeks resulted in production of 52 young, 44 of which survived 1 week and 11 of which survived 3 weeks. Mammary development was retarded, and a few rats had ovarian cysts. No lesions were found in other organs.

A "foreign body-type reaction" in perigonadal fat and the reversible formation of lipogranulomas were observed in rats fed 50 g/kg/day Stearic Acid for 24 weeks.[151] Anorexia, severe pulmonary infection, and high mortality were observed in rats fed diets containing 3000 ppm Stearic Acid for 30 weeks.[152]

Dermal Toxicity Studies

Acute Dermal Toxicity

Oleic, Palmitic, and Stearic Acids were tested for acute dermal toxicity after topical application and intradermal administration to the skin of guinea pigs, rabbits, and mice (Table 14).

In one study, application of commercial grade Oleic Acid to the skin of guinea pigs produced no deaths and no signs of toxicity. The number of applications was not stated.[153] Marked irritation characterized by crusting, ulceration, and thickening of the skin was observed following topical application of commercial grade Oleic Acid to the skin of rabbits, guinea pigs, and

TABLE 12. Acute Oral Toxicity Studies

Fatty acid tested	*Dose*	*Species (No. per group)*	*Results*	*Reference*
Oleic Acid[a]	5.0 g/kg	5 albino rats (bodyweight 193–217 g)	Range of BW after 7 days—235–273 g. No deaths. Signs of toxicity not reported. Oleic Acid classified "slightly toxic by ingestion"	129
Oleic Acid[b]	0.464, 1.00, 2.15, 4.64, 10.0, 21.5 ml/kg	5 male albino rats (BW 214–220 g)	LD_{50} > 21.5 ml/kg. Range in avg. BW gains 65–99. No deaths in any group	130
Oleic Acid—5.0% in cream formulation	5 ml/kg of cream	10 Fischer 344 rats (BW 135–175 g)	No deaths. Transient leg weakness, colored urine and feces	131
Lauric Acid[a]	0.464, 1.00, 2.15, 4.64, 10.0 g/kg	5 male albino rats (BW 221–247 g)	Range, avg. BW gain—73–99 g. One death in group given 10.0 g/kg dose on 1st postdosage day	130
Lauric Acid—8.7% in product formulation	5.0 g/kg of product	5 albino rats (BW 155–160 g)	BW range after 7 days—209–230 g. No deaths. Signs of toxicity not reported. Lauric Acid classified "slightly toxic by ingestion"	132
Palmitic Acid[a]	0.464, 1.00, 2.15, 4.64, 10.0 g/kg	5 male albino rats (BW 209–254 g)	Range, avg. BW gain—65–92 g. No deaths	130
Palmitic Acid—2.2% in shave cream formulation	5 g/kg of cream	≥ 10 albino rats (BW 200–300 g)	Formulation classified "non-toxic." No data or procedures (other than administration by gavage) reported; reference for test method - 16 CFR 1500.3(b)(6)(i)(A)	133
Myristic Acid[a]	0.464, 1.00, 2.15, 4.64, 10.0 g/kg	5 male albino rats (BW 208–211 g)	Range, avg. BW gain—75–95 g. No deaths	130
Stearic Acid (eutectic)[a]	0.464, 1.00, 2.15, 4.64, 10.0 g/kg	5 male albino rats (BW 213–223 g)	Range, avg. BW gain—71–101 g. One death in 4.64 g/kg dose group on day of dosage; one death in 4.64 g/kg dose group on final day of study	130

Stearic Acid—25% (w/v) in corn oil	0.464, 1.00, 2.15, 4.64, 10.0 g/kg	5 male albino rats (BW 216–225 g)	Range, avg. BW gain—90–104 g at lower doses, 77 g at 10.0 g/kg dose. One death in 10.0 g/kg on Day 7 of study	130
Stearic Acid—65% in ethylene oxide, diluted 1 : 3 in water	5 and 10 g/kg	10 male young adult ARS/Sprague-Dawley albino rats (BW 215–239 g)	Final avg. BW 5 g/kg group—317 g; 10 g/kg group—258 g. One death in 10 g/kg dose group on Day 5 following dosage. No pharmacotoxical signs noted. No remarkable alterations at necropsy	134
Stearic Acid—13% in face cream formulation	5 g/kg face cream	≥ 10 albino rats (BW 200–300 g)	Formulation classified "non-toxic." No procedures (other than administration by gavage) or data reported. Reference for test method - 21 CFR 1500.3(b)(6)(i)(A)	135
Stearic Acid—2.8% in skin lotion formulation	15 g/kg skin lotion	10(5M, 5F)albino rats (BW 206–258 g)	Final BW range—228–378 g. One death on Day 2	136
Stearic Acid—2.8% in skin lotion formulation	15 g/kg skin lotion	10(5M, 5F)albino rats (BW 218–254 g)	Final BW range—198–414 g. No deaths	137
Stearic Acid—2.8% in skin lotion formulation	5 g/kg skin lotion	10(5M, 5F)albino rats (BW 184–238 g)	Final BW range—174–386 g. Two deaths on Days 9 and 10	138
Stearic Acid—2.8% in skin lotion formulation	5 g/kg skin lotion	10(5M, 5F)albino rats (BW 202–264 g)	Final BW range—210–430 g. One female rat died on Day 7 postdosage. All rats appeared normal throughout study. At necropsy, fibrous tissue was observed encasing heart and lungs of rat that died and no gross changes were observed in other rats	139
Stearic Acid—2.8% in skin lotion formulation	5.0 ml/kg skin lotion	10 Sprague-Dawley rats (BW 200–254 g)	Range in BW gain—75–127 g. No deaths. All rats appeared normal throughout study. At necropsy, thoracic and abdominal organs appeared normal.	140
Stearic Acid—2.8% in skin lotion formulation	5.0 ml/kg skin lotion	10 Sprague-Dawley rats (BW 174–200 g)	Range in BW gain—85–118 g. No deaths. All rats appeared normal throughout study. At necropsy, thoracic and abdominal organs appeared normal	141
Stearic Acid—2.8% in skin lotion formulation	5.0 ml/kg skin lotion	10 Sprague-Dawley rats (BW 175–189 g)	Range in BW gain—42–118 g. No deaths. All rats appeared normal throughout study. At necropsy, thoracic and abdominal organs appeared normal	142
Stearic Acid—2.8% in skin lotion formulation	5.0 ml/kg skin lotion	6 Sprague-Dawley rats (BW 205–214 g)	Range in BW gain—102–129 g. No deaths. All rats appeared normal throughout study. At necropsy, thoracic and abdominal organs appeared normal	143
Stearic Acid	5 g/kg	rat	No deaths	45

[a]Fatty acid commercially supplied.

[b]These studies were cited in reviews for the safety assessment of particular fatty acids as they are used in foods[44–47, 68] and in fragrances.[69]

TABLE 13. Subchronic and Chronic Oral Toxicity Studies[a]

Study type	*Fatty acid tested*	*Species*	*Results*	*Reference*
Subchronic feeding study (4 weeks)	Stearic Acid—50% in diet	Chick	No adverse effects	145, 146
Subchronic feeding study (4 weeks)	Oleic Acid—5% in diet	Chick	No adverse effects	145
Subchronic feeding study (6 weeks)	Stearic Acid—5% in high-fat diet	Rat	Decreased clotting time, moderate hyperlipemia, severe phlebothrombosis after initiation with *S. typhosa* lipopolysaccharide (LPS)	147, 148
Subchronic feeding study (6 weeks)	Palmitic Acid—4.6 g/kg/day in diet	Rat	Most hyperlipemic of all fatty acids tested (versus Lauric, Myristic, and Stearic Acids). Second to Stearic Acid in thrombogenic effect	148
Subchronic feeding study (8 weeks)	Stearic Acid—50% in diet	Rat	Microscopic foreign body type reaction in excised fat. No reaction in controls	149
Subchronic feeding study (9 weeks)	Stearic Acid—6% in high-fat diet	Rat	Severe aortic atherosclerosis, high mortality, severe thrombosis after *S. typhosa* LPS initiation	147
Subchronic feeding study (10 weeks)	Oleic Acid—15% in diet	Rat	Normal appearance. Mammary gland underdeveloped; few rats with ovarian cysts. No lesions in non-reproductive organs. Production of 52 young by 7 adult females—11/52 survived by 3rd week	150
Chronic feeding study (16 weeks)	Oleic Acid 15% in diet	Rat	No impairment of males' fertility. 4/4 females became pregnant; 2/4 deaths at parturition; 1 litter died within 3 days of birth	150
Chronic feeding study (20 weeks)	Oleic Acid—15% in diet	Rat	Normal growth observed	150
Chronic feeding study (24 weeks)	Stearic Acid—50 g/kg/day in diet	Rat	4/5 rats had foreign body type reaction in perigonadal fat. Lipogranulomas observed. Reversible effects	151
Chronic feeding study (30 weeks)	Stearic Acid—3000 ppm in diet	Rat	Anorexia, severe pulmonary infection, high mortality. No significant pathological lesions	152

[a] These studies were cited in reviews for the safety assessment of particular fatty acids as they are used in foods[44–47, 68] and in fragrances.[69]

TABLE 14. Acute Dermal Toxicity Studies[a]

Fatty acid tested	*Dose*	*Species (No. per group)*	*Results*	*Reference*
Oleic Acid[c]	3.0 g/kg	6 guinea pigs	No deaths. Oleic Acid classified "non-toxic"	153
Oleic Acid[c]	1–2 ml	5 rabbits	Potent depilatory agent. Marked irritation. Microscopic hyper-	154[b]
	1 ml	2 guinea pigs	keratosis, acanthosis. (Observations in all 3 species)	
	0.3 ml	12 mice		
Oleic Acid—50% in mineral oil	1 ml	16 HRS/J mice	Epidermal hyperplasia and hyperkeratosis	155
Oleic Acid—25, 50, 75% in peanut oil	0.1 ml (intradermal)	2 guinea pigs	Local inflammation and necrosis. No alterations in controls given peanut oil	156[b]
Palmitic Acid—2.2% in shave cream formulation	2 g/kg	≥ 10 rabbits	No deaths. Formulation considered "non-toxic"	133
Stearic Acid—10–100 m*M* in olive oil	10–100 m*M* (intradermal)	guinea pigs rabbits	Mild erythema and slight induration of skin	157[b]

[a]Methods of most studies involved topical application of fatty acids. Intradermal administration noted parenthetically.

[b]Data from these studies were obtained from reviews for the safety assessment of particular fatty acids in foods[46, 47, 68] and fragrances.[69]

[c]Fatty acid as commercially supplied.

mice.[154] Microscopically, hyperkeratosis, pronounced acanthosis, follicular keratotic plugs, hyperplasia of sebaceous glands, and loss of hair shafts from follicles were observed. Treated skin returned to normal when treatment was discontinued.

Local skin inflammation and necrosis were observed at sites on the backs of guinea pigs receiving 0.1 ml intradermal injections of 25, 50, and 75% Oleic Acid in peanut oil and Oleic Acid as commercially supplied. No alterations were observed at sites injected with peanut oil alone.[156]

Epidermal hyperplasia and hyperkeratosis were observed in the skin of mice after topical application of 50% Oleic Acid in mineral oil.[155]

Application of a 2 g/kg dose of a shave cream formulation containing 2.2% Palmitic Acid was considered nontoxic to rabbits.[133,158]

Concentrations from 10 to 100 m*M* Stearic Acid in olive oil applied to the skin of guinea pigs and rabbits produced mild erythema and slight induration.[157]

Short-Term Dermal Toxicity

Follicular–keratogenic properties of Oleic, Lauric, Palmitic, Myristic, and Stearic Acids were studied after topical application to the skin of the external ear canal of 4 albino rabbits[159] (Table 15). A 5% (w/v) alcohol solution of Stearic Acid and alcohol solutions of the other fatty acids equimolar with the Stearic Acid solution were prepared [5% (w/v) Stearic Acid ~ 18 mmol% Stearic Acid]. A dose of 3 ml of each of the fatty acid solutions was applied once daily, 5 days per week, for 6 weeks. Controls in one group received similar treatment with absolute alcohol and those in another group received no treatment. Myristic and Palmitic Acids produced transient slight erythema and desquamation in the first 2 weeks of application. No clear alterations were observed after Stearic Acid treatment. One day after treatment with Oleic and Lauric Acids, erythema was observed. The intensity of the redness increased over the following few days and desquamation developed. Distinct follicular keratosis was observed within 1 month. After discontinuation of the applications, the erythema and scaling gradually disappeared, but the keratosis was discernible after 6 weeks.

Follicular epidermal hyperplasia was produced after topical application of undiluted commercial grade Oleic Acid (unspecified dose) to the backs of white mice 6 times per week for 1 month.[160]

In a recent study, no adverse effects were produced from subchronic topical application of Myristic Acid to rabbit skin.[161] One-half milliliter of a 30% preparation of Myristic Acid in ether and propylene glycol (solvents at a 1:1 ratio in concentration) was massaged into the depilated skin of the flanks of 5 rabbits daily for 30 days. The opposite flank of the rabbits was depilated and treated with solvent only. No significant macroscopic changes were observed. Microscopic lesions included thinning of collagen fibers in the superficial layers of the dermis after 10 days and a loose dermal infiltrate of lymphomononuclear cells and histiocytes after 20 and 30 days.

Stearic Acid application had little effect on the epidermis of rats.[72] Hair on the dorsa of albino or Long-Evans rats had been closely clipped before an unspecified dose of Stearic Acid was swabbed on the treatment sites once daily for 5 days to 2 weeks.

TABLE 15. Short-term Dermal Toxicity Studies

Fatty acid tested	*Dose*	*Species*	*Method Notes*[a]	*Results*	*Reference*
Oleic Acid—~ 18 mmol% in alcohol	3 ml	4 rabbits	External ear canal, 6 weeks	Erythema, desquamation, follicular keratosis	159[b]
Oleic Acid		Mice	Dorsa for 1 month	Epidermal hyperplasia	160[b]
Lauric Acid—~ 18 mmol% in alcohol	3 ml	4 rabbits	External ear canal, 6 weeks	Results similar to those after Oleic Acid application. Follicular keratosis persisted after treatment	159[b]
Palmitic Acid—~ 18 mmol% in alcohol	3 ml	4 rabbits	External ear canal, 6 weeks	Slight irritation for first 2 weeks	159[b]
Myristic Acid—~ 18 mmol% in alcohol	3 ml	4 rabbits	External ear canal, 6 weeks	Slight irritation for first 2 weeks	159[b]
Myristic Acid—30% in ether : propylene-glycol	0.5 ml	5 rabbits	Flank, 30 days	Microscopic thinning of dermal collagen. Cellular infiltration	161
Stearic Acid—50% (w/v) in alcohol	3 ml	4 rabbits	External ear canal, 6 weeks	No alterations	159[b]
Stearic Acid—20% in product formulation	2 ml/kg of product	6 rabbits	Abraded/intact sites on back, 4 weeks	No deaths. Slight edema, desquamation	162
Stearic Acid—20% in product formulation	2 ml/kg of product	6 rabbits	Abraded/intact sites on back, 4 weeks	No deaths. Slight edema, desquamation	163

[a]All methods involved repeated topical application to noted sites.
[b]Data from these studies were obtained from reviews for safety assessment of particular fatty acids in foods[46, 47, 68] and fragrances.[69]

Stearic Acid, at a concentration of 2.0% in 2 cosmetic product formulations was tested for subchronic dermal toxicity using groups of 6 New Zealand strain albino rabbits.[162,163] Hair was clipped from the backs of the rabbits, and the skin was either abraded or left intact. Doses of 2 ml/kg of the product formulations were applied to the sites daily, 5 days per week, for a total of 20 applications. The rabbits in the untreated control group had no signs of skin irritation. No mortalities were observed in the 2 groups of rabbits receiving applications of either formulation.

In the first group, the mean percentage gain in body weight was 33%, and the skin of all 6 rabbits was slightly edematous; edema was observed in 3/6 rabbits after the first week, 1/6 rabbits during the third week, and 2/6 rabbits during the fourth week. The skin of 5 of the 6 rabbits remained edematous for the duration of the study. Two of the rabbits had slight local desquamation of the skin that was of irregular duration. The brown color of the product obscured scoring of treatment sites for erythema. Both abraded and intact skin had similar reactions to treatment with the product. Individual fluctuations in hematological values were noted in animals of all groups including controls. Slight differences in serum glutamic-pyruvic transaminase values were observed that were considered unrelated to treatment. At necropsy, organ weights of the treated group were comparable to those of controls, and the pulmonary hemorrhages observed in 1 male were considered unrelated to treatment and common in New Zealand strain rabbits. Discharge from the left eye of 1 male rabbit was noted. No significant microscopic lesions considered to be treatment-related were noted.

In the second group of 6 NZW rabbits that received applications of a product formulation containing 2.0% Stearic Acid for 4 weeks, the mean body weight gain was 18%. The skin of all 6 rabbits was slightly edematous; edema was observed in 1/6 rabbits during the first week, 1/6 rabbits during the second week, and 4/6 rabbits during the fourth week. The edema observed in the skin of the first 2 rabbits disappeared after a few days, recurring in 1 during the fourth week. One rabbit had slight atonia during the second week only. Four rabbits during the second week and 2 rabbits during the third week developed slight desquamation of the skin at treatment sites, which returned to normal. Slight scaling of the skin was observed for the duration of the study. The brown-colored product obscured scoring of treatment sites for erythema. Clinical signs of toxicity included nasal discharge in 2 male rabbits (on days 18–28 and on days 10 and 11) and scabs on the back of a female rabbit (on days 7–28). Both intact and abraded sites had similar reactions to the treatment. No distinct treatment-related effects were noted in hematological, biochemical, or organ weight values. There were no significant gross or microscopic alterations.

A facial skin care product formulation containing 5.0% Stearic Acid was applied to the shaved dorsal skin of 15 female rats of the Crl:COBS CD(SD)BR strain in a 13-week dermal toxicity study.[164] Daily doses of 4.0 ml/kg of the product were applied 5 days per week for a total of 65 applications. The treatment was estimated to provide a dose 100-fold greater than the daily exposure to humans. Controls received no treatment. There were no deaths in the treatment group and one death in the control group. No major changes in

appearance or behavior were observed that were treatment-related, although minimal to moderate skin irritation was observed in all rabbits throughout the study. Statistically significant ($p < 0.05$) changes included decreased glucose and increased serum glutamic-pyruvic transaminase concentrations during the 7th week, and decreased hemoglobin, hematocrit, mean corpuscular volume, and total erythrocyte count during the 13th week. Urinalysis values were within normal limits. At necropsy, increases in absolute weights of the liver, heart, kidneys, and adrenals and in liver/body weight ratios were statistically significant ($p < 0.05$). The apparent statistical significance between hematological, biochemical, and organ weight values of treated and control groups was within normal limits. Subclinical bronchitis and "focal interstitial mononuclear cell infiltration into the kidneys, liver and heart" were noted in an unspecified number of rats. Grade 1 hyperkeratosis was observed in 5 of 15 treated rats.

A concealing cream product formulation containing 2.4% Stearic Acid was applied to the shaved dorsal skin of 15 female Sprague-Dawley rats in a 13-week dermal toxicity study.[165] Daily doses of 227 mg/kg of the product were applied 5 days a week for a total of 65 applications. As in the preceding study,[164] the treatment was estimated to provide a dose 100 times greater than the typical human exposure. Controls received no treatment. There were no deaths or significant differences in growth rates. Sporadic and transient skin irritation was observed in the treatment group throughout the study. Statistically significant ($p < 0.05$) differences between treatment and control groups in mean hematology values (decreased hemoglobin during weeks 7 and 13, decreased hematocrit during week 7, increased mean corpuscular volume during week 13, and decreased total erythrocyte count during weeks 7 and 13) and mean serum chemistry values (decreased serum alkaline phosphatase during week 13) were within normal limits. Urinalysis values were considered normal. At necropsy, changes in mean absolute organ weight (brain) and mean relative organ weights (liver/body, spleen/body) were considered toxicologically insignificant. Minimal hyperkeratosis of the epidermis was observed in some rats.

Administration of subcutaneous Oleic Acid injections at volumes increasing from 0.25 to 0.5 ml for 400 days had no adverse effects in the growth of albino mice. The life duration of mice of both sexes was lower than that expected for normal mice.[166]

Primary Skin Irritation

The fatty acids, Oleic, Lauric, Palmitic, Myristic, and Stearic Acid, were tested for primary skin irritation from topical application to the skin of rabbits (Table 16).

In a single insult occlusive patch test (SIOPT) with 6 albino rabbits, administration of a 0.5 ml dose of Oleic Acid, as commercially supplied, resulted in a primary irritation index (PII) of 0.5 (max PII = 8.0) and mild erythema 24 h after treatment.[130] In a Repeat Open Patch study with 6 rabbits (specific procedure not reported), application of commercial grade Oleic Acid produced mild to moderate erythema after 24 h, mild to marked erythema after 48 h, and moderate to marked erythema after 72 h. Slight to moderate

TABLE 16. Primary Skin Irritation Studies

Fatty acid tested	*Dose*	*No. of Rabbits*	*Method*	*Results*	*Reference*
Oleic Acid, as commercially supplied	0.5 ml	6	SIOPT,[a] I/A[b]	PII[c] 0.50. Minimal erythema at 24 h	130
Oleic Acid, as commercially supplied	~ 0.5 ml	6	Repeat Open Patch, 24, 48, 72 h patches	Cumulative irritation increasing from mild erythema and no edema at 24 h to marked erythema and moderate edema in some rabbits at 72 h	167
Oleic Acid—5.08% in product formulation	0.5 g of product	6	Modified Draize, 3 open patches	Minimal erythema after 72 h	169
Oleic Acid—5.08% in product formulation	0.5 g of product	6	See preceding entry	Minimal erythema in 3 rabbits after 72 h	170
Oleic Acid 5 % in product formulation	0.5 ml of product	6	Daily, 14 d	PII 2.3. Slight irritation after 4–7 days	131
Lauric Acid, as commercially supplied	0.5 ml	6	SIOPT, I/A	PII 1.12. Minimal erythema after 24 h. Minimal edema at few A sites after 72 h	130
Lauric Acid—8.7% in product formulation	0.5% of product in water	6	SIOPT, I/A	PII 0. No irritation	171
Palmitic Acid, as commercially supplied	0.5 ml	6	SIOPT, I/A	PII 0. No irritation	130
Palmitic Acid—74% "plus other fatty acids"	0.5 g	6	SIOPT, I/A 4-h exposure	PII 0.2. Very slight erythema at few I and at all A sites after 4 h	172
Palmitic Acid—4.4% in product formulation	0.5 ml of product	9	SIOPT	PII 1.00. Mild erythema after 2 h. Minimal to mild erythema after 24 h	173
Palmitic Acid—4.4% in product formulation	~ 0.5 ml of product	9	SIOPT	PII 1.00. See preceding entry	174
Palmitic Acid—2.2% in product formulation	0.5 g of product	≥ 6	SIOPT, I/A	"Non-irritating." No other data or specific procedures reported	133
Myristic Acid, as commercially supplied	0.5 ml	6	SIOPT, I/A	PII 0. No irritation	130
Myristic Acid, as commercially supplied	~ 0.5 g	6	Repeat Open Patch	Cumulative irritation increasing from no to mild/moderate erythema from 24 to 72 h	175

Stearic Acid, as commercially supplied	0.5 ml	6	SIOPT, I/A	PII 0. No irritation	130
Stearic Acid (eutectic), as commercially supplied	0.5 ml	6	SIOPT, I/A	PII 0. No irritation	130
Stearic Acid, as commercially supplied	~ 0.5 ml	9	SIOPT, 2-h exposure	PII 0.33. Few rabbits with barely perceptible erythema after 24 h	176
Stearic Acid—65% in ethylene oxide	0.5 g	6	SIOPT, I/A	PII 3.00. Defined erythema and slight edema after 24 and 72 h	134
Stearic Acid—59% "plus other fatty acids"	0.5 g	6	SIOPT, I/A, 4-h exposure	PII 0. No irritation	172
Stearic Acid—45% "plus other fatty acids"	0.5 g	6	SIOPT, I/A, 4-h exposure	PII 0. No irritation	172
Stearic Acid—50% in petrolatum	~ 0.5 ml	9	SIOPT, 2-h exposure	PII 0.56. Few with mild erythema after 2 h; decreased to barely perceptible erythema after 24 h	177
Stearic Acid—35% in water	~ 0.5 ml	9	SIOPT, 2-h exposure	PII 0.33. Few with barely perceptible erythema after 2 h	178
Stearic Acid—13% in product formulation	0.5 g of product	≥ 6	SIOPT, I/A	"Non-irritating." No other data or procedures reported	179
Stearic Acid—2.8% in product formulation	0.5 ml of product	6	SIOPT, I/A	PII 1.00. Transient minimal erythema after 24 h	138
Stearic Acid—2.8% in product formulation	0.5 ml of product	6	SIOPT, I/A	PII 1.05. Transient irritation after 24 h	139
Stearic Acid—2.8% in product formulation	0.5 g of product	6	SIOPT, I/A	PII 0.92. Very slight erythema after 24 and 72 h, persisting at most A sites. Transient minimal edema	140
Stearic Acid—2.8% in product formulation	0.5 ml of product	6	SIOPT, I/A	PII 1.45. Transient minimal to defined erythema and edema after 24 h. Dry skin noted	136
Stearic Acid—2.8% in product formulation	0.5 g of product	4	SIOPT, I/A	PII 0.63. Transient very slight erythema after 24 h	143
Stearic Acid—1.0% in product formulation	0.5 ml of product	6	SIOPT, I/A	PII 2.2. Transient defined erythema and edema after 24 h	180
Stearic Acid—1.0% in product formulation	0.5 ml of product	6	SIOPT, I/A	PII 2.0. Barely perceptible erythema, transient edema after 24 h	180

[a]SIOPT, single insult occlusive patch test, usually 24 h exposure period.
[b]I/A, patches applied to intact and abraded skin sites.
[c]PII, primary irritation index (max = 8.00).

edema was observed after 72 h.[167] In Modified Draize tests,[168] 3 repeated open patch topical applications of cream blush formulations containing 5.08% Oleic Acid produced mild erythema in 6 female NZW rabbits after 72 h. The formulations were not primary skin irritants.[169,170] In a 14-day study with 6 NZW rabbits, the daily topical applications of a red cream formulation containing 5% Oleic Acid produced slight to well-defined erythema and slight

In an SIOPT, commercial grade Lauric Acid applied to intact and abraded sites of the skin of 6 albino rabbits produced slight erythema at both sites after 24 h, which subsided by 72 h, minimal edema after 72 h, and a PII of 1.12. Blanching and some coriaceous tissue were noted at a few abraded sites.[130] In an SIOPT, a 5% aqueous preparation of a product formulation containing 8.7% Lauric Acid applied to intact and abraded skin of 6 albino rabbits resulted in a PII of 0.[171]

A dose of 0.5 ml of commercial grade Palmitic Acid applied to intact and abraded sites on the skin of 6 albino rabbits in an SIOPT resulted in a PII of 0.[130] Administration of product formulations containing 2.2–74% Palmitic Acid produced minimal erythema and no edema 2–24 h after application to the skin of albino rabbits.[133,172–174]

In an SIOPT, commercial grade Myristic Acid was applied to intact and abraded sites on the skin of 6 albino rabbits, and the PII was 0.[130] In a Repeat Open Patch test using commercial grade Myristic Acid, all 6 treated albino rabbits developed mild to moderate erythema from 24 to 72 h. One rabbit developed very slight edema after the 72-h scoring.[175]

No irritation was observed at intact or abraded sites of the skin of albino rabbits in two SIOPT studies involving a commercial grade Stearic Acid.[130] In an SIOPT of commercial grade Stearic Acid, transient minimal erythema and no edema were noted in 9 albino rabbits after a 2-h exposure period.[176]

A preparation of 65% Stearic Acid in ethylene oxide produced erythema and minimal edema 24 and 72 h after application to intact and abraded sites on the skin of 6 NZW rabbits. The PII for this SIOPT was 3.00.[134] No irritation was observed in SIOPT studies involving 4-h exposures of intact and abraded skin of 6 albino rabbits to 45 and 59% Stearic Acid in combination with "other fatty acids."[172] Two-hour exposures of the skin of 9 albino rabbits to 35.0% Stearic Acid in water and 50% Stearic Acid in petrolatum resulted in respective PIIs of 0.33 and 0.56. Transient mild erythema and no edema were observed in both SIOPT studies.[177,178]

SIOPT studies with lotion and cream formulations containing 1.0–13% Stearic Acid resulted in PIIs, ranging from 0.63 to 2.2, that were not directly related to Stearic Acid concentration. A face cream formulation containing 13% Stearic Acid was determined "non-irritating" in a 24-h SIOPT of the fatty acid applied to intact and abraded sites on the skin of at least 6 albino rabbits. The use of a standard procedure was reported,[158] and no additional data were recorded.[179]

In a 24-h SIOPT of a skin lotion formulation containing 2.8% Stearic Acid, the PII was 1.00, and barely perceptible erythema and edema were observed at most intact and abraded sites of 6 NZW rabbits after 24 h. Irritation had subsided after 72 h.[138]

Transient irritation was also observed in a 24-h SIOPT to intact and abraded sites of the skin of 6 NZW rabbits treated with a skin lotion formulation containing 2.8% Stearic Acid. Very slight to well-defined erythema was observed at both sites, and very slight edema was observed at some intact and all abraded sites after 24 h.[139]

A skin lotion formulation containing 2.8% Stearic Acid produced very slight erythema at both intact and abraded treatment sites and transient minimal edema at a few sites 1 day after a 24-h SIOPT.[140]

A skin lotion formulation containing 2.8% Stearic Acid produced minimal to well-defined erythema and edema at both intact and abraded sites of 6 NZW rabbits 24 h after treatment. Very slight erythema was observed at some of the sites after 72 h.[136] Dry skin was noted in all rabbits.

A skin lotion formulation containing 2.8% Stearic Acid produced very slight to well-defined erythema and edema at intact and abraded sites of 6 NZW rabbits 24 h after treatment. Very slight erythema was observed at a few sites, and there was no edema 48 h later.[137] Dry skin was noted at treatment sites of all rabbits.

Intact and abraded sites on the skin of 4 male albino rabbits were treated with a skin lotion formulation containing 2.8% Stearic Acid in a 24-h SIOPT study. Transient minimal erythema was observed after 24 h. One abraded site had very slight edema after 24 h.[143]

Intact and abraded sites on the skin of 6 NZW rabbits were treated with lotion formulations containing 1.0% Stearic Acid in two 24-h SIOPT studies.[180] Treatment with one formulation produced defined erythema and edema at both sites after 24 h, which had subsided by 72 h posttreatment.

Skin Sensitization

A cream blush formulation containing 5.08% Oleic Acid was tested for sensitization using a group of 24 female Hartley guinea pigs weighing 300–500 g.[181] In a maximization test,[182] single intradermal injections of 0.1 ml of 5% Freund complete adjuvant in water, of a 5% solution of the formulation in water, and of a 5% solution of the formulation, water, and Freund adjuvant were administered in rows along the dorsal midline of the guinea pigs. Seven days after the injections, a 10% preparation of sodium lauryl sulfate in petrolatum was topically applied to the clipped dorsal area. Twenty-four hours later, 1 g of the undiluted formulation was applied to the treatment sites under an occlusive patch. The challenge patch, 1 g of the undiluted formulation in a Duhring chamber (aluminum disk with diameter of 18 mm and 2 mm elevated flange), was topically applied under an occlusive wrapping 14 days after topical induction (22 days after the intradermal injection). After a 24-h exposure, the challenge patch was removed. Sites were scored at patch removal and 48 h later. None of the guinea pigs had reactions to the challenge patches. Although no other data were reported, the formulation was considered a weak, grade I, sensitizer.

A suntan lotion formulation containing 1.0% Stearic Acid was tested for sensitization on 22 young adult female Hartley guinea pigs[183] using the same

procedure as in the preceding study.[181] There was one sensitization reaction to the occlusive challenge patch of 1 g of the formulation in a Duhring chamber among the 22 treated guinea pigs. The formulation was considered a weak, grade I, sensitizer.

In a maximization study,[182] a cosmetic product formulation containing 3.5% Stearic Acid was tested for allergic contact sensitization using a group of 10 female guinea pigs.[184] Intradermal injections of 50% aqueous Freund complete adjuvant, 50% formulation in propylene glycol, and 50% formulation in 50% aqueous Freund adjuvant at each of three sites along the upper backs of the guinea pigs were followed 1 week later by a topical booster of a slightly irritating concentration of the formulation in petrolatum. A topical application of 10% sodium lauryl sulfate in petrolatum was made 24 h before the topical booster if the formulation was not sufficiently irritating. Guinea pigs in the control group received induction injections of 50% aqueous Freund complete adjuvant, propylene glycol, and a 1:1 preparation of propylene glycol and 50% aqueous Freund adjuvant along the upper back and topical booster applications of petrolatum. Two weeks after the topical booster application, occlusive challenge patches containing 50 or 100% of the formulation were applied to control and treated guinea pigs. Sites were scored 48 and 72 h later. Five of 10 treatment sites had minimal faint erythema, and 1 of 10 sites had mild erythema 48 h after challenge with the 100% concentration. There were 3 sites with minimal faint erythema after 72 h, 2 of which had signs of desquamation. Other treatment sites had no signs of sensitization. Challenge of the treatment sites with the 50% formulation preparation resulted in minimal faint erythema at 1 of 10 sites after 48 h, which was visible after 72 h. All other treatment sites challenged with the 50% concentration had no signs of sensitization. Two control guinea pigs died, and 4 of the remaining 8 sites challenged with the 100% formulation patch had minimal faint erythema after 48 h. Two of 8 sites challenged with the 50% concentration had minimal faint erythema, and desquamation was observed at another site after 72 h.

Photosensitization

Two skin lotion formulations containing 2.8% Stearic Acid were tested for phototoxicity.[185,186] Aqueous preparations of the formulations, 100, 75, 50, and 25%, were applied to four different sites on the backs of 10 male Hartley albino guinea pigs weighing 324–486 g[185] and 284–452 g.[186] These sites were exposed to UVA radiation. Ten control guinea pigs weighing 268–434 g[185] and 344–464 g[186] received the same topical applications but no UVA irradiation. Sites were evaluated 1 and 24 h after treatment. Neither formulation was considered phototoxic to the guinea pigs under these conditions because the control group had signs of irritation that were comparable to the irradiated test group. One guinea pig in the control group of one study died.[185] The test groups' reactions ranged from questionable to moderate erythema at 6 (50% preparation) to all 10 sites (75%, 100% preparations). The 25% preparations produced no signs of phototoxicity in either study. The control groups in both studies had questionable to moderate (50–100% sites,[185] 50–75% sites[186]) or considerable erythema (100% site[186]). No irritation was observed at control sites treated with the 25% preparations.

Two skin lotion formulations containing 2.8% Stearic Acid were tested for photoallergy using 12 male Hartley albino guinea pigs weighing 378–516 g[186] and 330–404 g.[185] Each guinea pig received 10 topical induction applications of the undiluted formulations. Two weeks after the last application, challenge applications of 10, 20, and 100% (w/v) preparations were made to two separate sites, one of which was irradiated. Control groups of 12 male guinea pigs (360–440 g,[185] 358–492 g[186]) received no induction applications and were treated as test animals in the challenge phase. Induction sites were evaluated daily and challenge sites were evaluated 24 and 48 h after treatment. In one study, 1 test animal died during the induction phase and 2 animals died during the challenge phase.[185] Neither formulation was considered photoallergenic to the guinea pigs under these conditions because the control group had signs of irritation comparable to the test group. Questionable to moderate erythema was observed at up to 11 of 12 sites by the second application of the induction phase. During the challenge phase, no irritation was observed at either irradiated or nonirradiated sites of guinea pigs in control and test groups at the 10 and 20% concentrations. Questionable to minimal erythema was observed at one or two nonirradiated sites and at five irradiated sites of the test group challenged with the undiluted formulation. In the control group, four to seven nonirradiated sites and five to six irradiated sites had questionable to minimal erythema after challenge with the undiluted formulation.

Comedogenicity

The comedogenicity of UVA-irradiated and nonirradiated Oleic Acid was evaluated.[24] A significant increase in lipid peroxide level of Oleic Acid was observed after 18 h of UVA irradiation. Daily applications of the nonirradiated Oleic Acid (approximately 2 ml of 99% Oleic Acid) for 2 weeks were made on the ventral surface of one ear of Japanese and New Zealand White rabbits. An equal volume of irradiated Oleic Acid was applied to the other ear. Both Oleic Acid and its peroxides induced fairly large comedones in both species of rabbit. The lipid peroxide concentration was positively correlated with the degree of comedo formation.

Ocular Irritation Studies

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids were tested for ocular irritation (Table 17).

No or minimal conjunctival irritation was produced in eyes of 6 albino rabbits treated with 0.1 ml of Oleic Acid as commercially supplied. Using the Draize Method,[168] the single instillation was not rinsed from the eyes. Untreated eyes served as controls.[130,187,188] In other Draize studies, 0.1 ml of mascara and cream product formulations containing 2–5% Oleic Acid produced no or slight conjunctival irritation in the eyes of rabbits within 2 days of treatment.[131,191–192] No irritation was observed in eyes that had been irrigated 20 sec after treatment with 20 ml lukewarm water.[190] No irritation was observed in rinsed and unrinsed eyes of rhesus monkeys treated with a mascara formulation containing 6% Oleic Acid.[189]

TABLE 17. Ocular Irritation Studies

Fatty acid tested	*Species (no. per group)*	*Methods*[a]	*Results*	*Reference*
Oleic Acid, as commercially supplied	6 albino rabbits	Draize	Mean score 2 after 24 h; 1 after 48 and 72 h (max = 110). Mild conjunctivitis	130
Oleic Acid, as commercially supplied	3 albino rabbits	Draize	No irritation	187
Oleic Acid, as commercially supplied	3 albino rabbits	Draize	Total mean score 1 after 1 and 2 days; 0 after 3 days. Grade 2 conjunctival irritation	188
Oleic Acid—6% in mascara formulation	3 rhesus monkeys	Draize, ± rinse	No irritation in either group	189
Oleic Acid—5% in cream formulation	6 NZW rabbits	14 daily instillations, no rinse	Intermittent slight conjunctivitis during 1st week	131
Oleic Acid—3% in mascara formulation	3 albino rabbits	Draize, ± rinse	Grade 1 conjunctival erythema in unrinsed treated eyes clearing by 2nd day	190
Oleic Acid—2% in mascara formulation	3 albino rabbits	Draize, ± rinse	No irritation	191
Oleic Acid—2% in mascara formulation	6 albino rabbits	Draize	Mean score 0.66 after 24 h; 0.33 after 48 h. Grade 1 conjunctival erythema in 1 rabbit only	192
Lauric Acid, as commercially supplied	6 albino rabbits	Draize	Mean score 35 after 24 h; 39 after 48 h; 41 after 72 h. Persistent corneal opacity, mild conjunctivitis, iritis	130
Lauric Acid—8.7% in product formulation, 8.0% aqueous dilution tested	6 albino rabbits	Draize	No irritation	193
Lauric Acid—1.95% in soap formulation, 1% aqueous dilution tested	6 NZW rabbits (rinse group) 3 NZW rabbits (no rinse group)	Draize, ± rinse	Max. mean score 0.3 for unrinsed eyes; 0.7 for rinsed eyes. Grade 1 conjunctival erythema	194
Palmitic Acid, as commercially supplied	6 albino rabbits	Draize	No irritation	130
Palmitic Acid—19.4% in product formulation	6 albino rabbits	3 instillations, no rinse	Total mean score 3 after 1 and 2 days. No irritation after 3 days. Primarily conjunctival irritation	195

Palmitic Acid—19.4% in product formulation, 75% solution in corn oil	6 albino rabbits	Draize	Total mean score 1 after 1 day; 6 after 2 days; 1 after 3 days. No irritation after 4 days. Mild irritation of cornea, iris, and conjunctivae	196
Palmitic Acid—4.4% in product formulation	6 albino rabbits	Draize	No irritation	197
Palmitic Acid—4.4% in product formulation	6 albino rabbits	Draize	No irritation	198
Palmitic Acid—2.2% in product formulation	6 albino rabbits	Draize	No irritation	133
Myristic Acid, as commercially supplied	6 albino rabbits	Draize	Grade 1 conjunctival erythema in 3 rabbits after 24 h	130
Myristic Acid—50% in petrolatum	3 albino rabbits	Draize	Total mean score 2 after 1 day; 1 after 2 and 3 days; 0 after 4 days. Grade 2–4 conjunctival irritation	199
Myristic Acid—1.5% in product formulation	6 NZW rabbits (no rinse) 3 NZW rabbits (rinse)	Draize, ±rinse	Max. mean score 1.3 for unrinsed; 0.7 for rinsed treated eyes. Conjunctival erythema up to 72 h later	200
Myristic Acid—1.5% in product formulation	See preceding entry	Draize, ±rinse	Max. mean score 0.7 for unrinsed; 1.3 for rinsed treated eyes. Conjunctival erythema 24–48 h later	201
Stearic Acid, as commercially supplied	6 albino rabbits	Draize	No irritation	130
Stearic Acid (eutectic), as commercially supplied	6 albino rabbits	Draize	Mild conjunctival erythema in 2 rabbits, subsiding by 72 h	130
Stearic Acid—65% in ethylene oxide	6 NZW rabbits	Draize	No irritation	134
Stearic Acid—50% in petrolatum	6 albino rabbits	Draize	Total mean score 4 after 1 day. Conjunctival irritation subsided after 2 days	202
Stearic Acid—35% in corn oil	6 albino rabbits	Draize	Total mean score 1. Mild conjunctival irritation subsided after 2 days	203
Stearic Acid—13% in product formulation	6 albino rabbits	Draize	Iritis in 1 rabbit	179

TABLE 17. (Continued)

Fatty acid tested	*Species (no. per group)*	*Methods*[a]	*Results*	*Reference*
Stearic Acid—2.8% in product formulation	6 NZW rabbits (no rinse) 3 NZW rabbits (rinse)	Draize, ±rinse	Mean total score 0.7 for unrinsed treated eyes after 1 day; conjunctival erythema subsided after 2 days. No irritation in rinsed treated eyes	138
Stearic Acid—2.8% in product formulation	6 NZW rabbits	Draize	No irritation	139
Stearic Acid—2.8% in product formulation	3 NZW rabbits	Draize	Max. mean score 3.3; conjunctival irritation after 1 and 24 h, subsiding after 48 h	140
Stearic Acid—2.8% in product formulation	6 NZW rabbits (no rinse) 3 NZW rabbits (rinse)	Draize, ±rinse	Mean total score 0.7 after 48 h, 0.3 after 72 h and 4 days for unrinsed eyes. Similar scores for rinsed eyes. Slight conjunctival erythema	136
Stearic Acid—2.8% in product formulation	See preceding entry	Draize, ±rinse	Mean total score 0.7 after 24 h in both groups. Slight conjunctival erythema	137
Stearic Acid—2.8% in product formulation	3 NZW rabbits	Draize	Max. mean score 6.0 after 1 h. Conjunctival irritation in all rabbits, subsiding after 24 h	141
Stearic Acid—2.8% in product formulation	3 NZW rabbits	Draize	Max. mean score 6.0 after 1 h. Conjunctival irritation persisting up to 24 h	142
Stearic Acid—2.8% in product formulation	3 NZW rabbits	Draize	Max. mean score 4.0 after 1 h. Slight conjunctival erythema persisting up to 24 h	143
Stearic Acid—1% in product formulation	4 albino rabbits	Draize	Max. mean score 6.0 after 1 h. Slight conjunctival irritation, 2 rabbits had corneal irritation. Subsided by 24 h	204
Stearic Acid—1% in product formulation	6 albino rabbits	Draize	Max. mean score 2.83 after 1 h. Slight conjunctival irritation and iritis in 1–3 rabbits	153

[a]Draize Method[168] used in most studies: usually single instillation of 0.1 ml volume into 1 eye (untreated eye = control). Variant methods (e.g., "rinse" denoting rinsing of treated eyes or "±rinse" denoting that treated eyes of animals in 1 group were rinsed, while those of animals in other group left unrinsed) are noted.

Instillation of commercial grade Lauric Acid into the eyes of 6 albino rabbits produced corneal opacity, mild conjunctivitis, and iritis throughout the 72-h observation period.[130] An aqueous dilution of a product formulation containing 8.7% Lauric Acid produced no ocular irritation in 6 albino rabbits.[193] A 1% aqueous preparation of a soap formulation containing 1.95% Lauric Acid was not irritating to treated unrinsed eyes of rabbits. The preparation was minimally irritating to treated eyes that had been rinsed 30 sec after instillation with 20 ml deionized water at room temperature.[194]

Administration of commercial grade Palmitic Acid to the eyes of 6 albino rabbits produced no irritation.[130] Mild to moderate ocular irritation was produced in rabbits by product formulations containing 19.4% Palmitic Acid. One of these formulations had been diluted to 75% with corn oil.[195,196] Cosmetic product formulations containing 2.2 and 4.4% Palmitic Acid produced no ocular irritation in 6 albino rabbits.[133,197,198]

Slight conjunctival irritation was produced in the eyes of albino rabbits 1 day after instillation of commercial grade Myristic Acid[130] and 50% Myristic Acid in petrolatum.[199] Lotion formulations containing 1.5% Myristic Acid were minimally irritating to rinsed (20 ml ionized water at room temperature, 30 sec after instillation) and unrinsed treated eyes of rabbits.[200,201]

No ocular irritation was produced in 6 albino rabbits by commercial grade Stearic Acid, whereas mild conjunctival erythema was produced in 3 of 6 albino rabbits by commercial grade eutectic (triple-pressed) Stearic Acid.[130] Treatment with 65% Stearic Acid in ethylene oxide resulted in no ocular irritation.[134] Treatment with 35% Stearic Acid in corn oil and 50% Stearic Acid in petrolatum was "practically non-irritating," primarily producing mild conjunctival erythema, which had subsided within 2 days.[202,203]

Iritis was observed in 1 of 6 albino rabbits treated with a face cream formulation containing 13% Stearic Acid.[179] No irritation[139] or mild conjunctival irritation after 1 and 24 h[136–138,141–143,153,204] was observed in the unrinsed eyes of albino rabbits treated with lotion formulations containing 1 and 2.8% Stearic Acid. Mild iritis was also observed in one study.[153] Eyes of rabbits that had been irrigated with water after treatment with a skin lotion formulation containing 2.8% Stearic Acid had no signs of irritation[138] or slight conjunctival erythema after 24 and 48 h.[136,137]

MUTAGENICITY

Oleic, Lauric, and Stearic Acids were assayed for their abilities to induce mitotic aneuploidy and crossing-over of chromosomes in the D_6 strain of *Saccharomyces cerevisiae.(205)* Concentrations of Oleic Acid from 100 to 500 μg/ml and of Lauric Acid from 10 to 200 μg/ml increased aneuploidy, whereas Stearic Acid at concentrations up to 500 μg/ml was inactive. None of the fatty acids tested increased the frequency of mitotic crossing-over events; concentrations of Oleic and Lauric Acids up to 50 μg/ml and of Stearic Acid up to 500 μg/ml were used.

Stearic Acid was tested for mutagenicity using the Ames test[206] with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538.[207]

Spot tests were performed using 50 mg/ml Stearic Acid suspensions in distilled water (50 μg/plate) with and without microsomal activation from hepatic S9 fractions from rats induced with Aroclor 1254 (50 μg/plate). Positive controls were 2-aminoanthracene and 4-nitro-o-phenylenediamine in dimethyl sulfoxide, 9-aminoacridine in ethanol, and sodium azide in distilled water. Stearic Acid had no mutagenic activity over background in the strains tested with and without metabolic activation.

The genotoxicity of Oleic Acid was studied using V79 Chinese hamster lung fibroblasts.[208] The three tested concentrations of Oleic Acid, 2.5, 5.0, and 10.0 μg/ml, produced a mean number of sister chromatid exchanges per metaphase that was similar to controls. Higher incidences of aneuploidy were observed in cultures at all three concentrations. The 2.5 μg/ml Oleic Acid-treated culture had a higher incidence of tetraploidy when compared to controls.

Isomers of Oleic Acid, *cis*-12- and *cis*-13-octadecenoic acids, produced a greater increase in mitochondrial DNA mutation in *S. cerevisiae* than did Oleic Acid.[209]

Inhibition of Mutagenesis

Oleic, Lauric, Stearic, and Palmitic Acids were tested for their inhibitory action on the mutagenicity of several compounds using two bacterial systems, *Escherichia coli* and *Salmonella typhimurium*. These studies and their results are summarized in Table 18.

In the *S. typhimurium* system, a modified Ames test[206] was used involving preincubation of a mixture containing the mutagen, dimethylsulfoxide (DMSO), fatty acid, S9, and bacteria before plating. A phosphate buffer at pH 6 was used for the preincubation mixture in the *E. coli* system. A significant decrease in the number of revertants compared to negative controls in both tests was interpreted as inhibition by the fatty acid. Positive controls with mutagen alone were done to determine maximum numbers of revertants.

Oleic Acid was toxic to *S. typhimurium* TA 100,[211] and Lauric Acid was toxic to *E. coli* WP2 uvrA/pKM101 in the absence of S9. In the presence of S9, Lauric Acid had a strong inhibitory effect on all N-nitrosodialkylamines tested.[212]

Mechanisms for Oleic and Lauric Acid-inhibition of potent mutagens have been discussed, and results of several bacterial tests for fatty acid inhibition of mutagenesis have been reported.[214]

CARCINOGENICITY

Oleic, Lauric, Palmitic, and Stearic Acids have been tested for carcinogenic activity. The studies were reviewed in the safety assessment of particular fatty acids (and their salts) as they are used in foods[44–47,68] and in fragrances.[69] Data and results from these and additional studies are summarized in Table 19.

TABLE 18. Inhibition of Mutagenicity by Fatty Acids

Fatty acid tested	*Bacterial system used*	*Metabolic activation*	*Results*	*Reference*
Oleic Acid isolated from fecal extract	*Salmonella typhimurium* TA98	S9 from livers of rats induced with poly-chlorinated biphenyl (PCB)	Inhibition of mutagenicity of: 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole; 2-amino-6-methyl-dipyrido[1,2-a:3',2'-d]imidazole; 2-amino-9H-pyrido[2,3-b]indole; 2-amino-3-methyl-imidazo[4,5-d]-quinoline; benzo[a]pyrene (amino acid pyrolysis products) and aflatoxin B_1 Degree of inhibition increased with decreasing pH. I_{50}, 0.02–0.08 mg; I_{95}, 0.05–0.38 mg	210
Oleic Acid	*Escherichia coli* WP2 try, hcr	S9-phenobarbital-induced rat liver	Inhibition: 140 μmol N-nitrosodimethylamine (NDMA); 14 μmol N-nitrosodiethylamine (NDEA); 4 μmol N-nitrosodibutylamine (NDBA); 35 μmol N-nitrosopyrrolidine (NPYR); 35 μmol N-nitroso morpholine (NMOR). Dose-related inhibition observed No inhibition: 2 μmol N-methyl-N'-nitro-N-nitrosoguanidine (NMMG)	211
Oleic Acid	*E. coli* WP2 uvrA/pkM 101	S9-phenobarbital-induced hamster liver	Inhibition: NDMA	212
Lauric Acid	*S. typhimurium* TA100	None reported	Inhibition: sodium azide, 4-nitro-o-phenylene-diamine, N-amino-morpholine, ethylmethane-sulfonate	213
Lauric Acid	*E. coli* WP2 uvrA/pKM 101	S9-phenobarbital-induced hamster liver	Inhibition: NDMA, NDEA, NDBA, N-nitroso-piperidine, NMOR. Cytotoxic in N-methylnitroso-urea cultures	212
		S9-PCB-induced rat liver	Inhibition: benzo[a]pyrene No inhibition: 2-aminoanthracene	
Palmitic Acid	*S. typhimurium* TA98	S9-PCB-induced rat liver	No inhibition: amino acid pyrolysis products, aflatoxin B_1	210
Stearic Acid isolated from fecal extract	*S. typhimurium* TA98	S9-PCB-induced rat liver	No inhibition: amino acid pyrolysis products, aflatoxin B_1	210
Stearic Acid	*E. coli* WP2 try, hcr	S9-phenobarbital-induced rat liver	No inhibition: NDMA	211

I_n, amount of fatty acid needed to produce a percent inhibition.

TABLE 19. Carcinogenicity Studies on Fatty Acids

Fatty acid tested	*Dose*	*Animal*	*Method*	*Results and conclusions*	*Reference*
Oleic Acid in tricaprylin	1–16.5 mg	Mouse (BALB/c, CFW)	Repeated subcutaneous injections. Two experiments: (1) 0.1 mg Oleic Acid in 0.1 ml tricaprylin 3 injections/week, total of 10 injections (2) 0.5 mg Oleic Acid in 0.1 ml tricaprylin 2 injections/week, total of 33 injections	Not carcinogenic (1) 1/15 mice alive at 18 months. No subcutaneous sarcomas (2) 4/16 mice alive at 18 months. No subcutaneous sarcomas, 1 breast carcinoma at 9 months	215[a]
Oleic Acid with linoleic acid in corn oil in diet	150–200 mg/mouse/day of 1.5% fatty acids in in refined corn oil	Mouse (T.M. strain)	Feeding study—dietary supplement. Several groups: (1) Control—chow only ($n = 623$) (2) Refined corn oil supplement ($n = 375$) (3) Refined corn oil + 1.5% free fatty acid supplement (oleic and linoleic acids) ($n = 329$)	(1) Controls— < 20% total tumor incidence mainly lung tumors (2) Incidence of lung and brain nerve cell tumors, lymphosarcomas similar to Group 3. Incidence gastric tumors lower than Group 3. 1 heart tumor found (3) High incidence of lung (48.5%), stomach (27.4% forestomach papillomas, 12.5% pyloric tumors), and brain nerve cell (11%) tumors. Low incidence of mammary carcinomas, myomas, lymphosarcomas. 1 heart tumor found	216
Oleic Acid with linoleic acid in corn oil in diet	200 mg/mouse/day of 1.5% fatty acids in refined corn oil	Mouse	Feeding study—dietary supplement. Several groups (1) Control—chow only ($n = 195$) (2) Refined corn oil supplement ($n = 209$) (3) Crude corn oil supplement ($n = 196$)	Number of tumors Groups: (1) (2) (3) (4) Forestomach papillomas 2 6 49 87 Squamous cell carcinomas 1 1 6 10 Pyloric tumors 0 2 9 41 No intestinal polyps or adenocarcinomas	217

			(4) Refined corn oil + free fatty acid supplement (oleic and linoleic acids) ($n = 328$)		
Oleic Acid in corn oil diet	10 g of 1.5% (w/w) in corn oil in chow	Mouse (C57BL/1 strain)	Feeding study—dietary supplement. 2 groups (1) Control—chow only ($n = 36$) (2) Corn oil + Oleic Acid ($n = 55$)	(1) Incidence of tumorigenesis not reported for controls (2) Metastatic colon adenocarcinomas in 8% of mice. Polycystic kidney in 1 mouse No corn oil in chow group (i.e., treated control) C57BL/1 strain reported to be generally resistant to tumor formation	218
Oleic Acid	Unspecified	Mouse	Unspecified method—biweekly applications for 40 weeks. Series of experiments	No malignant tumors. In 3 experiments: 0/100 mice with tumors 1/200 mice with benign tumor at week 35 1/100 mice with benign tumor at week 15 No change to malignancy	219[a]
Lauric Acid in tricaprylin	25 and 50 mg	Mouse (BALB/c; CFW)	Repeated subcutaneous injections. Two experiments: (1) 1.0 mg Lauric Acid in 0.1 ml tricaprylin. 2 injections/week, total 25 injections (2) 5.0 mg Lauric Acid in 0.1 ml tricaprylin. 3 injections/week, total 10 injections	Not carcinogenic (1) 5/16 mice alive at 18 months. 1 subcutaneous sarcoma, 1 pulmonary tumor, 2 leukemia–lymphomas (4, 5 months) (2) 8/15 mice alive at 18 months. No subcutaneous sarcomas; 1 pulmonary tumor; 1 leukemia-lymphoma (23 months)	215[a]
Palmitic Acid in tricaprylin	25 and 50 mg	Mouse (BALB/c; CFW)	Repeated subcutaneous injections. Two experiments: (1) 1.0 mg Palmitic Acid in 0.1 ml tricaprylin. 2 injections/week, total of 25 injections (2) 5.0 mg Palmitic Acid in 0.1 ml tricaprylin. 3 injections, total of 10 injections	(1) 5/16 mice alive at 18 months. 1 subcutaneous sarcoma (8 months); 2 breast carcinomas (18 months); 1 leukemia -lymphoma (12 months) (2) 6/16 mice alive at 18 months. 1 subcutaneous sarcoma (19 months); 2 pulmonary tumors (19, 22 months); 1 breast carcinoma (22 months)	216[a]
Palmitic Acid in diet	50 g/kg/day	Rat (Holtzman)	Feeding study—dietary supplement	Lipogranulomas observed in fat associated with testis or ovary—reversible upon diet substitution Conclusion: effect due to dietary imbalance	151[a]

TABLE 19. (Continued)

Fatty acid tested	*Dose*	*Animal*	*Method*	*Results and conclusions*	*Reference*
Stearic Acid in olive oil	Unspecified	Mouse	Single subcutaneous injection	No sarcomas observed. Used as a control in study on cholesterol carcinogenicity	220[a]
Stearic Acid in tricaprylin	1.3–82 mg	Mouse (BALB/c and CFW Swiss Webster)	Repeated subcutaneous injections. Series of expts. using 0.05–1.0 mg Stearic Acid in 0.1 ml tricaprylin. 1–3 injections per week, total of 10–114 injections per study	7–90% of mice were alive at 18 months ($n = 10–16$). Only 1 group (0.05 mg, 2x/week, 114 injections) had subcutaneous sarcomas (4 in 4 survivors). 1 adrenal carcinoma, 1 leukemia–lymphoma, 3 pulmonary tumors in total of 92 mice (in entire series)	215[a]
Stearic Acid in tricaprylin	1.3–13 mg	Mouse (ICR/Ha Swiss Millerton and CFW Swiss Webster)	Repeated subcutaneous injections. Series of expts. using 0.05 or 0.5 mg Stearic Acid in 0.1 ml tricaprylin 1 injection per week, 26 weeks	1–3 deaths within 6 months ($n = 15–16$). No sarcomas at injection site. No carcinogenic activity	221[a]
Stearic Acid in diet	0.3%	Rat	Feeding study. Dietary supplement for 209 days	No carcinogenic activity	152[a]
Stearic Acid in diet	50 g/kg/day	Rat (Holtzman)	Feeding study–dietary supplement	Lipogranulomas observed in fat associated with testis or ovary—reversible upon diet substitution. Concluded that effect due to dietary imbalance rather than Stearic Acid-related	151[a]

[a] These studies appeared in reviews for the safety assessment of particular fatty acids as they are used in food[44–47] and in fragrance.[69]

The carcinogenicity of Oleic, Lauric, Palmitic, and Stearic Acids was studied from 1964 to 1967 in a series of experiments with female BALB/c or Swiss-Webster mice.[215] Subcutaneous injections were administered in the inguinal area 3 times per week for 4 weeks. Materials that were administered daily or for longer than 4 weeks were given in inguinal and axillary areas to prevent their accumulation into deposits of unabsorbed oil. The vehicle for the injections was tricaprylin, and the volume per injection was 0.1 ml. One group of control mice was administered tricaprylin alone; the other control group received no treatment. Mice were observed twice weekly for the appearance of subcutaneous neoplasms. Animals with neoplasms or those in poor condition were killed and necropsied.

Oleic Acid was administered to 15 Swiss-Webster mice at a dose of 0.1 mg 3 times per week for a total of 10 injections.[215] The total dose administered in the study was 1.0 mg Oleic Acid per 1 ml tricaprylin. Nine mice were alive after 12 months, and 1 was alive after 18 months. No neoplasms were observed after this treatment. Another group of 16 Swiss-Webster mice received 2 injections of 0.5 mg Oleic Acid per week for a total of 33 injections. The total dose administered was 11.5 mg per 2.3 ml tricaprylin. Eight mice were alive after 12 months, and 4 were alive after 18 months. One mammary gland carcinoma was found after 9 months.

Lauric Acid was administered to 15 Swiss-Webster mice at a dose of 1.0 mg 3 times per week for a total of 12 injections (total dose, 12 mg Lauric Acid/1.2 ml tricaprylin).[215] Thirteen mice were alive after 12 months, and 8 mice were alive after 18 months. One pulmonary neoplasm and 1 "leukemia–lymphoma" were found after 23 months. Another group of 16 Swiss-Webster mice received 2 injections of 5.0 mg weekly for a total of 25 injections (total dose, 125 mg Lauric Acid/2.5 ml tricaprylin). After 12 months, 8 mice were alive, and after 18 months, 5 were alive. One subcutaneous sarcoma and 1 pulmonary neoplasm were found after 18 months. Two "leukemia–lymphomas" were found after the fourth and fifth months.

Palmitic Acid was administered to 16 Swiss-Webster mice at a dose of 1.0 mg 3 times per week for a total of 10 injections (total dose, 10 mg Palmitic Acid/1 ml tricaprylin).[215] Eight mice were alive after 12 months, and 6 were alive after 18 months. One subcutaneous sarcoma was found after 19 months, 2 pulmonary neoplasms were found after 19 and 22 months, and 1 breast carcinoma was found after 22 months. Another group of 16 Swiss-Webster mice received two injections of 5.0 mg weekly for a total of 25 injections (total dose, 125 mg Palmitic Acid/2.5 ml tricaprylin). Eight mice were alive after 12 months, and 5 were alive after 18 months. A subcutaneous sarcoma was found after 8 months, 2 breast carcinomas were found after 18 months, and 1 "leukemia–lymphoma" was found after 12 months.

Stearic Acid was administered to groups of 16 Swiss-Webster mice at doses of 0.05 mg and 0.5 mg weekly for a total of 26 injections.[215] After 18 months, 10 mice were alive in the group given the lower dose, and 6 mice were alive in the group given the higher dose. A third group of 15 Swiss-Webster mice was given injections of 1.0 mg Stearic Acid 3 times per week for a total of 10 injections. Eight mice were alive after 12 months, and 1 was alive after 18 months. A fourth group of 10 BALB/c mice was given injections of 1.0 mg

Stearic Acid twice weekly for a total of 82 injections. Seven mice were alive after 18 months. No neoplasms were found in these four groups.

Neoplasms were found in three other groups of BALB/c mice administered Stearic Acid.[215] The first group of 15 mice was injected with 0.05 mg Stearic Acid twice weekly for a total of 104 injections. Thirteen mice were alive after 18 months, and 1 pulmonary neoplasm was found after 19 months. The second group of 10 mice received injections of 0.05 mg Stearic Acid twice weekly for a total of 114 injections. Four mice were alive after 18 months. Four subcutaneous sarcomas (1 after 6 months, 2 after 10 months, and 1 after 12 months), 1 pulmonary neoplasm (after 19 months), and 1 "leukemia–lymphoma" (after 19 months) were found. The 10 mice in the third group received 0.5 mg Stearic Acid per injection twice weekly for a total of 114 injections. Nine mice were alive after 18 months. After 21 months, 1 pulmonary neoplasm and 1 adrenal carcinoma were found.

In a study modeled after the Swern et al.[215] study, Van Duuren et al.[221] found Stearic Acid to be noncarcinogenic, confirming the previous study's conclusion (see Table 14 for details of study). Investigators in both studies indicated that a compound's carcinogenic activity was assessed by its ability to induce sarcomas at the injection site.

Statistical techniques were used to determine possible associations between dietary fatty acids in triglycerides and the incidence of spontaneous mammary tumors in C3H mice.[222] Eleven natural fats and oils and their mixtures were used to obtain 20 substances with varying concentrations of different fatty acids that were fed to mice. The saturated fatty acids, Lauric, Myristic, and Palmitic Acids, had little effect on tumor incidence or the time needed for a tumor to appear. The concentration of Stearic Acid was calculated to be inversely related to tumor incidence and directly related to the time for tumor appearance. Oleic Acid produced no significant effect on tumor incidence.

The effects of free fatty acids fed as dietary supplement to mice of the T.M. strain were studied.[216] Refined corn oil (free fatty acid content, approximately 1.5%, removed during refining process) fed to the mice at a rate of 150–200 mg/mouse/day contained 1.5% free fatty acids, Oleic and linoleic Acids. Feeding of the refined corn oil plus free fatty acid diet resulted in a high incidence of lung (48.5%), stomach (27.4% forestomach papillomas, 12.5% pyloric tumors), and brain nerve cell (11%) tumors and a low incidence of mammary carcinomas, myomas, and lymphosarcomas. Feeding of the refined corn oil diet resulted in a high incidence of lung and brain nerve cell tumors, lymphosarcomas, and a lower incidence of gastric tumors. One heart tumor was found in each treated group ($n = 329$ in refined corn oil plus free fatty acids group, $n = 375$ in refined corn oil group). Controls fed the standard diet ($n = 623$) had a total tumor incidence of less than 20%; tumors were mainly located in the lung.

A later study was done to determine the types of gastrointestinal tumors induced in the T.M. strain mice fed a standard diet supplemented with refined corn oil, crude corn oil (contains 1.5% free fatty acids), or refined corn oil plus the fatty acids, Oleic Acid and linoleic acid, at concentrations up to 1.5%.[217] These corn oil supplements were given to the mice in daily amounts of 200

mg/mouse. Controls were fed the standard diet. Mice were killed when they began to lose weight rapidly. The average age of the control mice was 645 days, and that of the treated mice was 454–540 days. In the group fed the refined corn oil plus fatty acid diet, 138 gastric tumors were found in 328 treated mice. In the refined corn oil diet group, 9 gastric tumors were found in 209 treated mice. The crude corn oil diet group had 63 gastric tumors in 196 treated mice. Three gastric tumors were observed in the 195 control mice. No intestinal polyps or adenocarcinomas were observed in control or treated mice. The types of induced gastric tumors included papillomas and squamous cell carcinomas.

The carcinogenic activity of a feed supplement of Oleic Acid in corn oil was studied using C57BL/1 black strain mice that were "generally resistant to tumor formation."[218] Control animals from a different supplier were fed chow alone, and the 55 treated mice were fed a diet consisting of 10 g of a mixture of 1.5 g Oleic Acid/100 g corn oil dispersed in 100 g of laboratory chow to which water was added. Throughout the study, randomly selected mice were killed and examined after 6, 12, 18, 21, and 24 months. Colon adenocarcinomas, which metastasized to the lung and muscle, were found in 8% (3/36) of the treated mice. Lipid profiles of the livers and pituitary glands of the mice were obtained. Results for the 2 groups of mice were compared and discussed.

Tumor-Promoting and Cocarcinogenic Activity

In 1932, Twort and Bottomley reported that the induction of nonmalignant skin tumors by chrysene was increased in mice when Oleic Acid was used as the solvent compared to liquid paraffin or benzene. In a later study comparing the induction of skin tumors in mice by carcinogenic hydrocarbons dissolved in various solvents, chrysene induced more tumors when dissolved in Oleic Acid than in chloroform, but benzo(a)pyrene and fractions of synthetic tar induced fewer tumors when dissolved in Oleic Acid.[223] Also, in that study, induction of benign tumors, but not malignant tumors, increased when 1,2,5,6-dibenzanthracene was dissolved in Oleic Acid, compared to liquid paraffin. Use of chloroform as the solvent increased the incidence of malignant tumors.

Shubik[224] tested Oleic Acid as a tumor promoter for 9,10-dimethyl-1,2-benzanthracene-initiated mouse skin. Oleic Acid was administered twice weekly for 20 weeks but did not promote tumors. Gwynn and Salaman[225] also reported negative results for the promotion of 9,10-dimethyl-1,2-benzanthracene-initiated mouse skin tumors when Oleic Acid was administered twice weekly for 12 weeks or weekly for 15 weeks. Holsti[226] demonstrated that more frequent administration of Oleic Acid could promote 9,10-dimethyl-1,2-benzanthracene-initiated skin papillomas in mice; 2 of 40 mice developed papillomas when undiluted Oleic Acid was administered twice weekly, but 27 of 44 mice developed such tumors when Oleic Acid was administered daily for 6 days a week. Oleic Acid or Lauric Acid, but neither Palmitic Acid nor Stearic Acid, dissolved in chloroform also stimulated the

formation of skin papillomas. No malignant tumors were seen in any of the mice treated with any of the fatty acids.

Van Duuren and Goldschmidt[227] tested Oleic Acid and Stearic Acid as cocarcinogens in groups of 50 mice each. Benzo(a)pyrene, administered in acetone, induced 26 papillomas in 16 mice and squamous cell carcinomas in 12 mice. Mice that received the benzo(a)pyrene and 25 mg of Oleic Acid in acetone 3 times a week for 440 days developed no skin tumors, benign or malignant. Benzo(a)pyrene and 4 mg of Stearic Acid, administered 3 times a week for 440 days, resulted in 38 papillomas in 25 mice, but only 7 mice had squamous cell carcinomas, fewer than the controls. The results were considered inconclusive for Stearic Acid but supportive of the possibility that Oleic Acid is not a cocarcinogen.

Hogan and Shamsuddin[228] studied the tumor-promoting properties of *cis*- and *trans*-Oleic Acid on the induction of intestinal cancer by azoxymethane. *cis*-Oleic Acid had no promoting effect; *trans*-Oleic Acid (elaidic acid) had a small promoting effect. Both *cis*- and *trans*-Oleic Acids increased the incidence of nephroblastomas and squamous ear duct tumors from 3/30 to 6/30 rats. No tumors were seen in rats fed a diet containing 25% *cis*-Oleic Acid without azoxymethane for 20 weeks.

Promotion of mammary gland carcinomas has been observed in mice and rats fed diets containing unsaturated fats, particularly polyunsaturated fats.[229]

Several fats, oils, and fatty acids, including Lauric and Oleic Acids, produced acanthosis in guinea pig skin.[230] The acanthosis gradually receded with continued topical application. Oleic Acid has been found to enhance proliferation of both normal and cancer cells in vitro.[231–233] Myristic, Palmitic, and Stearic Acids had an inhibitory effect on normal smooth muscle cell proliferation; ability to inhibit proliferation was observed to increase with increasing chain length.[234] Traul et al.[235] reported that Oleic Acid and Lauric Acid can enhance the transforming ability of 3-methylcholanthrene in Rauscher murine leukemia virus-infected rat embryo cells.

Numerous mechanisms for the role of fatty acids in tumorigenesis have been studied and reviewed. Hypotheses include indirect effects on gene expression, the endocrine system, and the immune system and direct effects on tumor cells, such as alterations in cellular metabolism, membrane fatty acid composition, and intercellular cooperation.[236,237]

Antitumorigenicity

The antitumor activity of Oleic, Lauric, Myristic, Palmitic, and Stearic Acids was studied in vivo using Ehrlich ascites and solid carcinomas implanted into Swiss albino mice of strain ddY.[238] Suspensions of the fatty acids in Tween 80 and distilled water were administered 24 h after tumor implantation and were continued daily for 5 consecutive days. Commercial fatty acid preparations used in the study were not purified, and no analysis of components was performed. Treated mice were killed 30 days after implantation and examined for tumors. Doses of 8 mg/mouse/day of Lauric and Myristic Acids were effective inhibitors against Ehrlich ascites tumor, more than doubling the survival time of treated versus control mice. Similar doses of Palmitic, Stearic,

and Oleic Acids were relatively ineffective against Erhlich ascites tumor. The mode of administration for these fatty acids was not stated.

Several modes of administration were tested using a 1:1 mixture of Oleic and linoleic Acids in the same dosage regimen.[238] Linoleic acid alone was an effective ascites tumor inhibitor. Intraperitoneal administration of the mixture was the most effective against the ascites tumor, and subcutaneous administration inhibited as much as 60% of the weight gain of the solid tumor.

Oleic Acid, at a concentration of 10 μM, inhibited the growth of rat neuroblastoma cells (cell line B104) in serum-free supplemented media.[239] At least a 50% decrease in cell number relative to controls was observed.

The antitumor activity of palmitoleic (*cis*-9-hexadecanoic) acid was compared to that of Oleic Acid using Erhlich ascites tumors in female ICR strain mice.[240] The fatty acids were dissolved in a 0.15 M sodium chloride (NaCl) solution containing 0.2% Tween 80 and, 24 h after tumor inoculation, were injected intraperitoneally once daily for 10 consecutive days. The experiment was terminated on day 60 after tumor inoculation. Control mice received the same volume of the NaCl plus Tween 80 solution. Significant inhibition of tumor growth was observed in Oleic Acid-treated mice at doses ranging from 37.5 to 300 mg/kg/day when compared to control mice. Palmitoleic Acid was more effective than Oleic Acid, inducing complete regression of the tumor in 5 of 10 treated mice at a dose of 75 mg/kg/day.

A diet supplement of Oleic Acid, at a daily dose of 1 mg per rat, failed to protect Sprague-Dawley rats from colon carcinoma caused by 1,2-dimethyl hydrazine (DMH).[241] All rats (22 rats per group) were killed 22 weeks after the first subcutaneous DMH injection and were examined for colon tumors. Control rats fed chow alone and injected with 15 mg/kg DMH weekly for 16 weeks developed 77 colon tumors, whereas those fed chow plus Oleic Acid before and during the DMH injections developed 90 colon tumors.

TERATOGENICITY

Food and fragrance safety evaluation reports on Oleic and Stearic Acids contained no data on their teratogenicity.[44,45,69] Reviews of the scientific literature from 1920 to 1973 were used for the final food safety assessments.[46,47]

Although placental transfer of fatty acids has been documented in several species and fetal lipid metabolism has been studied,[87,242] no studies on the teratogenicity of fatty acids were found.

CLINICAL ASSESSMENT OF SAFETY

A health hazard evaluation report was prepared by the National Institute for Occupational Safety and Health (NIOSH) after environmental and medical observations and examinations of 7 employees exposed to Lauric Acid.[243] Investigators found no significant decreases in pulmonary function, but interviews with workers indicated that Lauric Acid exposure caused local

irritation of moist body surfaces (eye, nose, throat, sweaty skin). Severe irritation was reported by 1 worker after exposure of moist occluded skin areas to Lauric Acid. The suggested reason for the observed irritation was the acidity of Lauric Acid.

Skin Irritation Studies

In a single insult occlusive patch test (SIOPT), commercial grade Oleic Acid produced no irritation in 18 and minimal erythema in 2 of the 20 panelists. The primary irritation index (PII) was 0.05 and Oleic Acid was considered "practically nonirritating"[244] (Table 20).

A 30% preparation of Oleic Acid in water produced barely perceptible erythema in 2, mild erythema in 1, and moderate erythema in 1 of 21 panelists in an SIOPT. There were no signs of irritation in 17 panelists. The PII was 0.19 and Oleic Acid was considered "practically nonirritating."[245]

In a soap chamber test,[251] 0.2 ml of a 50% solution of Oleic Acid in mineral oil was applied to the ventral skin of the forearm of 16 human subjects once daily for 5 days using the Duhring chamber, an aluminum cup with a 12 mm diameter, fitted with nonocclusive tape. The first exposure was usually 24 h long. Successive exposures to the same sites were for 6 h. The erythema score was 0.22 on a scale of 0 to 5. Oleic Acid was considered "non-irritating under conditions of this test."[246]

Several bar soap formulations with concentrations of Oleic Acid ranging from 2.53 to 92.7% were tested for skin irritation using 16 human subjects. A 0.2 ml volume of 8% aqueous preparations was applied to the ventral skin of the forearm under occlusive patches once daily for 5 days using the Frosch and Kligman soap chamber test.[251] The formulations were considered "slightly" to "moderately irritating." The erythema scores ranged from 1.41 to 3.21 on a scale of 0 to 5 and were not directly related to Oleic Acid concentrations in the formulations.[247–249,271]

In a cumulative irritation study, approximately 9.3 ml of each of 2 mascara formulations, a black cream and a brown cream, containing 6% Oleic Acid were applied to the backs of 14 female and 1 male panelist using closed patches.[250] The panelists removed the patches after 23 h and bathed. Reactions were scored 24 h after sample application. The samples were reapplied daily to the same test sites for 21 consecutive days or until irritation scores of 3, corresponding to erythema and papules, were observed.[252] Up to 7 panelists had minimum scores of 1 or slight erythema by the 5th application, and 3 to 4 panelists had maximal scores of 3 and 4 for erythema, papules, or edema by the 14th application. The total irritation scores for the formulations, a summation of the scores over the number of applications and panelists, were 212 and 204 compared with a maximal score of 945. Mean scores were 14.1 and 13.6 compared with a maximal score of 63. The positive control, an aerosol deodorant concentrate, had a total score of 828 and mean score of 55.2. The negative control, a clear liquid baby oil formulation, had a total score of 18 and a mean score of 1.2. The formulations were considered "slightly irritating."

A red paste cosmetic product formulation containing 5% Oleic Acid was tested for cumulative irritation on the skin of 10 human subjects.[255] Each of

TABLE 20. Clinical Skin Irritation Studies

Fatty acid tested	*Concentration*	*No. of subjects*	*Methods*	*Results*	*Reference*
Oleic Acid	As commercially supplied	20	SIOPT[a]	PII[b] 0.05. "Practically non-irritating"	244
	30%	21	SIOPT	PII 0.19. "Practically non-irritating"	245
	0.2 ml of 50% in mineral oil	16	Soap chamber test,[c] 5 daily occlusive patches	Erythema score 0.22. "Non-irritating"	246
	8% (92.7%)[c] in bar soap formulation	16	See preceding entry	Erythema score 2.13. "Moderately irritating"	247
	8% (2.53–41%) in 13 bar soap formulations	16	See preceding entry	Erythema scores ranged from 1.41 to 3.21 (slight to intense erythema). Scores not correlated with Oleic Acid concentration	248, 249
	6% in 2 mascara formulations	15	21-day cumulative irritation test[d]	CIS[e] 204 and 212 (max. 945). Mean irritation score 14 (max. 63). "Irritating"	250
	5% in product formulation	10	See preceding entry	CIS 95 (max. 630). "Probably mild..."	255
	2% in 3 mascara formulations	13	See preceding entry	One faint erythemal reaction to 4th patch of 1 formulation	256
Palmitic Acid	2.2% in shave cream formulation	101	Single patches, open and occlusive	No irritation	257
	2.2% in shave cream formulation	60	4-week controlled use[f]	"Non-irritating"	258
Myristic Acid	As commercially supplied	20	SIOPT	PII 0.2. "Practically non-irritating"	259
	50% in mineral oil	16	Soap chamber test[c]	Erythema score 0.48. "Non-irritating"	260
	8% (10–91%) in 3 bar soap formulations	16	Soap chamber test[c]	Erythema scores ranged from 1.41 to 1.95 (slight to moderate erythema)	261–263
	5% in cleanser lotion formulation	12	21-day cumulative irritation[d]	CIS 609 (max. 756). "Highly irritating"	264

TABLE 20. (Continued)

Fatty acid tested	*Concentration*	*No. of subjects*	*Methods*	*Results*	*Reference*
Stearic Acid	40% in mineral oil	21	SIOPT	No irritation	265
	13% in face cream formulation	101	Single patches, open and occlusive	Mild erythema to occlusive patch in 4 subjects. "Non-irritating"	266
	13% in face cream formulation	105	4-week controlled use[f]	"Non-irritating"	267
	8% in shave cream formulation	100	Single 48-h occlusive patch and 2–4 week daily home use	No reactions to patch. Complaints of minor pruritus from 2 subjects during home use unsubstantiated	268
	2.8% in liquid eyeliner formulation	13	21-day cumulative irritation[d]	CIS 216 (max. 675). "Moderately irritating"	269
	2.6% in 2 moisturizer formulations	12	See preceding entry	CIS 28 and 56. "Basically non-irritating"	270

[a]SIOPT, single insult occlusive patch test.
[b]PII, primary irritation index; maximum possible value 8.00.
[c]In Soap Chamber Test[251] volume of 0.2 ml usually applied; 8% aqueous preparations of bar soap formulations were tested and noted in Concentration column. Erythema scores reported—scale from 0–5.
[d]Ref. 252. Daily 23-h patches to same site. Some studies modified by Ref. 253.
[e]CIS, cumulative irritation scores; maximum possible score noted in parenthesis following CIS.
[f]Ref. 254.

the 21 consecutive closed-patch applications remained in contact with the skin for 23 h. Scoring for irritation and reapplication to the same test site was done 24 h after the preceding application.[252,253] The total irritation score for all subjects for all 21 applications of the formulation was 95 of a maximal possible score of 630. The total scores for the negative and positive controls were 7 and 554, respectively. The formulation was considered "probably mild in normal use."

Three mascara formulations containing 2% Oleic Acid were tested for cumulative irritation on the skin of 13 human subjects.[256] The closed patches were applied for 21 days, but no applications were made on weekends.[253] One of the 13 subjects had a single equivocal erythema reaction (scored ±) after the fourth application of one of the formulations. No other reactions were observed.

Shave cream formulations containing 2.2% Palmitic Acid were considered "non-irritating" to the skin of 101 panelists treated with closed and open patch applications[257] and to facial skin of 60 panelists in a 4-week controlled-use study.[254,258] Although the former skin irritation study was part of a prophetic patch test[272] in which patches usually remain in place for 24 h, no specific procedure was outlined.

In an SIOPT, commercial grade Myristic Acid produced no irritation in 17, mild erythema in 2, and moderate erythema in 1 of 20 panelists. The primary irritation index was 0.2, and Myristic Acid was considered "practically non-irritating."[259]

In a soap chamber test,[251] 0.2 ml of a 50% solution of Myristic Acid in mineral oil was applied to the ventral skin of the forearm of 16 human subjects once daily for 5 days.[260] The erythema score was 0.48 on a scale of 0 to 5. Myristic Acid was considered "non-irritating under conditions of this test."

Several bar soap formulations with concentrations of Myristic Acid of 10,[261] 22.1,[263] and 91%[262] were tested for skin irritation using 16 human subjects. A 0.2 ml volume of an 8% aqueous preparation was applied to the ventral skin of the forearm under occlusive patches once daily for 5 days using the Frosch-Kligman soap chamber test.[251] The formulations were considered "slightly"[261] to "moderately irritating,"[262] and erythema scores were 1.41, 1.73, and 1.95 on a scale of 0 to 5 for the formulations containing 10, 22.1, and 91% Myristic Acid, respectively.

A white cleanser lotion formulation containing 5% Myristic Acid was tested for cumulative irritation on the skin of 12 human subjects using a 21-day consecutive closed-patch test.[252,253] The total irritation score for all subjects for all 21 applications of the formulation was 609 of a maximal possible score of 756. The formulation was considered "highly irritating."[264]

In an SIOPT, 40% Stearic Acid in mineral oil produced no irritation in 21 panelists.[265]

A face cream formulation containing 13% Stearic Acid was considered "non-irritating" to the skin of 101 panelists treated with single 24-h closed and open patch applications. Four of the 101 panelists had mild erythemal reactions to the closed patch application; no other reactions were observed.[266]

A face cream formulation containing 13% Stearic Acid was tested for irritation of the facial skin of 105 panelists in a 4-week controlled-use study.[254] Under these conditions, the formulation was considered "non-irritating."[267]

As part of a Modified Schwartz/Peck prophetic patch study,[272] a shave foam formulation containing 8% Stearic Acid was tested for irritation of the dorsal skin of 100 male subjects.[268] The formulation was applied to subjects' backs for 48 h, then washed from the area. Subjects then used the formulation to shave at least once daily for 2–4 weeks. No irritation was observed after the 48-h occlusive patch, and the complaints of minor pruritus by 2 subjects during the home-use part of the study were not recorded because no clinical signs of erythema or other evidence of itching were noted.

A gray liquid eyeliner formulation containing 2.8% Stearic Acid was tested for cumulative irritation on the skin of 13 human subjects using a 21-day consecutive closed-patch test.[252,253] The total irritation score for all subjects for all 21 applications of the formulation was 216 of a maximal possible score of 675. The formulation was considered "moderately irritating."[269]

Two moisturizer product formulations containing 2.6% Stearic Acid were tested for cumulative irritation on the skin of 12 human subjects.[270] Occlusive patches were applied for 24 h to the skin of the scapular or interscapular area daily for 21 days. Scoring on a scale of 0 to 4 for erythema and edema was done after each patch was removed and before the next application. Markers of results after treatment with 0.5% and 2% sodium lauryl sulfate were used for comparison with sample treatment. Total irritation scores for the formulations from all 12 subjects for all 21 applications were 28 and 56, lower than the score of 67 obtained after treatment with 0.5% sodium lauryl sulfate. The 2% sodium lauryl sulfate score was 298. Both formulations were considered "basically non-irritating."

Skin Sensitization Studies

The maximization test[182] was used to test a black cream mascara formulation containing 6% Oleic Acid for contact sensitization (Table 21).[273] Induction sites on the volar aspect of the 14 subjects' forearms were pretreated with single 24-h occlusive patches of 5% aqueous sodium lauryl sulfate (SLS). Five alternate-day 48-h occlusive induction patches were followed by a 10–14-day nontreatment period. After pretreatment of new sites with single 30-min occlusive patches of 2% aqueous SLS, single 48-h occlusive challenge patches were applied. Results for the sites treated with the formulation were similar to those for control sites treated with petrolatum alone and petrolatum plus SLS, respectively. There was "no significant irritation or evidence of contact sensitization."

In a repeated insult patch test (RIPT), 200 human volunteers were tested for contact sensitization of a purple wax cosmetic formulation containing 5.0% Oleic Acid.[274] Nine 24-h closed induction patches containing 0.3 ml of the formulation were applied to sites on the volar forearm on Mondays, Wednesdays, and Fridays of 3 consecutive weeks during the induction phase of the study. Signs of irritation were scored 48 or 72 h after the application. After a 10–14 day nontreatment period, a single 48-h challenge patch was

made to a separate site, and the site was scored 48-h and 72-h to 96-h after application. Of the 200 subjects, 153 completed the study. Slight irritation was observed in 1 to 3 subjects during the induction phase, and 1 subject reacted slightly to the challenge patch after 48 h. "No contact sensitization" was produced by the formulation under the conditions of this study.

A mascara formulation containing 3.0% Oleic Acid was tested for irritation and sensitization using an RIPT and 222 human subjects, 200 of whom completed the study.[275] Ten occlusive induction patches were applied for 24 h to sites on the upper back on Mondays, Wednesdays, and Fridays. Sites were scored before application of the next induction patch. After a 2-week nontreatment period, 2 48-h challenge patches were applied 1 week apart. Challenge sites were scored after patch removal. Mild erythemal reactions to single induction patches were observed and considered toxicologically insignificant due to their transient nature. Three subjects reacted with mild erythema to the 2nd challenge patch after 48 h. Two different subjects with mild erythemal reactions 72 h after the 2nd challenge patch was applied were challenged again. One of the 2 had a mild reaction to this 3rd challenge patch. The formulation was considered "not irritating or allergenic."

A mascara formulation containing 2.0% Oleic Acid was tested for irritation and sensitization using an RIPT and 222 human subjects, 205 of whom completed the study.[276] The 10 semiocclusive induction patches, applied for 24 h, and the 2-week nontreatment phases were followed by 2 48-h challenge patches applied to a new site, 1 week apart. No irritation or sensitization was observed.

In a modified Draize RIPT[10] with 14 human subjects, there was "no evidence of allergic contact sensitization" produced by a mascara formulation containing 2.0% Oleic Acid.[277] The formulation had been applied to the skin of the upper arms or backs (unspecified) of subjects during the 9 occlusive patch induction phase (3 times weekly for 3 weeks) and after a 2-week nontreatment period during the single patch challenge phase. Induction and challenge patches remained in contact with the skin for 48 h or 72 h. One equivocal reaction to the challenge was observed. There was "no evidence of allergic contact sensitization."

In a modified Shelanski RIPT of a 1% aqueous dilution of a liquid soap formulation containing 1.95% Lauric Acid on intact and abraded skin of the backs of 52 human subjects, no primary or cumulative skin irritation and no sensitization were observed.[278] Approximately 0.2 ml of the preparation was applied to occlusive induction and challenge patches. A total of 12 24-h induction patches were were administered for 3 weeks, 4 times per week from Monday through Thursday. Sites were scored before application of the next patch. No patches were applied from Friday to Sunday of each week. A total of 4 24-h challenge patches were applied to a new site on the 4th week, after a 72-h nontreatment period, from Monday through Thursday. Of the 52 subjects who began the study, 46 subjects were present for the completion of the study.

In a prophetic patch test,[272] a shave cream formulation containing 2.2% Palmitic Acid was tested for irritation and sensitization of the skin of 101 human subjects.[257] Two 24-h closed and open patches are usually applied to

TABLE 21. Clinical Skin Sensitization Studies (Product Formulation Data Only)

Fatty acid tested	*Concentration*	*No. of subjects*	*Methods*	*Results*	*Reference*
Oleic Acid	6% in mascara formulation	23	Maximization	Similar results for treated and control sites. "No significant irritation or evidence of contact sensitization"	273
	5% in product formulation	153	RIPT[a]	Faint reactions to induction in 1–3 subjects. Slight reaction to challenge in 1 subject	274
	3% in mascara formulation	200	RIPT	Isolated irritation reactions. Mild reactions to 2nd challenge patch	275
	2% in mascara formulation	205	RIPT	No irritation or sensitization	276
	2% in mascara formulation	14	RIPT	Equivocal reaction to challenge in 1 subject	277
Lauric Acid	1% (1.95%)[b] in liquid soap formulation	46–48	RIPT, I/A[c]	No irritation or sensitization	278
Palmitic Acid	2.2% in shave cream formulation	101	Prophetic Patch, O/C[d]	Erythema to closed challenge patch in 3 subjects. No other reactions	257
	2.2% in shave cream formulation	52	RIPT, O/C	No irritation or sensitization	257
Stearic Acid	13% in face cream formulation	101	Prophetic Patch, O/C	Mild reactions to closed induction and challenge patch(es) in few subjects	266
	13% in face cream formulation	52	RIPT, O/C	Mild reactions to closed induction patches in few subjects. No reactions to challenge	266
	10% in product formulation	116	RIPT	Mild to moderate erythema to 2 induction patches in 1 subject. No reactions to challenge	279
	10% in mascara formulation	206	RIPT	Reactions to induction and 48–72 h after challenge. Cumulative irritation in 3 subjects	280
	8% in shave foam formulation	101	Prophetic Patch and In-Use Testing	Several reactions 48 h after induction and challenge, fewer 72 h later. No reactions during In-Use phase	22
	8% in shave foam formulation	100	See preceding entry	No reactions to induction or challenge. Complaints of minor pruritis from 2 subjects during In-Use phase	268

7.7% in mascara formulation	101	RIPT	1 subject had reaction to 8th induction patch. No reactions to challenge	281
5% in mascara formulation	205	RIPT, semiocclusive patches	No irritation or sensitization	282
4% in product formulation	48	RIPT	No irritation or sensitization	283
2.8% in hand lotion formulation	51	RIPT	Transient slight induction reactions in 2 subjects. No reactions to challenge at original or untreated site	284
2.8% in 2 skin lotion formulations	57	RIPT, 48-h patches	Reactions to induction in 1–5 subjects. Slight reactions 72 h after challenge	285
2.66% in eyeliner formulation	200	RIPT	Definite erythema to isolated induction patches in few subjects. No reactions to challenge	286
2.6% in moisturizer formulation	204	RIPT	Mild to intense reactions to induction and challenge. "Mild irritant under occlusion patch"	287
2.6% in moisturizer formulation	203	RIPT	Isolated, mild erythema to induction. Few intense reactions to challenge but none to repatching	288
2.6% in sun lotion formulations	208	RIPT, semiocclusive patches	No irritation or sensitization	289
2.6% in sun lotion formulations	208	RIPT, semiocclusive patches	Few subjects with isolated reactions to induction and challenge	290
2.6% in sun block formulations	208	RIPT, semiocclusive patches	Few subjects with isolated reactions to induction. No reactions to challenge	291
1.0% in hand lotion formulation	76	RIPT	Minimal to definite erythema in few subjects to induction and challenge at same site. No reactions to challenge at untreated site	292
1.0% in hand lotion formulation	76	RIPT	Minimal to moderate irritation to induction in few subjects. No reactions to challenge	292
1.0% in suntan lotion formulation	184	RIPT	No reactions to induction or challenge	293
1% (23%)[b] in bar soap formulation	25	Maximization	No contact sensitization	294
0.5% (25%) in product formulation	99	RIPT	Equivocal induction reaction in 1 subject	295

[a]RIPT, repeat insult patch test.
[b]0.5 or 1.0% aqueous dilutions of formulation containing percentage of fatty acid (percentage in parentheses).
[c]I/A, patches applied at intact and abraded sites.
[d]O/C, 2 series of patches, open and closed, applied at separate sites.

the skin 10–14 days apart in the standard Schwartz-Peck procedure. There were 3 reactions of mild to intense erythema to the closed challenge patch and the formulation was considered "nonirritating and nonsensitizing."

A modified Shelanski RIPT[296] in 52 human subjects involved 10 alternate-day 24-h induction patches, a 2- to 3-week nontreatment phase and a single 48-h challenge patch.[257] Closed and open patches with the same shave cream formulation containing 2.2% Palmitic Acid were applied. No irritation or sensitization was observed.

A face cream formulation containing 13% Stearic Acid was tested for photosensitization using a prophetic patch test[272] in 101 subjects and a modified RIPT in 52 subjects.[266] There were mild reactions in a few subjects to closed induction and challenge patches. The formulation was considered "nonirritating and nonsensitizing."

Approximately 0.1 ml of a cosmetic product formulation containing 10% Stearic Acid was tested for irritation and sensitization of sites on the upper back of 116 human subjects with an RIPT involving 9 alternate-day 24-h occlusive induction patches, a 3-week nontreatment period, and a single 24-h challenge patch at a new site.[279] Moderate erythema was observed in 1 subject after the 5th and 6th induction patches and the 7th induction patch at an adjacent site; the remaining 2 induction patches were eliminated. There were no other reactions to induction and no reactions to challenge.

In a modified Draize-Shelanski RIPT,[168,296] approximately 0.1 g of a mascara formulation containing 10% Stearic Acid produced mild to moderate irritation in a few subjects during induction.[280] Signs of erythema, edema, and induration or vesiculation were observed in 1 to 4 subjects 48 and 72 h after challenge application. The 206 subjects had received 10 alternate day 24-h occlusive induction patches and single 48-h occlusive challenge patches following a 2-week nontreatment period.

In a prophetic patch and in-use testing study, application of single 48-h occlusive induction patches was followed by a 4-week period of daily home use and single 48-h occlusive challenge patches of a shave foam formulation containing 8% Stearic Acid.[268] There were no reactions to induction or challenge patches, and 2 of the 100 subjects complained of minor pruritus during the in-use part of the study. However, there was no erythema or itching.

Several 1 + and a few 2 + reactions were observed 48 h after application of induction and challenge patches in another prophetic patch and in-use testing study.[22] Fewer reactions were noted after 72 h. No significant product-related reactions were reported during the in-use phase of the study.

In a modified Draize RIPT,[168] a mascara formulation containing 7.7% Stearic Acid was tested for irritation and sensitization in 101 human subjects.[281] Approximately 0.2 g was applied to upper arm sites with 24-h occlusive patches on Mondays, Wednesdays, and Fridays for 3 weeks during the induction phase and with single 48-h patches during the challenge phase, following a 2-week nontreatment period. One subject had minimal erythema after the 8th induction patch. There were no other reactions to induction and no reactions to challenge patches.

No irritation and no sensitization were noted in RIPTs of cosmetic product formulations containing 4%[283] and 5%[282] Stearic Acid. The 4% formulation

was tested using the 10 alternate-day 24-h occlusive induction patches followed by a single 24-h occlusive challenge patch to a separate site. The 5% formulation involved 10 alternate-day 24-h semiocclusive induction patches and 2 48-h semiocclusive challenge patches 1 week apart. Both studies had a 2-week nontreatment period between induction and challenge phases.

Although slight transient reactions were observed, a hand lotion formulation containing 2.8% Stearic Acid was considered nonirritating and nonsensitizing.[284] In an RIPT, 0.2 ml of the formulation was applied to the skin of 57 human subjects via 10 alternate-day 24-h occlusive induction patches and single 24-h challenge patches to the same site and to a new site following a 10–14-day nontreatment period.

In RIPTs of two skin lotion formulations containing 2.8% Stearic Acid, 9 consecutive 48-h induction patches, followed by a single 48-h challenge patch after a 13-day nontreatment period, were applied to the skin of 57 human subjects.[285] One to five reactions of barely perceptible to mild erythema were observed throughout the induction phase. Application of one lotion produced erythema and minimal edema to the induction patch and 1 reaction to the challenge patch 72 h after its application in 1 subject.

Several cosmetic product formulations containing 0.13% (0.5% aqueous dilution of formulation containing 25%[295]) to 2.66%[286] Stearic Acid were tested for irritation and sensitization in 76 to 208 human subjects. RIPTs involving 9 to 10 alternate-day 24-h occlusive (semiocclusive patches used in 1 study[289]) induction patches, a 13-day to 2-week nontreatment period, and single 48-h challenge patches[286,292,294,295] or 2 48-h challenge patches administered 1 week apart[287–291,293,296] resulted in isolated 1 + irritation reactions in few subjects during the induction phase. These occasional reactions were considered nonspecific; no cumulative irritation was produced. There were no or very few reactions to challenge patches, and the formulations were considered nonsensitizing.

No contact sensitization was produced in 25 human subjects tested with a 1% aqueous dilution of a bar soap formulation containing 23% Stearic Acid in a maximization study.[182] Five 48-h occlusive induction patches applied to volar forearm sites were followed by a single 48-h occlusive challenge patch. Sodium Lauryl Sulfate was used at concentrations of 2% for pretreatment of induction sites and 10% for the 1-h pretreatment of challenge sites.

Photosensitization Studies

Two makeup formulations containing 5.08%[298] and 1.5%[299] Oleic Acid were tested for photosensitization using the skin of the backs of 25 human subjects. A Xenon Arc Solar Simulator (150 W), which was filtered to produce a continuous emission spectrum in the ultraviolet region ranging from 290 to 400 nm (UVA and UVB), was used. Individual minimal erythemal dose (MED) values were determined.[300] Six alternate-day induction patches were applied, each left in place for 24 h, scored, irradiated with 3 MED using the full source spectrum, and scored again 48 h after the application. After a 10-day nontreatment period, single 24-h occlusive challenge patches were applied to new sites. Sites were scored, irradiated for 3 min, using a Schott WG345 filter over the light source, then scored again 15 min and 24, 48, and 72 h after

irradiation. There were no "reactions" to either formulation recorded. The liquid makeup formulation was considered nonphotosensitizing[299] and the blusher formulation nonphotoallergenic.[298] No data were presented to distinguish between "phototoxic reactions" and "photoallergic reactions."

The phototoxicity of a shave cream formulation containing 2.2% Palmitic Acid was tested in 101 human subjects using single 24-h closed and open patches.[257] Sites were UV-irradiated (wavelength and dosage unspecified) after patch removal. Irritation was observed at 1 site tested with a closed patch.

In a photosensitization study with 52 human subjects, sites under 4 induction patches and 1 challenge patch containing the shave cream formulation with 2.2% Palmitic Acid were UV-irradiated (wavelength and dosage unspecified) after patch removal.[257] Both closed and open patches were used. There were no reactions during induction or challenge phases, and the formulation was considered "non-photosensitizing."

No phototoxicity was observed in 101 human subjects exposed to UVA irradiation and single closed or open patches with a face cream formulation containing 13% Stearic Acid.[266]

Minimal to mild erythema was observed at a few sites after treatment with a lotion formulation containing 2.8% Stearic Acid or a 1% aqueous dilution of a bar soap formulation containing 23% Stearic Acid followed by UVA irradiation.[301,302] The lotion formulation was applied via 24-h occlusive patches to the forearm, and treatment sites were irradiated with UVA light for 15 min at a distance of approximately 10 cm, receiving a dose of 4400 $\mu W/cm^2$. The bar soap formulation was applied via 24-h occlusive patches to the infrascapular region of the back, and treatment sites were irradiated with UVA light from Xenon Arc Solar Simulator (150 W) with a Schott WG345 filter for 12 min. Similar results were observed at control sites that had received UVA irradiation alone.

A face cream formulation containing 13% Stearic Acid was tested for photosensitization using 52 human subjects and 4 induction patches and 1 challenge patch.[266] Closed and open 24-h patches were applied, and treated sites were irradiated with the full Xenon UV light spectrum at 3 times the individuals' predetermined MED after removal of each patch and 48 h later. After the 24-h challenge patch, treated sites were irradiated with UVA light (Xenon source plus Schott WG345 filter) for 3 min. There were no reactions observed at sites under closed or open patches at either induction or challenge sites.

No reactions were observed in 100 human subjects of a photosensitization study testing an eyeliner formulation containing 2.66% Stearic Acid.[286] In a 10 induction, 1 challenge occlusive patch RIPT, treated sites were irradiated with UV light from a Hanovia Tanette Mark 1 light source for 1 min at a distance of 1 foot after removal of the 1st, 4th, 7th, and 10th induction patches and after the challenge patch. Approximately 50% of the subjects were designated as "sensitive subjects" because of past experiences of rash or irritation from the use of facial products or because of reaction to a previous patch test with a facial product.

Most of the 30 human subjects tested with 2 lotion formulations had no photosensitization reactions.[303,304] Subjects had been treated with 10 24-h

occlusive induction patches, each patch followed by UVA irradiation of the site for 15 min at a distance of 10 cm from the source for a dosage of 4400 $\mu W/cm^2$. The single 24-h challenge patch was also UVA irradiated. Nonirradiated controls had isolated reactions of minimal erythema.

No reactions were observed in similar photosensitization studies testing suntan lotion,[305,308] moisturizing lotion,[306] and facial lotion[307] formulations containing 1% Stearic Acid in 20–27 human subjects. No other data were included in these studies.

Table 22 summarizes clinical photosensitization studies.

Ocular Irritation Studies

To evaluate ocular irritation produced by eye area cosmetics in contact lens and noncontact lens wearers, female volunteers participated in a 3-week exaggerated-use study. After a brief medical history with emphasis on ocular details (e.g., history of eye diseases, use of contact lenses and eye area cosmetics) and an eye examination, each subject was instructed to use assigned kits of test cosmetics twice daily (morning and early evening) for 3 weeks. The wearers of contact lenses were to handle, wear, and disinfect their contact lenses normally and to apply cosmetics after lens insertion into the eye. Examinations were performed on the 7th, 14th, and 21st days of the study. Eye area cosmetics in the test kits included mascaras containing 2–3% Oleic Acid and eye shadows.[309,310]

There were no product-related findings of irritation in any of the 23 subjects after daily use of a mascara formulation containing 2% Oleic Acid.[309] Investigators considered the "risk of any significant eye area irritation and/or ocular damage minimal, if existent at all."

Similar results were obtained in another 3-week exaggerated use study, with 35 female subjects testing mascara formulations containing 2% and 3% Oleic Acid in combination with eye shadow formulations.[310]

Other Studies

Graded intraduodenal administration of 5–40 ml of Oleic Acid in humans inhibited pentagastrin-stimulated gastric acid secretion.[311,312] Intracolonic infusion of Oleic Acid (117 cal., pH 7.4) into human subjects decreased pancreatic enzyme concentrations and bicarbonate ion output and inhibited biliary secretion.[313]

SUMMARY

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are fatty acids with hydrocarbon chains ranging in length from 12 to 18 carbons with a terminal carboxyl group. The saturated fatty acids, Lauric(12C), Palmitic(16C), Myristic(14C), and Stearic(18C) Acids, are solids and the *cis*-9,10 monounsaturated Oleic Acid(18C) is a liquid at standard temperature and pressure.

The fatty acids are obtained by the hydrolysis of animal fats and vegetable oils. Cosmetic grade fatty acids occur as mixtures of several fatty acids, the

TABLE 22. Clinical Photosensitization Studies

Fatty acid tested	*Concentration*	*No. of subjects*	*Study type*	*Results*	*Reference*
Oleic Acid	5.08% in blusher formulation	25	Photosensitization	No photoallergic reactions	298
	1.5% in liquid makeup formulation	25	Photosensitization	No indication of photosensitization	299
Palmitic Acid	2.2% in shave cream formulation	101	Phototoxicity	Phototoxic reaction to single closed patch in 1 subject	257
	2.2% in shave cream formulation	52	Photosensitization	No photosensitization reactions to closed or open patches	257
Stearic Acid	13% in face cream formulation	101	Phototoxicity	No phototoxic reactions to closed or open patches	266
	2.8% in lotion formulation	10	Phototoxicity	Minimal erythema after 48 h in 2 subjects similar to control group. No irritation after 1 week	301
	1.0% (23%)[a] in bar soap formulation	10	Phototoxicity	Mild erythema at all irradiated sites—both treated and control	302
	13% in face cream formulation	52	Photosensitization	No photosensitization reactions to closed or open patches	266
	2.66% in eyeliner formulation	200	Photosensitization	No reactions	286
	2.8% in lotion formulation	30	Photoallergy	No photoallergic reactions in most subjects. Non-irradiated control sites had isolated minimal erythema reactions	303
	2.8% in skin lotion formulation	30	Photoallergy	Minimal erythema at irradiated and nonirradiated control sites in 1–2 subjects	304
	1.0% in suntan lotion formulation	25	Photosensitization	No reactions. No other data included	305
	1.0% in moisturizing lotion formulation	27	Photosensitization	No reactions. No other data included	306
	1.0% in facial lotion formulation	27	Photosensitization	No reactions. No other data included	307
	1.0% in suntan lotion formulation	20	Photosensitization	No reactions. No other data included	308

[a] 1.0% aqueous dilution of bar soap formulation containing 23% Stearic Acid tested.

content varying with method of manufacture and source. Fatty acid preparations may include up to 1.5% unsaponifiable matter, glyceryl monoesters of fatty acids, and butylated hydroxytoluene. Gas chromatography is the predominant analytical method for fatty acid identification.

The fatty acids are primarily used as intermediates of fatty acid salts. These salts are used as emulsifiers, emollients, and lubricants in cosmetic creams, cakes, soaps, lotions, and pastes that are slightly alkaline, ranging in pH from 7.5 to 9.5. In product formulation data voluntarily filed in 1981 with FDA by the cosmetic industry, 424 products contained Oleic Acid, 22 contained Lauric Acid, 29 contained Palmitic Acid, 36 contained Myristic Acid, and 2465 contained Stearic Acid at concentrations ranging from 0.1 to 25%.

Fatty acids are absorbed, digested, and transported in animals and humans. Radioactivity from labeled fatty acids administered orally, intravenously, intraperitoneally, and intraduodenally has been found in various tissues and in blood and lymph. β-Oxidation of the fatty acids involves serial oxidation and reduction reactions yielding acetyl-CoA. Although placental transfer of fatty acids has been documented in several species and fetal lipid metabolism has been studied, no studies on the teratogenicity of Oleic, Lauric, Palmitic, Myristic, or Stearic Acids were found. High intake of dietary saturated fatty acids has been associated with the incidence of atherosclerosis and thrombosis.

Little acute toxicity was observed when Oleic, Lauric, Palmitic, Myristic, or Stearic Acid, or cosmetic formulations containing these fatty acids at concentrations of 2.2–13% were given to rats orally at doses of 15–19 g/kg body weight.

In subchronic oral toxicity studies, Oleic, Palmitic, and Stearic Acids were fed to rats in diets at doses ranging from 5 to 50%. Thrombosis, aortic atherosclerosis, anorexia, and mortality were observed. In a subchronic study, no signs of toxicity were observed in chicks fed 5% dietary Stearic and Oleic Acids. Feeding of 15% dietary Oleic Acid to rats in a chronic study resulted in normal growth and general health, but reproductive capacity of female rats was impaired.

Results from topical application of Oleic Acid (at concentrations from 50% Oleic Acid to commercial grade Oleic Acid) to the skin of mice, rabbits, and guinea pigs ranged from no toxicity to signs of erythema, hyperkeratosis, and hyperplasia. Intradermal administration to guinea pigs of 25% Oleic Acid to commercial grade Oleic Acid resulted in local inflammation and necrosis. A formulation containing 2.2% Palmitic Acid was considered nontoxic to rabbits. A topically applied dose of 5 g/kg commercial grade Stearic Acid was not toxic to rabbits. Intradermal administration of 10–100 m*M* Stearic Acid to guinea pigs and rabbits resulted in mild erythema and slight induration.

Eighteen mmol% concentrations of the fatty acids topically applied to the skin of the external ear canals of albino rabbits for 6 weeks produced a range of responses, varying from no irritation with Stearic Acid to slight irritation with Myristic and Palmitic Acids to defined erythema, desquamation, and persistent follicular keratosis with Oleic and Lauric Acids. Slight local edema and no deaths were observed among NZW rabbits after 4 weeks of topical administration of product formulations containing 2.0% Stearic Acid.

In 13-week dermal toxicity studies, 2 cosmetic product formulations containing, at most, 5% Stearic Acid produced moderate skin irritation in rats receiving 4.0 ml/kg and 227 mg/kg doses. All other physiological parameters were normal.

In single insult occlusive patch tests for primary irritation, commercial grades of all 5 fatty acids, at doses of 35–65% in vehicles (Stearic Acid only) and at 1–13% in cosmetic product formulations (other fatty acids), produced no to moderate erythema and slight, if any, edema in the skin of rabbits. Slight increases in irritation were observed in the short-term repeated patch tests (daily for 3–14 days) of Oleic and Myristic Acids.

In maximization studies with 2 cosmetic product formulations containing 5.08% Oleic Acid and 1.0% Stearic Acid, slight reactions were observed to challenge patches. These formulations were considered weak, grade I, sensitizers. In another maximization study, after intradermal induction and booster injections of a formulation containing 3.5% Stearic Acid, reactions to topical challenge applications of the formulation were few and minimal in intensity.

Skin lotion formulations containing 2.8% Stearic Acid were not photosensitizing to the skin of Hartley guinea pigs.

Oleic Acid and its UVA-induced peroxides were associated with increased comedo formation on the treated ears of two species of rabbits.

In ocular irritation studies, the fatty acids alone and at concentrations ranging from 1 to 19.4% in cosmetic product formulations produced no to minimal irritation after single and multiple (daily, 14-day) instillations into the eyes of albino rabbits. Irritation was primarily in the form of very slight conjunctival erythema. A single instillation of Lauric Acid also produced corneal opacity and iritis.

Although Oleic and Lauric Acids induced mitotic aneuploidy in in vitro mutagenicity tests, both have been indicated as inhibitors of mutagenicity produced by positive controls, such as N-nitrosopyrrolidine and sodium azide, in other tests. Stearic Acid was inactive in aneuploidy induction tests and in the Ames test, and it did not inhibit mutagenicity, as did Oleic and Lauric Acids. No increase of mitotic crossing-over events was induced by Oleic, Lauric, or Stearic Acids. Oleic Acid did not increase the number of sister chromatid exchanges over background.

In carcinogenicity studies, no malignant tumors were induced by repeated subcutaneous injections of 1–16.5 mg Oleic Acid in two species of mice. Intestinal and gastric tumors were found in mice receiving dietary Oleic Acid at daily concentrations up to 200 mg/mouse. Treatment of mice with repeated subcutaneous injections of 25 and 50 mg Lauric Acid was not carcinogenic. Low incidences of carcinomas, sarcomas, and lymphomas were observed in mice receiving single or repeated subcutaneous injections of 25 and 50 mg Palmitic and up to 82 mg Stearic Acid. Feeding of up to 50 g/kg/day dietary Stearic Acid to mice was not carcinogenic.

In clinical primary and cumulative irritation studies, Oleic, Myristic, and Stearic Acids at concentrations of 100% or 40–50% 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 (open, occlusive, and semiocclusive), maximization tests, and prophetic patch tests with cosmetic product formulations containing Oleic, Lauric, Palmitic, and Stearic Acids at concentrations ranging from <1 to 13%, no primary or cumulative irritation or sensitization was reported. A few subjects ($<5\%$ of the approximate 4000 subjects tested) reacted to a few, isolated induction patches. Slight, if any, reactions were observed after challenge patching at original or adjacent sites on the upper backs or forearms of some subjects ($\sim <2\%$). Intensity of observed reactions to the formulations was not directly related to the concentrations of the fatty acid ingredients.

Cosmetic product formulations containing 1–13% Oleic, Palmitic, or Stearic Acid produced no photosensitization in human subjects. There were slight reactions to a few induction patches.

There was no treatment-related ocular irritation in female subjects, some of whom were contact lens wearers, involved in two 3-week exaggerated-use studies of mascara formulations containing 2 and 3% Oleic Acid. These formulations were used in combination with other eye area cosmetics.

DISCUSSION

Although insufficient data were available for Myristic Acid, the Expert Panel included it in this safety assessment due to its structural similarity with the other fatty acids of this group.

Applications of Lauric and Oleic Acids to the skin of rabbits resulted in follicular keratosis and/or formation of comedones. These effects were considered by members of the Expert Panel in their safety assessment of the fatty acids reviewed in this report.

CONCLUSION

On the basis of available data from studies using animals and humans, the Expert Panel concludes that Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are safe in present practices of use and concentration in cosmetics.

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4

Final Report on the Safety Assessment of Sorbic Acid and Potassium Sorbate

Sorbic acid is a straight-chain monocarboxylic acid used in cosmetic formulations as a preservative at concentrations up to 1.0%.

Sorbic acid and potassium sorbate were practically nontoxic to rats and mice in acute oral toxicity studies. In subchronic studies no significant adverse effects were observed in rats, mice, or dogs when 10% sorbic acid was included in the diet.

Sorbic acid and potassium sorbate at concentrations up to 10% were practically nonirritating to the rabbit eye. Both ingredients at concentrations up to 10% were at most only slightly irritating.

Sorbic acid and potassium sorbate have been tested for mutagenic effects using the Ames test, genetic recombination tests, reversion assays, *rec* assays, tests for chromosomal aberrations, sister chromatid exchanges, and gene mutations. Results have been both positive and negative.

Potassium sorbate at 0.1% in the diet or 0.3% in drinking water of rats for up to 100 weeks produced no neoplasms. In other chronic studies, no carcinogenic effect was demonstrated by sorbic acid in rats or mice fed diets containing up to 10% sorbic acid.

No teratogenic effects have been observed in pregnant mice and rats administered potassium sorbate.

In three repeat insult patch tests, sorbic acid had overall sensitization rates of 0, 0.33, and 0.8%. All of the subjects sensitized were inducted with 20% sorbic acid and challenged with 5% sorbic acid. Formulations containing up to 0.5% sorbic acid and or potassium sorbate were not significant primary or cumulative irritants and not sensitizers at this test concentration. A formulation containing 0.01% sorbic acid was not a photosensitizer.

On the basis of the available data, it is concluded that sorbic acid and potassium sorbate are safe as cosmetic ingredients in the present practices of use and concentration.

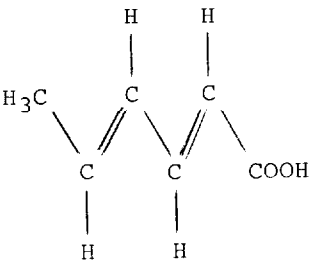
INTRODUCTION

The literature on sorbic acid and potassium sorbate dating from 1920 to 1975 has been previously reviewed in a generally recognized as safe

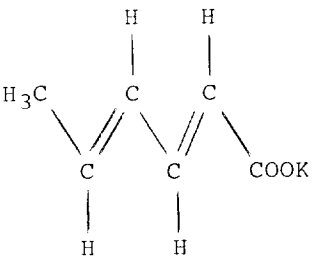
(GRAS) report and evaluation and is only briefly summarized here.[1,2] A survey of the most recent literature, pertinent articles not included in the GRAS report and evaluation, and the unpublished industry data have been incorporated here.

CHEMICAL AND PHYSICAL PROPERTIES

Sorbic acid is a straight-chain monocarboxylic acid also known as 2,4-hexadienoic acid. Potassium sorbate is the potassium salt of sorbic acid.[3,4] These ingredients conform to the following structures[4]:



SORBIC ACID



POTASSIUM SORBATE

Sorbic acid is a white, free-flowing, crystalline powder that is relatively soluble in alcohol and ether and only slightly soluble in water. It has a faint characteristic odor and a slightly acrid taste. Potassium sorbate is a white crystalline powder or white granules or pellets with no or slight odor. It is soluble in alcohol and freely soluble in water.[5-10] The physicochemical properties of sorbic acid and potassium sorbate are presented in Table 1.

Sorbic acid occurs naturally as the lactone, parasorbic acid, in berries of the mountain ash, *Sorbus aucuparia* L., *Rosaceae*. It can be synthesized by various processes, which include condensation of crotonaldehyde and acetic or malonic acid in pyridine solution,[6,10,11] condensation of crotonaldehyde and ketene in the presence of boron trifluoride,[4,10] preparation from 1,1,3,5-

TABLE 1. Physicochemical Properties

Property	*Values*	
	Sorbic acid	*Potassium sorbate*
Appearance	White, free-flowing powder[5,8]	White crystalline powder[5,8]
Odor, taste	Faint characteristic, odor,[3] slightly acrid taste[9]	No or slight odor[9]
Molecular weight	112.13[5,13]	150.22[5,13]
Boiling point (°C)	228 (decomposes)[10,14]	—
Melting point (°C)	134.5[10,14,15]	270 (decomposes)[4,5,10,14]
Flash point (°C)	127[10,14]	—
Ionization constant (at 25°C)	1.73×10^{-5}[1]	—
Density (19/4°C)	1.204 (19/4°C)[15]	1.36 (25/20°C)[14]
Maximum absorption (chloroform)	260 nm ($E = 2400$)[16]	—
pH	3.3 (0.20%)[17]	8.0 (0.3%)[18]
Solubility (%)		
Water	0.25 (at 30°C)[10] 58.2 (at 20°C)[10] 3.8 (at 100°C)[10]	
Propylene glycol	5.5 (at 20°C)[10]	—
Ethanol or methanol	12.90[10]	6.5[10]
Ethanol, 20%	0.29[10]	—
Glacial acetic acid	11.5[10]	—
Acetone	9.2[10]	—
Benzene	2.3[10]	—
Carbon tetrachloride	1.3[10]	—
Cyclohexane	0.28[10]	—
Dioxane	11.0[10]	—
Propanol	8.4[10]	—
Isopropyl ether	2.7[10]	—
Methyl acetate	6.1[10]	—
Toluene	1.9[10]	—
Chloroform	Relatively soluble[4]	Relatively insoluble[4]
Ether	Relatively soluble[4,8]	Relatively insoluble[4]
Loss on drying (% maximum)	0.5[5,13]	1.0[4,8,9,13]
Residue on ignition (% maximum)	0.2[5,8,9,13]	—
Arsenic as As (maximum)	3 ppm[5]	3 ppm[5]
Lead as Pb (maximum)	10 ppm,[5] 20 ppm[8]	10 ppm,[5] 20 ppm[8]

tetraalkoxyhexane,[10] and dealkanolation and hydrolysis of a 3,5-dialkoxyhexanal dialkyl acetal under oxidative conditions.[4] The trans,transisomer is usually obtained and is the commercial product.[10] Potassium sorbate is prepared by reacting sorbic acid with an equimolar portion of potassium hydroxide. The resulting potassium sorbate may be crystallized from aqueous ethanol.[4,6,10]

Numerous studies have been conducted on the stability of sorbic acid and its salts. From a study on the effects of acids, heavy metal ions, and sodium chloride on the autoxidation of sorbic acid in aqueous solution, it was determined that acetaldehyde and fumaraldehydic acid were formed as reaction products. Solutions of sorbic acid salts were stabilized against atmospheric oxidation by the inclusion of gluconic acid, δ-lactone, citric acid, EDTA, or erysorbic acid and its alkaline salts. Propyl gallate was also an effective antioxidant for sorbic acid solutions.[12]

McCarthy et al.[19] found that both temperature and type of container affected the breakdown of sorbic acid. Aqueous solutions of sorbic acid (0.1% w/v) stored for 12 weeks in polypropylene, polyvinyl chloride, polyethylene, and glass containers all had significant loss on storage, except when refrigerated or in the presence of an antioxidant (as occurs in polyethylene-92.2% sorbic acid remaining). The mechanism of decomposition was uncertain and in polyvinyl chloride and glass (at 50°C) was not linear. Although some solutions became increasingly acidic with time, leading to improved contact killing times, both dilution tests confirmed a loss in potency. These losses were not always proportional to the spectrophotometric results.

Gruntova et al.[20] also studied the stability of sorbic acid in aqueous and polysorbate solutions; sorbic acid was oxidized more readily in the polysorbate solutions, with the rate influenced by the packaging material. Kondrat'eva et al.[21] found that the amount of sorbic acid in petrolatum and emulsified bases stored at room temperature in metal containers started to decrease within 1 month and reached 60–80% of the initial content of the bases. They concluded that sorbic acid does not react with sodium lauryl sulfate or diethylene glycol stearate. Nielsen[22] found that sorbic acid incorporated in a cough syrup formulation did not decompose after 26 months of storage at room temperature.

Sorbic acid formed complexes with various starches by interacting with the amylose fraction of the starch. Sorbic acid complexed with acacia in aqueous solution and was also absorbed by nylon and cellulose acetate. The degree of sorbic acid uptake by nylon increased with both temperature and time and was dependent on the pH of the solution, indicating the undissociated molecule was the preferentially absorbed form.[12]

Shihab et al.[23] reported that urea, methylurea, ethylurea, 1,3-dimethylurea, an 1,3-diethylurea increased the solubility of sorbic acid in water. The ureas decreased the hydrophobic attraction between the acid molecules, thus allowing the formation of hydrogen bonds between the acid and water molecules. In another study on the solubility of sorbic acid in the presence of 12 macromolecules, it was found that the amount of solubilization was greatest with polysorbates.[12]

Sorbic acid and potassium sorbate are analyzed primarily by chromatographic techniques, including high-pressure liquid chromatography,[24–26] thin-layer chromatography (TLC),[27–29] gas-liquid chromatography,[30–32] gas chromatography,[33–35] and a combination of gas chromatography and mass spectrometry.[36] Other methods of analysis include ultraviolet spectrophotometry,[37–39] colorimetry,[37] and an isotachophoretic separation based on different electrophoretic mobilities.[40] Both sorbic acid and potassium sorbate can be identified by close matching to standard infrared spectra with no indication of foreign materials.[8]

There has been some concern in the past that sorbic acid may be contaminated with trace amounts of its isomer, parasorbic acid (5-hydroxy-2-hexanoic acid δ-lactone), which is a suspected carcinogen.[41] Stafford et al.,[42] using a new method combining column chromatography, thin-layer chromatography, and gas chromatography-mass spectral analysis, found no parasorbic acid in several food-grade samples of sorbic acid [method sensitive

down to concentrations of 20 ppm (20 mg/kg)]. Murphy and Wardleworth[43] described a more sensitive method in which parasorbic acid was extracted from aqueous potassium sorbate with dichloromethane and determined by gas chromatography using a flame ionization detector. They found no evidence of parasorbic acid down to a concentration of 0.5 mg/kg in the few samples of sorbic acid examined.

USE

Cosmetic

Sorbic acid and potassium sorbate are used in cosmetics and toiletries as preservatives and antimicrobials.[44-46] The 1986 U.S. Food and Drug Administration (FDA) data show that sorbic acid was used in a total of 445 products, including primarily makeup (44%), skin care (19%), eye makeup (16%), hair (7%), and bath (4%) preparations. Of these formulations, 62% incorporated sorbic acid at concentrations of $\leq 0.1\%$; 37% incorporated sorbic acid at concentrations of > 0.1–1%. Potassium sorbate was reported in 117 products, primarily skin care (including suntan preparations) (44%), hair (34%), and makeup (8%) preparations. Of the formulations, 56% incorporated potassium sorbate at concentrations of > 0.1–1%; 44% incorporated potassium sorbate at concentrations of $\leq 0.1\%$.[44]

The FDA cosmetic product formulation data presented in Table 2 are compiled through voluntary filing of such data in accordance with Title 21 Part 720.4 (d)(1) of the Code of Federal Regulations (1979). Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration is a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

The formulation data presented in Table 2 indicate that cosmetic products containing sorbic acid and potassium sorbate may contact all external body surfaces and hair, as well as ocular and vaginal mucosae. Sorbic acid additionally may contact the oral mucosae. These products may be used daily or occasionally over a period of up to several years. The frequency and length of application can result in continuous exposure.

Noncosmetic

Sorbic acid and potassium sorbate are effective preservatives at low concentration for the control of mold and yeast in cheese products, based goods, fruit juices, fresh fruits and vegetables, wines, soft drinks, pickles, sauerkraut, and certain fish and meat products.[3] These ingredients are generally recog-

TABLE 2. Product Formulation Data

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%) > 1–5	> 0.1–1	≤ 0.1
	Sorbic acid				
Baby products	55	4		3	1
Bubble baths and other bath preparations	771	20		18	2
Eyeliner	235	12		1	11
Eye shadow	1406	26		2	24
Eye makeup remover	77	8		5	3
Mascara	325	10		4	6
Other eye makeup preparations	156	15		4	11
Fragrance preparations	1848	10		5	5
Powders (dusting, face, and talcum, excluding aftershave talc)	759	14		7	7
Hair conditioners, rinses, tonics and other hair-grooming aids	1204	25		15	10
Hair shampoos (noncoloring)	821	3		1	2
Hair shampoos (coloring)	27	3		3	
Blushers (all types)	472	19		14	5
Foundations	472	13		2	11
Lipstick	1552	32		32	
Makeup bases	462	106		1	105
Rouges	106	4		3	1
Other makeup preparations (not eye)	337	21		4	17
Manicuring preparations	77	3		1	2
Personal cleanliness products	506	6			6
Skin-cleansing preparations (cold creams, lotions, liquids, and pads)	1000	18	1	7	10
Face, body, and hand skin care preparations (excluding shaving) preparations)	1029	21		15	6
Moisturizing skin care preparations	802	23	1	13	9
Other skin care preparations	1042	22		7	15
Suntan preparations	202	7		1	6
1986 Totals		445	2	168	275
	Potassium sorbate				
Bubble baths and other bath preparations	478	4		3	1
Miscellaneous eye makeup	793	6		5	1
Hair conditioners	556	7		3	4
Hair shampoos (noncoloring)	838	18		4	14
Tonics, dressings, and other hair-grooming aids	350	3		2	1
Wave sets	160	12		2	10
Foundations	472	9		7	2
Skin and personal cleansing preparations	976	7		5	2
Face, body, and hand skin care preparations, including suntan preparations	3281	51		35	16
1986 Totals		117		66	51

Source: From Reference 44.

nized as safe direct food additives when used in accordance with good manufacturing practice.[47,48] Results of a survey of food manufacturers in 1970 indicated that the mean (weighted) level of the addition of sorbic acid to foods ranged from < 0.01 to 1.40% and that for potassium sorbate ranged from < 0.01 to 0.58%. The Grocery Manufactures of America has made an independent estimate of 0.5–0.3% for the range of sorbate addition to food.[2]

The Joint FAO–WHO (Food and Agriculture Organization–World Health Organization) Expert Committee on food additives has estimated the acceptable daily intake of sorbic acid and its salts (expressed as sorbic acid) as 25 mg/kg body weight.[49]

Potassium sorbate is also recognized as a GRAS indirect food additive as it migrates to food from paper and paperboard products used in food packaging.[50]

Sorbic acid and potassium sorbate are also used as preservatives in a variety of pharmaceuticals.[12,13,51–54] The Ophthalmic Advisory Review Panel of the FDA over-the-counter (OTC) drug review program has proposed that sorbic acid used alone in concentrations of 0.1–0.2% is not an effective antimicrobial agent because of its limited bactericidal effects. They also indicated that more data were required to establish the safety and effectiveness of sorbic acid used as a preservative in combination with other approved preservatives.[55,56] The OTC panel on contraceptives and other vaginal drug products has proposed that potassium sorbate at a concentration of 1–3% was safe and effective for OTC use as a vaginal douche for the relief of minor vaginal irritations. However, as potassium sorbate has not been marketed for this purpose to a material extent in the United States, it is considered by the FDA to be a new drug within the meaning of Section 201 (p) of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321 (p)].[57]

Sorbic acid and potassium sorbate also have various industrial uses. These ingredients are both used as preservatives in starch glue.[58,59] Sorbic acid is used to improve the characteristics of drying oils, the gloss in alkyd type coatings, and the milling characteristics of cold rubber.[10] Sorbic acid and potassium sorbate are used to prevent the premature sprouting of wheat seeds,[60,61] to dry out cut plants, such as alfalfa,[62] and in spray compositions applied to the foliage of crop plants to increase yield and growth.[63] Sorbic acid has also been used in insect disease control. Silkworm larvae consuming mulberry leaves that had been sprayed with 0.1–1.0% sorbic acid were protected from insect diseases caused by bacteria, fungi, and viruses.[64]

GENERAL BIOLOGY

Antimicrobial Effects

Sorbic acid and potassium sorbate have a broad spectrum of fungistatic activity but are less active against bacteria. Their antimicrobial activity depends upon the amount of undissociated acid, which is determined primarily by the dissociation constant (1.73×10^{-5} for sorbic acid) and the pH of the system. Optimum effectiveness is attained at pH values up to 6.5.[3,45] Table 3 gives the percentage of preservative undissociated related to pH value.

TABLE 3. Percentage of Sorbic Acid Undissociated Related to pH Value

pH	*% Undissociated*
3	98
4	86
5	37
6	6.0
7	0.6

Source: From References 45 and 65.

The anions of dissociated acids may be inactive owing to repulsion from the negatively charged microbial cell wall.[45,65] However, Eklund[66] has reported that the inhibition of bacteria by sorbic acid was due to both the undissociated and dissociated acid and has calculated the effect in accordance with a proposed mathematical model. Although the inhibitory action of the undissociated acid was 10–600 times greater than that of the dissociated acid, the latter was responsible for more than 50% of the growth inhibition of most of the organisms tested at pH levels above 6.

The antimicrobial activities of sorbic acid and potassium sorbate have been studied extensively. Bell et al.[67] tested sorbate against 66 species of filamentous fungi, 32 species of yeast, and 6 species of lactic acid bacteria. They reported that all organisms grew in media containing 0.1% sorbic acid at pH 7; however, this concentration of sorbic acid inhibited the yeasts and fungi at pH 4.5 and the bacteria at pH 3.5. Extensive tables on the antimicrobial spectrum of sorbic acid are found in the thesis by York.[68] The minimal inhibitory concentrations of sorbic acid for various common microbes are given in Table 4. The reader is referred to Woodford and Adams[12] and Sofos and Busta[69] for more in depth reviews of the antimicrobial effectiveness of sorbic acid and potassium sorbate.

Numerous mechanisms for microbial growth inhibition by sorbate are found in the literature; they indicate there was little or no agreement among

TABLE 4. Effective Concentrations of Sorbic Acid Against Common Microbials

Test organisms ($\sim 10^6$ colony-forming units per ml)	*Minimal inhibitory concentration (μg/ml) (serial dilution test; incubation times of 24 and 72 h; pH 6.0)*
Staphylococcus aureus	50–100
Clostridium sporogenes	100–500
Escherichia coli	50–100
Klebsiella pneumoniae	50–100
Pseudomonas aeruginosa	100–300
Pseudomonas fluorescens	100–300
Pseudomonas cepacia	50–100
Candida albicans	25–50
Saccharomyces cerevisiae	200–500
Aspergillus niger	200–500
Penicillium notatum	200–300

Source: *From Reference* 11.

scientists as to the manner in which sorbic acid inhibited microorganisms. Many investigators have suggested that sorbic acid works by inhibiting various enzyme systems and their reactions. Sorbic acid inhibition of sulfhydryl enzymes, including fumarase, aspartase, succinic dehydrogenase, and yeast alcohol dehydrogenase, has been noted.[69] Inhibition of the enzymes enolase,[11,69] proteinase,[70] catalase,[71] phosphopyruvic hydratase,[72,73] and cytochrome c oxidase[74] has also been reported. Reinhard and Radler[75] found that high concentrations of sorbic acid did not inhibit the enzymes aldolase, enolase, or pyruvate decarboxylase and assumed that sorbic acid inhibited the yeast cells mainly by influencing the cell membrane and its permeability. Cells of *Saccharomyces cerevisiae* rapidly adsorbed sorbic acid (primarily the undissociated form), and disturbances of cell growth may be caused by a reaction of sorbic acid with thiol groups of the surface of the yeast cell.[76] Harada et al.[77] suggested that sorbic acid inhibited the respiration of yeast through its competitive action with acetate at the site of acetyl-CoA formation. Deak and Novak[78] suggested that interference with active transport processes may play an important role in the inhibition of yeast by sorbic acid. Freese et al.[79] and Sheu et al.[80] generalized that lipophilic acid preservatives uncouple both substrate transport and oxidative phosphorylation from the electron transport system. Growth was inhibited by a reduction in cellular uptake of amino acids, organic acids, phosphate, and other compounds.

It has been variously reported that sorbic acid was both effective and ineffective as an antimicrobial in the presence of nonionic surfactants. Some of these discrepancies have been attributed to test conditions,[12] and it was generally accepted that sorbic acid was not strongly affected by the presence of nonionic surfactants.[81–84] In other interaction studies, pantothenic acid and biotin reduced the effectiveness of sorbate against *Verticillium dahliae* but thiamine did not.[85] The activity of sorbic acid may also be reduced by interaction with or loss through the containers.[45,86] Sorbic acid can also be degraded by microbes capable of using sorbic acid as a carbon source.[12,69,76] The mode of sorbic acid degradation has been postulated as being through decarboxylation.[87]

Potassium sorbate has synergistic antimicrobial activity with butylated hydroxyanisole and *tert*-butylhydroquinone against *Staphylococcus aureus* and *Salmonella typhimurium*.[88] It has acted synergistically with sodium nitrite and tripolyphosphate against *Clostridium botulinum*.[89] Potassium sorbate acted synergistically with heat to inactivate four types of molds; the addition of sucrose and sodium chloride further enhanced the inhibition of *Aspergillus flavus*.[90] The synergistic action of sorbate with sodium chloride[91–93] and sucrose[92] has been previously noted. A chlorine addition product of sorbic acid was more effective than sorbic acid alone and was less affected by pH.[45]

Biochemical and Cellular Effects

Sorbic acid did not affect the protein content or the biosynthesis of RNA and DNA in mouse embryo fibroblast cells in tissue culture.[94]

Sorbic acid (1.0 mmol/kg) and/or aminopyrine (0.4 mmol/kg) and sodium nitrite (1.0 mmol/kg) were orally administered to groups of five rats for 3

consecutive days. The rats were killed 24 h later and evaluated for alterations in biochemical parameters, namely, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase in serum, hepatic mincrosomal drug oxidation systems, and glucose-6-phosphate dehydrogenase and lysosomal enzymes in hepatic soluble fractions. The simultaneous administration of aminopyrine and sodium nitrite induced alterations in these parameters, believed to be due to the formation of *N*-nitrosodimethylamine. Sorbic acid did not inhibit the alterations produced by these chemicals, and when administered alone, did not significantly affect these parameters.[95]

Sorbic acid strongly inhibited both the peroxidase and oxidase activity of cabbage peroxidase and its isozymes. Sorbic acid produced a marked difference in action on the isozymes, being much more effective on isozyme III than I. Inhibition was noncompetitive, and the effectiveness depended on the concentration of sorbic acid, time of action, and pH of the medium. Inhibition increased with decreasing pH. Sorbic acid was the most effective organic acid assayed.[96]

Alimukhamedova and Mavlani[97] reported that sorbic acid affected the ultrastructural organization of yeast cells. *Saccharomyces vini* or *Rhodotorula glutinis* incubated with 250–500 mg sorbic acid had an accumulation of dense phospholipoprotein granules, numerous mitochondria of various sizes, and vacuoles within the cells, as well as the presence of irregular nuclei.

Sorbic acid was the second active compound of 35 food ingredients evaluated for a protective effect against cytogenetic radiation damages in the root tip of an onion (*Allium cepa*). Sorbic acid produced a 31% reduction in the rate of aberrant mitosis caused by 100 R irradiation.[98]

Sorbic acid combined in a 1:1 mixture with monolaurin effectively reduced (> 99.9%) the viability of the 14 human RNA and DNA enveloped viruses studied. The sorbic acid–monolaurin mixture was added to the cell culture at a concentration of 1% and incubated for 1 h at 23°C. The virucidal effect was attributed to the solubilization of the lipids and phospholipids in the envelope by the mixture, leading to a generalized disintegration of the viral envelope.[99] Similarly, sorbic acid enhanced the viral activity of the nucleopolyhedrosis virus in treated larvae of the gypsy moth *Lymantria dispar*.[100]

Sorbic acid had no inhibitory effect on the formation of plaque or the development of caries in rats; however, it did enhance the activity of dextranase on these factors.[101]

Metabolism and Excretion

The results of early metabolic studies indicated that sorbic acid was qualitatively metabolized in the same manner as the saturated or singly unsaturated fatty acids of the same C-atom number and was readily used as an energy source.[17]

The metabolism of [^{14}C] sorbic acid was studied after the administration by stomach tube of approximately 920 mg sorbic acid per kg body weight to female Sprague-Dawley rats. Within 4–10 h, 85% of the radioactivity was recovered in the expired carbon dioxide, 0.4% in the feces, 2% in the urine, 3%

in internal organs and blood, 3% in skeletal muscles, and 6.6% in other parts of the carcass. No radioactivity was in the liver or muscle glycogen, but some radioactivity was associated with the lipid fraction of the carcass, internal organs, and skin. The percentage of the radioactivity found in expired carbon dioxide was independent of dosage between 61 and 1213 mg sorbic acid per kg body weight. In similar tests, caproic acid was oxidized at the same rate and to the same extent.[2]

Sodium sorbate or sodium caproate was administered orally to fasted female rats at doses of 75 or 150 mg (calculated as acetone) per 100 m^2 of body surface. Administration was conducted daily in two divided doses of approximately 6 g sorbic acid per kg body weight; a similar proportion was excreted as ketone bodies. Sorbic acid and caproic acid were metabolized via acetone bodies, and under normal conditions sorbic acid was completely oxidized to carbon dioxide and water.[2]

In rabbits fed 3 g sorbic acid per kg body weight, the urine contained 0.1–0.2% *trans,trans*-muconic acid.[1,2] Small amounts of sorbic acid and muconic acid also have been found in the urine of mice orally administered aqueous solutions of sodium sorbate in doses of 40 and 3000 mg/kg body weight. Within 4 days, 81 ± 10% of the sorbic acid was oxidized to carbon dioxide and water; about 4% was found in urine, partially as muconic acid.[2] The metabolism of sorbic acid was identical in animals and humans.[1]

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The oral LD_{50} for sorbic acid in rats has ranged from 7.36 to 12.5 g/kg body weight.[1,11,102] In rats fasted for 18 h prior to the administration of sodium sorbate, the LD_{50} (calculated as sorbic acid) was 3.6 g/kg for females and 4.3 g/kg for males. In rats that had not been fasted, the LD_{50} was 5.9 g/kg (also calculated as sorbic acid). The lower LD_{50} value for sodium sorbate compared with that for sorbic acid was attributed to its more rapid absorption from the gut.[2] The oral LD_{50} for sorbic acid in mice was greater than 8.0 g/kg body weight.[103]

Verrett et al.[104] also evaluated the toxicity of potassium sorbate using embryonating chicken eggs. They injected up to 10.00 mg potassium sorbate (in aqueous solution) into the air sac of the egg at 96 h incubation. The LD_{50} was 2.44 mg potassium sorbate per egg.

A formulation containing 5% sorbic acid was administered by intubation at a dose of 7.0 g/kg to five rats of unspecified strain and sex, and the rats were observed for 7 days. One of the rats died 1 day after treatment. The four surviving rats gained weight during the 7 day observation period.[105]

A 26 ml/kg dose of a cosmetic containing 0.15% potassium sorbate was administered orally to five male and five female fasted Harlan-Wistar rats. There were no signs of toxicity, and weight gains were normal during the 7 day observation period.[106] Groups of five male and five female fasted

Harlan-Fischer 344 rats were given 13 ml/kg of a bronzer[107] and a moisturizer[108] containing 0.15% potassium sorbate by gavage, and the animals were observed for 2 weeks. There were no deaths and no signs of toxicity.

Intraperitoneal

Sparfel et al.[103] reported an intraperitoneal (IP) LD_{50} for sorbic acid in mice of 2800 mg/kg body weight. Five mice were used for each dose. Aqueous solutions of sorbic acid were brought to a pH of 6 with sodium carbonate before injection of 0.5 ml/20 g. An IP LD_{50} value of 2820 mg/kg has also been reported for sorbic acid in mice.[109]

Potassium sorbate had an IP LD_{50} of 1300 mg/kg in mice.[110]

Subcutaneous

Sorbic acid had a subcutaneous LD_{50} of 2820 mg/kg in mice.[109]

Short-Term to Subchronic Toxicity

Oral

Numerous studies have been conducted on the short-term to subchronic oral toxicity of sorbic acid and potassium sorbate when administered to mice, rats, guinea pigs, and dogs. Results have varied with dose and length of administration.

Groups of 5–10 male and female albino rats were fed diets containing 10% sorbic acid for 30–120 days. The test animals had a higher liver-body weight ratio than the controls. Liver homogenates of the first-generation rats fed sorbic acid had lower oxygen consumption than controls; homogenates of liver from second-generation rats had a statistically significant decrease in oxygen uptake. Feed intake and reproduction were normal.[1]

Rats fed an 8% sorbic acid diet for 90 days had no adverse effects other than a slight enlargement of the liver. A 4% diet did not cause hepatic enlargement. Similarly, no adverse effects were found upon histopathologic examination of three dogs fed a diet containing 4% sorbic acid for 3 months.[2]

Rats and dogs fed diets containing up to 8% sorbic acid for 3 months were not adversely affected.[1]

A diet containing 2% sorbic acid (about 2 g/kg body weight) was fed to 8-week-old Wistar rats for 10 weeks. Growth was unaffected. Livers were slightly enlarged, although no microscopic abnormalities were noted.[2]

Groups of rats were fed diets containing 2 or 0.25% sorbic acid or potassium sorbate for 3 months. At the 2% dose, slight increases in the bilirubin and cholesterol content of the bile were noted as well as decreased pancreatic chymotrypsin and amylase. Potassium sorbate also reduced the lipase activity. At the 0.25% level, an increase was seen in the pancreatic juice secretion, its protein content, and the activity of all its enzymes.[1] It was concluded that the significance of these findings could not be assessed from the data.[2]

A 1% oily solution of sorbic acid administered orally to guinea pigs for 20 days produced a four- to sixfold increase in phagocytosis of staphylococcus.[1]

Groups of 50 male and 50 female mice were orally administered sorbic acid at a dose of 80 mg/kg per day for 3 months. Groups of 25 male and 25 female mice were similarly administered sorbic acid at a dose of 40 mg/kg per day or a polymeric impurity obtained from it at doses of 0, 8, or 800 mg/kg per day for 2 months. The mice were observed for general condition and behavior, survival, feed consumption, and weight gain. Tests were also conducted to determine the effects of hunger, physical stress, and carbon tetrachloride poisoning on the test animals compared to controls. Those mice administered sorbic acid for 2 months did not differ significantly from controls in survival, feed consumption, or weight gain. Weight gain was significantly increased in mice receiving the 800 mg/kg dose of the sorbic acid polymeric impurity. Mice administered sorbic acid for 3 months had slightly decreased weight gains compared to controls. Generally, the test mice reacted as well or better than controls to conditions of stress, hunger, and administration of carbon tetrachloride.[111]

In another study, sorbic acid was administered for a period of 3 months at a dose of 40–80 mg/kg per day to 400 albino rats and 1900 mice. No toxic effects on weight gain, feed consumption, or survival rate and no deleterious effects on reactions to stress were produced. The immunobiologic activity and detoxifying action of the liver were increased.[1]

Groups of 10 rats (5 males and 5 females) were fed a diet containing 0, 1, 2, 5, or 10% potassium sorbate for 3 months. The weight gain of the female rats fed 5 and 10% potassium sorbate was decreased initially. Relative hepatic weights were the same in all groups; renal weights were increased in the rats fed 10% potassium sorbate, to a lesser degree in those fed 5% potassium sorbate. No controls for high potassium intake were described.[2]

Potassium sorbate was administered in the diet for 3 months at concentrations of 1 and 2% to two groups of eight dogs each. Weight gains were comparable to those of the control group of four dogs. No adverse effects attributable to potassium sorbate were found upon gross examination at necropsy.[2]

Dermal

A dose of 2 ml/kg of a formulation containing 0.5% sorbic acid (pH not specified) was applied by inunction 5 days/week for 4 weeks to the clipped skin of the backs of three male and three female New Zealand albino rabbits. Plastic collars were worn to prevent ingestion of the test material. The skin of three of the rabbits was abraded. A control group consisted of three male and three female rabbits. Hematologic and biochemical measurements were made during the study. At the end of the study, the rabbits were killed, and the internal organs were examined microscopically. No adverse effects were produced on physical appearance, behavior, body weights, or survival, and no systemic effects were attributed to the formulation. No gross or microscopic lesions were produced. The intact and the abraded skin responses were the same. Slight to moderate erythema and edema were observed in all the rabbits during the first week, and this continued throughout the study. Slight atonia

was observed in all the animals during the second week and continued to be observed in four animals throughout the study. Slight desquamation was observed in two animals during week 2 and in two other animals during week 3, and this continued until the end of the study. The skin had a mild intradermal inflammatory response.[112]

A cream containing 0.15% potassium sorbate (pH not specified) was applied daily for 90 days to the clipped backs of five male and five female New Zealand rabbits. The cream was applied with a spatula to 10% of the total body surface of each animal in a dose of 6 mg/cm^2. Collars were worn to prevent ingestion of the test material. There were five male and five female control rabbits. The animals were observed for local and systemic effects. They were killed at the end of the study, and gross and microscopic examinations were performed. Two control rabbits and one treated rabbit died during the study from causes not considered treatment related. Mean feed consumption, body weights, and organ weights were normal, as were values obtained for hematology, clinical chemistry, urinalyses, and light microscopic examination. Incidental lesions in treated rabbits included granulomatous meningoencephalitis and acute colitis. All treated animals developed slight to moderate erythema and edema during the first week, and this continued throughout the study. Desquamation was slight to moderate in all the rabbits. Four animals developed fine fissures during week 3, and one animal had cutaneous fissures and bleeding on days 46–48. Papillae were observed on the backs of two animals during week 12. Histologically, compound-related dermatitis was observed in 8 of the 10 treated rabbits. The dermatitis was mild and was characterized by the presence of a few inflammatory cells in the upper dermis. No erosion or ulceration of the dermis was observed.[113]

Chronic Toxicity

Oral

Sorbic acid was evaluated for chronic oral toxicity in Wistar rats by administration of 0, 1.5, or 10% sorbic acid in the diet for 2 years. Experimental groups consisted of 48 males and 48 females each. For a similar caloric intake in all groups, a mixture of corn oil and starch (1:1) was added to the 0 and 1.5% sorbic acid diets at 10 and 8.5%, respectively. Body weight, feed and water consumption, mortality, and hematologic and urinalysis parameters were monitored. The organs of all rats were weighed and examined microscopically. No changes in appearance or behavior were noted. Mortality was similar in test and control groups. No significant effects attributable to sorbic acid treatment were found in the hematologic and serum evaluations, urinalyses, or microscopic examination. The total incidence of neoplasms (malignant and nonmalignant), as well as the distribution of affected tissues, was not influenced by sorbic acid treatment. The body weight gain in rats of the 1.5% sorbic acid group did not differ significantly from that in controls; however, rats of the 10% sorbic acid group had a statistically significant reduction in body weight gain from weeks 26 and 39 on in the females and males, respectively. This difference was only 5–10% of the control weight and was

not considered to represent a serious toxic effect because it did not affect mortality. No consistent differences were noted in feed consumption; the mean daily intake was calculated as 0.63 and 4.33 g/kg in males and 0.85 and 5.69 g/kg in females fed the diets with 1.5 and 10% sorbic acid, respectively. Male rats of the high-dose group had higher thyroid gland weights and higher thyroid gland-body weight ratios. All these animals had signs of advanced renal disease, and as prolonged renal damage in the rat can result in parathyroid gland hyperplasia,[114] the increased thyroid weights were considered due to increased parathyroid gland weights. The investigators claimed that their evaluation was further supported since this condition was found in males only, as glomerulonephrosis is usually less severe in females. Both males and females of the 10% sorbic acid group had higher relative liver weights, and the females additionally had higher relative kidney, small intestine, and ovary weights. The higher relative liver and kidney weights were not considered indicative of a serious effect as they were not associated with microscopic changes. (The livers of the high-dose females had only a marginal increase in fatty change and focal necrosis attributed to increased intakes of fatty acid.) In conclusion, the investigators stated that sorbic acid had a no-effect level of at least 1.5% (~ 750 mg/kg per day), although the lack of a carcinogenic effect and the "doubtful nature" of the other effects at 10% (~ 5 g/kg per day) indicate that the no-effect level may be closer to 5%.[115]

Sorbic acid was evaluated for chronic oral toxicity in a similar study in mice (strain ASH/CSI) by administration of 0, 1, 5, or 10% sorbic acid in the diet for 80 weeks. Experimental groups consisted of 48 male and 50 female mice each. To maintain the caloric intake in all groups, a mixture of corn oil and starch (1:1) was added to the 0, 1, and 5% sorbic acid diets at 10, 9, and 5%, respectively. Body weights, mortality, and hematologic parameters were monitored. At termination, the mice were killed and organs examined microscopically. Organ weights were also recorded. No adverse effects attributable to sorbic acid were noted on mortality, hematologic parameters, or the incidence of lesions, including neoplasms. A statistically significant reduction in body weight gain was noted in the males fed 5% sorbic acid and in both males and females fed the 10% sorbic acid diet; this reduction was more pronounced in the latter group. However, as mortality was unaffected and these mice had no other adverse effects, the lower weight was considered a "mildly unfavorable response." Statistically significant increases were noted in the relative organ weights of the brain, liver, kidney, stomach, and small intestine of males on both the 5 and 10% sorbic acid diets. All groups of females treated with sorbic acid had increased relative heart and liver weights, and females of the highest dietary group also had increased relative brain, small intestine, kidney, and spleen weights. The elevation in the relative weights of brain, spleen, stomach, and small intestine were not considered a toxic effect in that there were no significant differences in their absolute weights and no indication of microscopic change. The increased values for relative heart weights, occurring in females only, were not considered an effect of sorbic acid intake. The increased relative liver weights were considered to reflect an increase in metabolic demand resulting from increased fatty acid intake as there was a lower incidence of lesions in the livers of mice fed

sorbic acid than in controls. Similarly, the enlarged kidneys of these mice were not considered a serious toxic effect in that the incidence of lesions in the kidneys was significantly less in the treated mice than in controls. In conclusion, the investigators stated that the no-effect level of sorbic acid in mice may be considered 1% of the diet (~ 1400 g/kg per day), although because of the nature of the effects at concentrations up to 10%, the actual no-effect level may be substantially higher.[116]

Sorbic acid was administered in the diet at concentrations of 0, 0.1, 0.5, and 5.0% (0, 50, 250, and 2500 mg/kg per day) for a period of 1000 days to groups of 50 male and 50 female rats. No differences between test and control animals were noted in appearance, growth, mortality, or reproduction. Rats fed through the second generation a 0.1 or 0.5% sorbic acid diet had no signs of toxicity in respect to growth or reproduction. A group of 30 rats of the second generation maintained on a 5% sorbic acid diet for 252 days had no significant lesions. An unpublished report from the same laboratory described a study in which 50 male and 50 female rats were again fed 5% sorbic acid in the diet during their life span. Mortality was not significantly affected; the average life span of test males compared to control males was 811 and 709 days, and the test and control females lived an average of 789 and 804 days, respectively. No differences were reported in organ weights, and only two neoplasms were found in each of the control and test groups. No abnormalities were seen in the liver, kidneys, heart, or testes.[1,2]

Chronic oral administration of sorbic acid at concentrations of 1–500 times the amounts used in foods had no adverse effect on the blood or internal organs of rats, guinea pigs, rabbits, or dogs.[117]

Shtenberg and Ignatev[111] studied the toxicologic effects of some combinations of preservatives on both mice and rats. Groups of 25 male and 25 female mice were administered 40 mg/kg per day sorbic acid or 40 mg/kg per day sorbic acid plus 2 mg/kg per day nisin as a paste prior to the main feed. Administration continued for 17 months. A control group was fed the basal diet only. The mice were observed for their general appearance and behavior, feed consumption, weight gain, and survival. Organ weights were also recorded at the end of the study. Some of these mice were tested for the effects of physical stress (swimming with a 2 g weight on the tail) and feed restriction. After 8 months on test, some mice from the groups receiving the preservative combination or control diets were mated and reproduction was studied over five generations. The test mice were given the same combination (40 mg sorbic acid and 2 mg nisin per kg per day) from weaning to mating; litters were monitored for weight gain for 3.5 months after weaning. Mice receiving the sorbic acid-nisin combination had a lower survival rate than controls. Relative weights of the liver, kidneys, and testes of mice receiving only sorbic acid were lower than those in all other groups; however, these were not considered adverse effects. The litters from the five-generation study administered the sorbic acid-nisin combination gained more weight than those receiving a benzoic acid-sodium bisulfite mixture. Those mice administered sorbic acid or sorbic acid-nisin also had better scores on the stress tests than those receiving benzoic acid or the benzoic acid-sodium bisulfite mixture. No neoplasms were found in the control or sorbic acid-nisin groups.

The rats of this study similarly received 40 mg/kg per day sorbic acid (groups of 10 males and 10 females) or 40 mg sorbic acid and 2 mg nisin per kg per day (groups of 50 males and 50 females). Other groups received benzoic acid and/or sodium bisulfite. Feed and water consumption, weight gain, and hematologic parameters were monitored. The effects of stress factors were also recorded. These consisted of feed restriction, cold stress, centrifugation, a carbon tetrachloride detoxication test, and a renal function test. Rats fed the sorbic acid-nisin mixture gained more weight and fared better than those on the benzoic acid-sodium bisulfite diet under all stress conditions except feed restriction. The results of the latter study were inconclusive as rats in all test groups survived longer than the controls.[111] (The results of this study were not analyzed statistically.)

Several additive toxicity tests have been conducted with sorbic acid and other preservatives. Ohno et al.[118] studied the additive toxicity of sorbic acid and benzoic acid in groups of 20 male and 20 female Sprague-Dawley rats. The rats were administered diets for 1 year containing concentrations of 5% sorbic acid, 0.5% benzoic acid, 2% benzoic acid, 5% sorbic acid plus 0.5% benzoic acid, 5% sorbic acid plus 2% benzoic acid, or a basal diet with no supplementation. A slight growth inhibition was noted in the female rats receiving 5% sorbic acid after 6 months; no effects were noted on the males. No significant effects were noted in the hematologic values or in the serum and urine analyses of the test rats when compared to controls. No distinctive microscopic changes were noted in any experimental group. Sorbic acid and benzoic acid did not produce additive toxicity in the rat.

Because of concern about the possible contamination of sorbic acid with parasorbic acid, two chronic oral toxicity studies were conducted in rats and mice using sorbic acid deliberately adulterated with 1000 ppm parasorbic acid. Groups of 48 male and 48 female Wistar rats were fed diets containing sorbic acid or the adulterated sorbic acid at concentrations of 1.2% for 2 years.[119] Similarly, groups of 48 male and 48 female mice were fed diets containing the same concentrations of sorbic acid and adulterated sorbic acid for 80 weeks.[120]

The inclusion of parasorbic acid in the diet of rats had no significant effect on feed and water consumption, weight gain, hematologic values, renal function, serum analyses, or the incidence of lesions, including neoplasm incidence. Mortality was slightly greater in the females of the parasorbic acid group, but this was attributed to five rats that died or were killed between weeks 58 and 80. No comparable difference was observed in the males. The liver weights and relative liver weights of the females in the parasorbic acid group were increased compared to those of the sorbic acid group; however, this was not considered significant. The investigators concluded the sorbic acid diet was not made more toxic by the inclusion of 1000 ppm parasorbic acid.[119]

The inclusion of parasorbic acid in the diet of mice produced no statistically significant effects on weight gain, hematologic values, organ weights, or lesions, including the incidence of neoplasms. Mortality was slightly higher in the females of the parasorbic acid group (statistically significant for the last 3 weeks of the study) but was not attributable to the administration of parasorbic acid. Three moribund mice were killed because of severe middle ear

infection, generalized lymphoblastoma, and papillary adenoma of the lungs; both of the latter are common in mice. The other deaths were of differing etiology. The females receiving sorbic acid had only an unusually low mortality rate compared with historic controls from the same laboratory, and the mortality rate of the females receiving parasorbic acid was within the normal range for this strain of mice. The prolonged feeding to mice of a sorbic acid diet adulterated with 1000 ppm parasorbic acid did not lead to an increase in the toxic effects of sorbic acid and did not have a carcinogenic effect.[120]

Irritation

Ocular

A modified Draize ocular irritation test was conducted to evaluate the irritancy of sorbic acid and potassium sorbate to the rabbit eye. Sorbic acid (in petrolatum) and potassium sorbate (in aqueous solution) were evaluated at concentrations of 1, 5, and 10% (pH not specified). Three rabbits were used for each dose group. Eyes were scored at 1, 2, and 24 h and daily thereafter until all irritation had disappeared. Sorbic acid at concentrations of 1, 5, and 10% had ocular irritation indices (at 24 h) of 0.7, 0.7, and 2 (maximum = 110), respectively. Potassium sorbate had an ocular irritation index of 0 at all concentrations (at 24 h). Sorbic acid and potassium sorbate caused practically no ocular irritation and were well tolerated under these conditions.[121]

A 1% aqueous solution of potassium sorbate (pH not specified) was placed in the conjunctival sacs of one eye of each of three male and three female rabbits of unspecified strain. The Draize irritation score, 1 day after test material administration, was 0; the solution had no potential for eye irritation.[122] A 0.1 ml dose of a potassium sorbate solution of unspecified concentration was instilled into the conjunctival sac of one eye of each of six New Zealand white rabbits. The Draize irritation score at 24 h ranged from 2 to 11, and the average of the Draize scores for 24, 48, and 72 h was 4.7. No irritation was observed on day 7 after exposure. Some of the conjunctival tissue in two female rabbits was bleached white on day 1, and this was also observed in one of these rabbits on day 2. Conjunctival petechial hemorrhage was observed in the third female on days 1–3; on day 7, this was no longer observed.[123]

An eye makeup remover containing 0.10% sorbic acid was placed in one eye of each of six albino rabbits. The Draize irritation score was 0; the formulation was nonirritating.[124]

A 0.1 ml dose of a cosmetic containing 0.15% potassium sorbate was instilled into one eye of each of six albino rabbits of unspecified strain, and the animals were observed for 7 days. Slight conjunctival redness was observed 1 h after treatment, but this cleared within 24 h. Cornea and iris were not affected.[106] Groups of six New Zealand albino rabbits were used to evaluate the acute ocular irritation potential of a bronzer[107] and a moisturizer[108] containing 0.15% potassium sorbate. The undiluted formulations were instilled into one eye of each rabbit in a dose of 0.1 ml, and irritation was scored on days 1, 2, 3, and 7. Slight conjunctival hyperemia was

observed 1 h after treatment with both formulations, and this cleared within 24 h. No other signs of irritation were observed.

Dermal

A modified Draize irritation test was conducted to evaluate the dermal irritancy of sorbic acid and potassium sorbate in rabbits. Sorbic acid (in petrolatum) and potassium sorbate (in aqueous solution) were evaluated at concentrations of 1, 5, and 10% (pH not specified). Three rabbits were used for each dose group. Patches were applied under semiocclusive conditions. Sorbic acid at concentrations of 1, 5, and 10% had irritancy indices of 0, 0.2, and 0.5 (maximum = 8), respectively. Potassium sorbate had an irritancy index of 0 at all concentrations. Sorbic acid and potassium sorbate cause practically no dermal irritation and were well tolerated under these conditions.[121]

The primary skin irritation of a 1% aqueous potassium sorbate solution (pH not specified) was evaluated with nine rabbits of unspecified strain. A single occlusive patch was applied, and erythema and edema were scored 2 and 24 h after removal. The primary irritation index (PII) for the test material was 0.6 of a maximum possible of 4.0; the material was practically nonirritating.[125]

Sorbic acid, at a concentration of 5% in a lanoline-petrolatum paste, was applied daily, 6 days/week for 3 weeks, to the shaved skin of three rats. The paste was massaged lightly into the skin for 2 minutes, and the area was then washed with water and any excess paste wiped away. Three rats received similar applications of the lanoline-petrolatum paste without sorbic acid as controls. Weight gain was monitored over the 3 week period. All of the rats gained weight, those receiving petrolatum only at a rate of 5% and those receiving sorbic acid at a rate of 3%. No irritation or other adverse effects were reported.[103]

The primary skin irritation of a product containing 0.5% sorbic acid (pH not specified) was evaluated with nine rabbits of unspecified strain. Frythema and edema were scored 2 and 24 h after a single occlusive patch was removed. The PII of the test material was 0.72 of a maximum possible of 8.0; the skin irritation potential of the material was minimal.[126] An eye makeup remover that contained 0.10% sorbic acid (pH not specified) was evaluated for dermal irritation with 24 h occlusive patches on the intact and abraded skin of rabbits. The formulation did not irritate rabbit skin.[124]

Sensitization

Maurer et al.[127] compared the results of several methods used to assess the contact allergy of weak allergens in guinea pigs with the known epidemiologic data on the occurrence of hypersensitivity reactions in humans. Sorbic acid was evaluated for sensitization by an optimization method in 10 male and 10 female Pirbright white strain guinea pigs at a concentration of 0.1% in physiologic saline. The first week of induction consisted of intracutaneous injections of 0.1 ml of the test solution on Monday (flank and back), Wednesday (back), and Friday (back). The guinea pigs were chemically depilated 21 h after each injection, and the reactions were assessed 3 h later. The diameters of the two largest erythematous reactions (in vertical alignment) and the

skinfold thickness (as measured with a skinfold gauge) were used to determine the individual reaction volume for each animal for each reaction. For induction weeks 2 and 3, 0.1 ml of a 1:1 mixture of sorbic acid (in saline) and adjuvant was injected intracutaneously into the nuchal skin of each guinea pig on Monday, Wednesday, and Friday. The first challenge was administered 2 weeks after the last induction dose. A volume of 0.1 ml of 0.1% sorbic acid in physiologic saline was injected into a previously untreated site on the flank of each animal. The diameter and increase in skinfold thickness of each reaction were measured 24 h later to determine the individual reaction volumes. For each animal, the reaction volume at challenge was compared to the mean plus the standard deviation of the first four induction doses (considered the individual threshold value). If the reaction volume upon challenge exceeded the corresponding threshold value, the animal was considered sensitized. A second epidermal challenge was administered 2 weeks after the intradermal challenge. Occlusive patches containing 1% sorbic acid in soft white petrolatum were applied for 24 h to a shorn, previously untreated site. The reaction sites were chemically depilated 21 h after patch removal and the extent of erythema and skinfold thickness determined 3 h later. An allergic reaction was considered a clearly discernible reddening of the reaction site. The number of positive reactions to the first (intradermal) challenge was 4 of 20 ($P = 0.053$); the second (epidermal) challenge produced no positive reactions.

REACTIONS WITH NITRITE

The potential formation of mutagenic or DNA-damaging reaction products in the presence of sorbic acid or potassium sorbate and sodium nitrite has been studied extensively. Conflicting results have been reported. High concentrations of sorbic acid and sodium nitrite, reacting under acidic conditions, in most cases produced ethylnitrolic acid (ENA).[128–130] ENA has been reported by some as mutagenic and a potent inhibitor of *Escherichia coli*.[128,131,132] However, the results of an extensive study by Difate[133] established that nitrite, not ENA, was the mutagenic compound. He attributed the mutagenicity of ENA reported in earlier studies to possible free nitrite contamination of the test solutions.

Robach et al.,[134] evaluating the mutagenicity of sorbic acid-sodium nitrite reaction products produced in bacon-curing brines, reported that ENA was not formed at nitrite concentrations < 250 ppm and at a pH of 3.4. ENA was not formed at higher pH values (6) even with a nitrite concentration of 500 ppm. All sorbate-nitrite solutions and their ether extracts were negative in the Ames *Salmonella* assays.

Osawa and Namiki[131] analyzed the reactants of sodium nitrite with some sorbic acid analogs for mutagenicity using the *rec* assay and the Ames test. By a large-scale reaction of sodium nitrite with sorbic acid methyl ester, they isolated and identified 5-nitro-2,4-hexadienoic acid methyl ester and ENA as the main mutagens. The investigators concluded that a nitro group adjacent to the double bond is an important factor for the development of mutagenicity.

Khoudokormoff and Gist-Brocades[135] studied the mutagenicity of several food preservatives under conditions of pH and nitrite concentrations approximating those used in preserved foods. *Bacillus subtilis* mutant strain M45 *rec*$^-$, unable to repair DNA damage, was used as the test organism, with the wild-type strain H17$^+$ as control. Sorbic acid (0.1% as potassium sorbate) solutions containing 100, 200, or 400 ppm nitrite at a pH range of 3.0–6.5 consistently had a mutagenic activity that increased as the pH decreased and persited for 8 weeks. No mutagenicity was detected at pH $\geq$ 6. The results of similar experiments carried out with concentrations of SO_2^- approximating those used in wine (150 mg/liter) were a weak mutagenic effect after 2 weeks of exposure. Sorbic acid, nitrite, or bisulfite, tested alone at any pH, did not have mutagenic activity. The sorbic acid-nitrite complex was not mutagenic in two other microbiologic systems using Ames *Salmonella* strains and *E. coli* WP2 and WP2uvra$^-$. Both these systems had media buffered at pH 7, a pH at which sorbate and nitrite exert no mutagenic activity.

Namiki et al.[136,137] studied the effects of reaction conditions on the induced mutagenic (and antibacterial) activities of sorbic acid-sodium nitrite reactants. The pH of the medium, relative molar ratio of nitrite to sorbic acid, and time and temperature were all varied. Mutagenicity was assayed by the *B. subtilis rec* assay and by the Ames assay using *Salmonella typhimurium* TA-98 and TA-100 strains without metabolic activation. The reaction mixture (20 mM sorbic acid and 160 mM sodium nitrite reacted at 60°C for 1 h) obtained at a pH above 6.0 was inactive in the *rec* assay. DNA-damaging activity was produced at a pH between 2 and 5, with the maximum at 3.5–4.2. Mutagenic activity reached a maximum at a sorbic acid-$NaNO_2$ molar ratio of 1:8 (20 mM/160 mM), even though the formation of mutagenic compounds was detected at a ratio of 1:0.5.[137] Heating the reaction mixture to 60°C produced maximal DNA-damaging activity within 30 minutes, which then decreased gradually over time; the reaction carried out at 4°C had activity increasing slowly with time and reaching a maximum level between 48 and 96 h. By use of TLC and/or column chromatography, five C-nitro and C-nitroso compounds were isolated. These compounds included ENA, product Y, determined to be 1,4-dinitro-2-methylpyrrole, product B (total structure unknown), and products F and pre-F, considered primary products of the reaction that would lead to secondary and tertiary products. Tested individually for mutagenic activity, 1,4-dinitro-2-methylpyrrole was highly mutagenic by both the *rec* assay and the Ames assay. ENA was highly active in the *rec* assay but had no activity with *S. typhimurium* strain TA-98 and only weak activity with strain TA-100. Product B had no mutagenicity by the Ames assay and weak activity by the *rec* assay; products F and pre-F were inactive by all bioassays. Sorbic acid (100 mM) and sodium nitrite (160–800 mM) had no mutagenicity by the *rec* assay at the concentrations used in these experiments, although sodium nitrite has had mutagenic activity in other systems.[136] The addition of ascorbic acid or cysteine effectively inhibited the mutagen formation in this reaction system.[137]

Tanaka et al.[138] also tested for mutagenic activity in sorbic acid-nitrite reaction products. A compound designated compound I (the same as product

pre-F just discussed), as well as an unidentified product, were examined by the Ames assay with *S. typhimurium* strains TA-98, TA-100, and TA-1538. Neither compound gave positive results with or without metabolic activation. These compounds also gave negative results when evaluated for DNA-damaging activity using the *B. subtilis rec* assay. The reaction mixture itself (10 mM sorbic acid and 100 mM $NaNO_2$, pH 1, 37°C) was negative by the Ames assay when evaluated without metabolic activation.

Because sorbic acid reacts readily with nitrite, it was postulated that sorbic acid would inhibit the formation of carcinogenic nitrosamines from amines and nitrite. Numerous investigators have studied this under varying experimental conditions. Tanaka et al.[138] found that sorbic acid (20 mM) inhibited the in vitro formation of *N*-nitrosodimethylamine from dimethylamine and nitrite (40 mM $NaNO_2$, pH 2) by up to 74%. Sorbic acid (20 mM) also inhibited the formation of *N*-nitrosomorpholine from morpholine and nitrite (20 mM, pH 2) by up to 72%. Sorbic acid had no effect on the nitrosation of *N*-methylaniline. Ascorbic acid, tested under similar conditions, was equally inhibiting to the formation of *N*-nitrosodimethylamine but was a much stronger inhibitor of the formation of the other nitrosamines.

Lathia and Schellhob[139] also investigated the inhibition of nitrosamine formation in vitro by sorbic acid and/or ascorbic acid. Sorbic acid (0.05 mM) inhibited the formation of *N*-methyl-*N*-nitrosoaniline by 54% and *N*-nitrosomorpholine by 77% from *N*-methylaniline and morpholine, respectively. Reactions were carried out at pH 2 with equimolar amounts of potassium nitrite with either *N*-methylaniline (0.1 mM) or morpholine (10 mM). Increasing the concentration of sorbic acid to 0.1 mM decreased the amount of inhibition for both nitrosamines. Sorbic acid and ascorbic acid synergistically inhibited the formation of *N*-methyl-*N*-nitrosoaniline but not *N*-nitrosomorpholine.

Massey et al.[140] studied the effects of sorbic acid (and ascorbic acid) on *N*-nitrosamine formation in a heterogeneous, protein-based model system containing a 20% nonaqueous phase (glycerol tributyrate). The reactions were carried out at 37°C with an aqueous phase pH of 5.25. Sorbic acid (0.05 M) reduced the formation of nitrosopyrrolidine from pyrrolidine (0.05 M) and sodium nitrite (0.1 M) by 50% in both the aqueous and nonaqueous phases of the system.

Amundson et al.[141] compared the nitrosamine formation in fat and lean bacons cured with 0.26% potassium sorbate and either 40 or 120 ppm sodium nitrite. Nitrosamine formation was suppressed, although not eliminated, by the sorbate cure in both types of bacon.

Kawanishi et al.[142] reported that sorbic acid had no effect on nitrosamine formation from either aminopyrine or aminocycline reacting with sodium nitrite in guinea pig or rat stomachs.

Sorbic acid inhibited the formation of *N*-nitrosodimethylamine in human saliva from the interaction (in vitro) of salivary nitrite with aminopyrine or oxytetracycline. Inhibition ranged from 24 to 45% with 1 mM of sorbic acid and from 51 to 81% with 10 mM sorbic acid. Inhibition was greater at pH 3 than at pH 4.[143]

Potassium sorbate has been incorporated into cosmetic formulations to minimize *N*-nitrosamine contamination.[144]

MUTAGENICITY

The results of genetic recombination tests indicated that sorbic acid had a deleterious effect on the genetic material of *B. subtilis* 168. At concentrations of 20 and 30 μg/ml, sorbic acid (pH adjusted to 7) decreased the frequency of transformations to 77 and 75%, respectively. Concentrations of 1–10 μg/ml sorbic acid produced at 90–91% frequency of transformation. In further testing, sorbic acid (10 μg/ml) did not influence the reversion of characteristic genetics in cells of *B. subtilis* strains 3308, 112, 566, or 168. Sorbic acid (10 μg/ml) was also nonmutagenic by the Ames test with *S. typhimurium* strains 1535 and 1537.[145]

Morita et al.[146] evaluated sorbic acid for mutagenicity using a *rec* assay with wild and recombination-deficient strains of *B. subtilis* and a reversion assay using *S. typhimurium* strains TA-98 and TA-100, both with and without metabolic activation. Sorbic acid was negative by both the *rec* assay and reversion assay at concentrations up to 5.0 mg per disk and 10 μg per plate, respectively. Kada[129] also reported that sorbic acid was negative in the *rec* assay.

Potassium sorbate was evaluated for mutagenicity in a series of short-term assays using *S. typhimurium* strains TA-100 and TA-98 and silkworms for mutations, *B. subtilis* for *rec* assay (without metabolic activation, pH 5), and hamster lung fibroblast cells for chromosomal aberrations and sister chromatid exchanges (SCE; without metabolic activation), as well as rat bone marrow cells for chromosomal aberrations. Potassium sorbate was positive for chromosomal aberrations in hamster fibroblast cells and in the *rec* assay with *B. subtilis*; all other results were negative. No quantitative results were given.[147]

Potassium sorbate was evaluated for chromosomal aberrations and sister chromatid exchanges in a pseudodiploid Chinese hamster cell line at concentrations ranging from 5×10^{-3} to 4×10^{-2} M (maximum concentration of 2×10^{-2} M for the SCE test). Potassium sorbate produced a significant increase in SCE ($p = 0.05$) at concentrations of 1 and 2×10^{-2} M when compared with the mean value for the saline solvent, although this was not considered a dosage effect. However, a dose-related increase in chromosomal aberrations was noted. The investigators concluded that potassium sorbate induced aberrations but did not cause a pronounced increase in SCE.[148]

Ishidate et al.[149] studied the induction of chromosomal aberrations using a Chinese hamster fibroblast cell line in vitro. No metabolic activation was used. Potassium sorbate (in saline) at a maximum tolerated dose of 4.0 mg/ml produced chromosomal aberrations (chromatid gaps, breaks, and translocations) in 11% of the cells within 48 h. This was considered a positive response. Sorbic acid (in dimethylsulfoxide) was negative, producing aberrations in only 3% of the cells at a maximum tolerated dose of 1.0 mg/ml.

Ishidate et al.[149] conducted further studies using the Ames test with *S. typhimurium* strains TA-92, TA-1535, TA-100, TA-1537, TA-94, and TA-98 both with and without metabolic activation. Potassium sorbate (in distilled water) at a maximum dose of 3.0 mg per plate was negative. Sorbic acid (in dimethylsulfoxide) at a maximum dose of 10.0 mg per plate was also negative.

Potassium sorbate was evaluated for mutagenicity in a series of microbial assays. The results of plate tests using *S. typhimurium* strains TA-1535, TA-1537, and TA-1538 with a concentration of 2.5% (w/v) potassium sorbate (in phosphate buffer, pH 7.4) were negative for reversions. Suspension tests using the same strains of *S. typhimurium* and *S. cerevisiae* strain D4 with concentrations of 2.5 and 5.0% potassium sorbate were negative both with and without metabolic activation.[150]

Hasegawa et al.[151] studied the potential of sorbic acid, potassium sorbate, and sodium sorbate to induce chromosomal aberrations, SCE, and gene mutations in cultured Chinese hamster V79 cells. Sorbic acid was tested at concentrations of 350, 700, and 1050 μg/ml; potassium sorbate was evaluated at concentrations of 5000, 10,000, 15,000, and 20,000 μg/ml. Sorbic acid and potassium sorbate induced chromosomal aberrations in a significant number of cells (21 and 28%, respectively) only at the highest doses tested. The effect of sorbic acid and potassium sorbate on SCE was very limited, although concentration dependent, with the highest doses tested resulting in numbers of SCE 1.2 times the control level. The increase in the numbers of SCE was statistically significant at concentrations of 1050 μg/ml sorbic acid and at all concentrations $\geq$ 10,000 μg/ml potassium sorbate. The same test concentrations of sorbic acid and potassium sorbate produced no 6-thioguanine-resistant mutations. The effects of change in osmotic pressure caused by the addition of sorbic acid and potassium sorbate were also evaluated by substituting sodium chloride and potassium chloride. The induction of chromosomal aberrations could be partially attributed to the change in osmotic pressure, whereas the latter did not affect the number of SCE. Sodium sorbate was substantially more genotoxic than either sorbic acid or potassium sorbate.

Tsuchiya and Yamaha[152–154] conducted a series of mutagenicity tests on mice administered sorbic acid or potassium sorbate. Five groups of 77–79 male mice were fed diets containing 0 (control), 1.34, 6.7, and 20.1% potassium sorbate as well as 15% sorbic acid for periods of time up to 15 months. The first test evaluated the mutagenicity of the intestinal contents of these mice. Small and large intestinal contents were removed from 5–10 mice of all groups at week 1 and after 1, 3, and 6 months. The samples of contents taken from mice of the same dose group at the same time were combined, homogenized, and extracted with diethyl ether. The ether layer was evaporated, and one part was dissolved in dimethyl sulfoxide at a concentration of 0.70 mg/ml for the mutagenicity assay test and the other part subjected to fractionation (basic, neutral, and acidic). Using a modified Ames assay both with and without metabolic activation, the ether extracts of week 1 and 1 and 3 months were nonmutagenic in *S. typhimurium* TA-100 (extensive killing of bacteria occurred at 6 months); the extracts of contents sampled up through 6 months were nonmutagenic in *S. typhimurium* TA-98. These tests were repeated with strain TA-98 using the acidic, basic, and neutral components obtained by

fractionating the intestinal contents sampled at 3 and 6 months from the control and sorbic acid groups only. The results with basic and neutral components were negative. The acidic components from both samples of the sorbic acid group were slightly mutagenic but only with metabolic activation. The mutagenic ratios of acidic components at 3 months were slightly higher than those at 6 months; the distribution of acidic components at 6 months was 1.5 times greater than that at 3 months. The investigators suggested that mutagens were gradually produced in the intestine and moved into the liver, the site of metabolic activation.[152]

The urine of these mice was collected at 6 months and 100 μl samples assayed for mutagenicity using the Ames test with *S. typhimurium* strains TA-98 and TA-100. All results were negative. Samples (200 μl) from the control, 15% sorbic acid, and 20.1% potassium sorbate groups were also assayed using the Ames preincubation method; results were negative. Urinary samples from these mice treated with or without β-glucuronidase were fractionated by XAO-2 column chromatography and assayed for mutagenicity with strain TA-98. Only those samples from the 15% sorbic acid group were mutagenic when metabolically activated. Mutagenic ratios were unaffected by treatment with β-glucuronidase. Urine from mice on the 15% sorbic acid diet for 12 months was also collected and fractionated. These samples were nonmutagenic in strain TA-100 but gave positive results in TA-98 with metabolic activation. A comparison of the volume and pH of the urine between mice of the control and 15% sorbic acid groups at 6 months indicated a slight increase in volume and decrease in pH of urine of the sorbic acid group.[153]

Tsuchiya and Yamaha[154] further studied the mutagenicity of the intestinal contents, the glutathione content in the liver, and the relative body weight-liver weight ratios in these mice after administration of sorbic acid or potassium sorbate for 12 months. The acidic components of the intestinal contents of 10 mice from each group were assayed for mutagenicity using *S. typhimurium* strain TA-98 both with and without metabolic activation. Those samples taken from the 15% sorbic acid group were mutagenic with metabolic activation (mutagenicity increased with increasing amount in milligrams of acidic components per plate); slight mutagenicity was noted in samples from the 20.1% potassium sorbate group (with metabolic activation). The glutathione content in the liver of the 15% sorbic acid group at 3 months was decreased by 60% compared with that of the controls. This low concentration was maintained for up to 12 months. A close correlation was noted between the extent of depletion of hepatic glutathione content and the concentration of sorbic acid in the diet. The relative body-liver weight ratios of animals of the 15% sorbic acid group were clearly increased compared with those of the other groups.

CARCINOGENICITY

Potassium sorbate was administered orally to six rats at a concentration of 0.1% in the diet and to another six rats at 0.3% in the drinking water. No induced hepatic tumors were detected by laparotomy in these animals at 65

weeks, and the oral administration was therefore continued for 100 weeks (when all had died). The animals were examined postmortem, with microscopic examination when appropriate; no induced tumors were found in any of the rats.[18]

No carcinogenic effect was demonstrated by sorbic acid in Wistar rats[115] or ASH/CSI mice[116] fed diets containing up to 10% sorbic acid for periods of 2 years and 80 weeks, respectively. (See Chronic Toxicity: Oral section for more details). Ishizawa et al.[155] have reported a carcinogenic effect on the liver of mice fed diets containing up to 15% sorbic acid for 88 weeks.

TERATOGENICITY

Potassium sorbate was evaluated for teratogenicity in groups of approximately 20 pregnant mice (CD-1) and rats (Wistar-derived stock). The mice were administered potassium sorbate as a water suspension at doses of 4.6, 21.4, 99.1, and 460.0 mg/kg body weight; the rats received doses of 3.4, 15.8, 73.3, and 340.0 mg/kg body weight. Doses were administered daily by oral intubation on days 6–15 of gestation. Both vehicle and positive (aspirin) controls were used. No significant effects were noted on nidation or on maternal or fetal survival in either mice or rats. The number of abnormalities seen in soft and skeletal tissues of the potassium sorbate groups did not differ from the number occurring spontaneously in the vehicular controls.[156]

CLINICAL IRRITATION AND SENSITIZATION

Clemmensen and Hjorth[157] patch tested 91 dermatologic patients on the upper back with concentrations of 0.1, 1.0, 5.0, and 10% sorbic acid and benzoic acid in petrolatum. Occlusive patches were applied for 20 minutes and reactions scored upon removal. Sorbic acid produced erythema in 19.8, 61.5, 64.8, and 67.4% of the patients at each of the four increasing concentrations, respectively. Edema was produced by sorbic acid in 0, 1.1, 7.7, and 9.0% of the patients at successively increasing concentrations, respectively. The investigators noted that the positive reactions seemed to follow a dose-response curve with a plateau at 1%. A group of 10 patients with positive reactions to sorbic acid was selected to test the effects of local application of an antihistamine prior to patch testing. Mepyramine (2% in water or gel) was applied as a closed patch test 3 h prior to patch testing with sorbic acid. Mepyramine produced a mean reduction in erythemal responses of 31.4% (range 2.2–65.6%), although in no patient was the reaction totally abolished. Prick tests with histamine produced no reactions in these patients (See Table 5 for clinical irritation and sensitization results.)

Soschin and Leyden[158] studied the effects of sorbic acid on different body regions when used as an ingredient in steroid preparations and other vehicles. Patches containing a 0.1 ml sample of sorbic acid at concentrations of 0.1, 0.5, and 1.0% in 2-isopropanol and water 1:1 were applied to the deltoid muscle, the volar aspect of the forearm, and the upper portion of the back of 15–17

TABLE 5. Clinical Irritation and Sensitization

Ingredient	*Test method*	*No. of subjects*	*Results*		*Reference*
Sorbic acid					
0.1% in petrolatum	Single occlusive	Dermatologic patients	Erythema in:	Edema in:	157
1.0%	patch on upper back	91	18 (19.8%)	0(0%)	
5.0%	(20 minutes)	91	56 (61.5%)	1 (1.1%)	
10.0%		91	59 (64.8%)	7 (7.7%)	
		89	60 (67.4%)	8 (9.0%)	
			Investigators noted a dose-response curve with a plateau at 1%		
In isopropanol and water	Single occlusive patch on deltoid muscle, forearm, and upper back (20 minutes)	15–17	High prevalence of either erythema or edema at all body sites, with dose response evident by the intensity of the reaction; reactions most intense on the face		158
0.1%					
0.5%					
1.0%					
In ethanol and water	Single occlusive patch on cheeks and forehead (20 minutes)	15			
0.05%					
0.1%					
0.5%					
1.0%					
In petrolatum	Draize RIPT[a]				
Induction					
10		93	Induction with 10%: 0 of 93 sensitized		159
20		33	Induction with 20%: 1 of 33 sensitized		
Challenge, 5			Overall sensitization rate of 0.8%		
In petrolatum	Draize RIPT				
Induction					
10%		181	Induction with 10%: 0 of 181 sensitized		160
20%		121	Induction with 20%: 1 of 121 sensitized		
Challenge, 5%			Overall sensitization rate of 0.33%		
1.0% in petrolatum	Draize-Shelanski RIPT	50	49 totally negative responses; 1 subject with 2+ reaction at application 3, given 1 day rest, administration continued with 0.5% sorbic acid, no further reactions		161
0.10% in eye makeup remover	Schwartz and Peck prophetic patch test, with and without UV	102	Eye makeup remover was nonirritating, nonsensitizing, and nonphotosensitizing		162
	Shelanski and Shelanski RIPT	Unspecified	Eye makeup remover was nonirritating, nonsensitizing, and nonphotosensitizing		162
	Controlled use study (4 weeks)	54	No irritation was observed		163

TABLE 5. Continued

Ingredient	*Test method*	*No. of subjects*	*Results*	*Reference*
0.5% in formation, tested as a 0.5% aqueous solution	RIPT	86	Formulation did not induce contact sensitization; minimal to mild irritation	164
0.2% in bubble bath	RIPT	78	Bubble bath did not induce allergic sensitization; minimal irritation in three subjects	165
0.2% in formulation	RIPT	52	Formulation did not induce allergic sensitization; minimal irritation	166
0.2% in facial conditioner	RIPT	84	Facial conditioner did not induce allergic sensitization; minimal to mild irritation	167
0.2% in formulation	RIPT	98	Formulation did not induce allergic sensitization; minimal irritation	168
Potassium sorbate				
0.15% in cream	Cumulative irritation test	12	Very mild cumulative irritation was observed	169
0.15% in moisturizer	Cumulative irritation test	12	Very mild cumulative irritation was observed	170
	Cumulative irritation test	12	No cumulative irritation was observed	171
0.15% in formulation	Shelanski-Jordan RIPT	209–210	Formulation was not a strong irritant or a strong contact sensitizer	172
0.15% in bronzer	Modified Draize-Shelanski RIPT	199–204	Bronzer was not a primary irritant or an allergic contact sensitizer	173
0.15% in moisturizer	Modified Draize-Shelanski RIPT	202–205	Moisturizer was not a primary irritant or an allergic contact sensitizer	174
0.1% in facial scrub, tested diluted 1:100 in deionized water	RIPT	53	Facial scrub was a very mild cumulative irritant but was not a primary irritant and did not induce sensitization	175
	RIPT	53	Facial scrub was a very mild cumulative irritant but was not a primary irritant and did not induce sensitization	176
	RIPT	56	Facial scrub did not induce dermal irritation or sensitization	177
	RIPT	47	Facial scrub was not a sensitizer	178

[a]Repeat insult patch test.

subjects. The patches were occluded for 20 minutes, and sites were scored 10 minutes after patch removal using a maximum scale of 4. Of these subjects, 15 received an additional application of sorbic acid at concentrations of 0.05, 0.1, 0.5, and 1.0% on the cheeks and forehead. A high prevalence of either erythema or edema was observed at all sites, with the dose response evident by the intensity of the reaction. Reactions were most intense on the face; the number of scores of 3 or 4 on the face was significantly increased compared with the other body sites at 0.1% sorbic acid. No significant differences in the rate or intensity of the reaction rate were noted at higher concentrations of sorbic acid.

Soschin and Leyden[158] also investigated the effect of sorbic acid-induced reactions on the anti-inflammatory effects of corticosteroid creams. They compared the dermal effects of 0.1% sorbic acid in ethanol and water with the effects of hydrocortisone cream containing 0.1% sorbic acid or potassium sorbate in 17 subjects. Each was applied to the cheek and forehead without occlusion for 20 minutes. The intensity of the reaction rate was significantly less in the topical steroid preparations than in the ethanol-water vehicle. The anti-inflammatory effect was not affected by sorbic acid-induced erythema.

The results of further studies showed that pretreatment of skin with topical steroids to induce vasoconstriction diminished the response to sorbic acid. Oral administration of aspirin blocked the erythematous component, and the investigators suggested that prostaglandins were therefore important mediators. Systemic steroids, antihistamines, and hydroxyzine failed to influence the erythema and edema produced by sorbic acid. In electron microscope studies of tissue from the sites on the upper back, it was concluded that erythema, edema, and flare in response to sorbic acid were not associated with mast cell degranulation.[158]

Marzulli and Maibach have conducted two Draize repeat insult patch tests (RIPT) of sorbic acid. In each test, sorbic acid was applied in petrolatum at concentrations of 10 and 20% during the induction period and 5% for the challenge. The induction period consisted of ten 48 h occlusive patches (72 h on the weekends) applied over a period of 3–5 weeks. Each patch contained 0.5 g of the test material and was applied to the same site on the lateral arm above the elbow. Following a 2 week rest period, a challenge patch was applied for 72 h. All reactions were scored upon patch removal on a scale of 1–4. The results of the first test were 0 in 93 sensitized when treated with 10% sorbic acid and 1 in 33 sensitized when treated with 20% sorbic acid. This gave an overall sensitization rate of 1 in 126, or 0.8%.[159] The results of the second test were 0 in 181 sensitized when treated with 10% sorbic acid and 1 in 121 sensitized when treated with 20% sorbic acid. This gave an overall sensitization rate of 1 in 302, or 0.33%. The sensitization rate of 0.8% for the subjects treated with 20% sorbic acid was not statistically significant.[160]

Klauder[161] conducted a Draize-Shelanski RIPT with sorbic acid at a concentration of 1% in petrolatum. Closed patches containing sorbic acid were applied at the same site every other day for a total of 12 applications. It was not specified whether the patches were occlusive or nonocclusive. After a 2 week rest, a challenge patch was applied to the same site. Of the 50 subjects completing the test, 49 had negative responses. A single subject had a

2+ reaction to the third induction patch; he was given 1 day of rest and then patched with 0.5% sorbic acid. All subsequent reactions were negative. The investigator noted that this was probably an irritation response and was consistent with the results of the pilot study (2 and 4% sorbic acid producing irritation in 2 of 12 and 4 of 10 subjects, respectively).

An eye makeup remover containing 0.10% sorbic acid was tested for skin irritation in the Schwartz and Peck prophetic patch test using 102 panelists. Open and closed patches were scored on a 1+ to 3+ scale, and the effect of ultraviolet radiation was also determined. There were no reactions to open patches. There were five 1+ reactions to closed patches on day 1, two 1+ reactions on day 2, one 2+ reaction on days 1 and 2, respectively, and one reaction on day 2 following ultraviolet radiation. The same formulation was also tested in a Shelanski and Shelanski RIPT with an unspecified number of panelists. There were no reactions to open patches, and there were no reactions following ultraviolet radiation. There were up to three 1+ reactions to closed patches each day during the 11 days of the study. The eye makeup remover was nonirritating, nonsensitizing, and nonphotosensitizing.[162]

An eye makeup containing 0.10% sorbic acid was tested for skin irritation in a controlled use study with 54 panelists. No irritation was observed in any panelist during the 4 weeks of the study. The eye makeup remover was nonirritating.[163]

A formulation containing 0.5% sorbic acid was tested for skin irritation and sensitization in an RIPT procedure using 86 panelists. The formulation was tested as a 0.5% aqueous solution. Occlusive 24 h induction patches were applied three times a week for 3 weeks to the upper backs of the subjects. An untreated site was challenged with a 24 h patch during week 6 of the study. Induction patches were each scored 24 h after removal, and the challenge patch was scored 24 and 48 h after removal. A total of 19 panelists reacted to induction patches: 7 had mild (pink uniform erythema covering most of the contact site) reactions, and 12 had at most barely perceptible (minimal faint uniform or spotty erythema) reactions. There were three barely perceptible reactions at the 24 h challenge reading and no reactions at the 48 h reading. The formulation under these test conditions did not induce contact sensitization.[164]

A bubble bath containing 0.2% sorbic acid was tested as a 0.25% aqueous solution for skin irritation and sensitization in an RIPT with 78 panelists. Three occlusive 24 h induction patches were applied to the upper back of each panelist each week for 3 weeks, and a 24 h challenge patch was applied to a previously untreated site after a 3 weeks rest. Reactions to induction patches were scored 24 h after patch removal, and reactions to challenge patches were scored 24 and 48 h after patch removal. Of the 78, 3 subjects had barely perceptible reactions to induction patches, and there were no reactions at challenge. The bubble bath formulation did not induce allergic sensitization.[165]

An RIPT was conducted with a formulation containing 0.2% sorbic acid. Occlusive 24 h induction patches were applied to the upper backs of 52 panelists three times a week for 3 weeks, and reactions were scored 24 h after the removal of each patch. A 24 h challenge patch was applied to an untreated site during week 6 of the study, and the reaction was scored 24 and

48 h after patch removal. Of 52 subjects, 9 had barely perceptible reactions to at least one induction patch. Another 2 subjects had barely perceptible reactions at one reading of the challenge site, 1 at 24 h and 1 at 48 h. Follow-up testing was performed with the subject with the reaction at the 48 h challenge reading; no reactions were observed. The original challenge reaction was of a nonspecific, irritant nature and was not due to allergy. Under these test conditions, this formulation did not induce allergic sensitization.[166]

The skin irritation and sensitization of a facial conditioner containing 0.2% sorbic acid was determined in an RIPT using 84 panelists. Three occlusive 24 h induction patches were applied to the upper back each week for 3 weeks, and a 24 h challenge patch was applied after a 3 week rest period. Induction reactions were scored 24 h after patch removal, and challenge reactions were scored 24 and 48 h after patch removal. Of 84 panelists, 23 had barely perceptible to mild reactions to at least one induction patch. A single panelist had a barely perceptible reaction, and another panelist had a mild reaction at the 24 h challenge reading. There were no reactions at the 48 h challenge reading. The facial conditioner did not induce allergic sensitization.[167]

A formulation that contained 0.2% sorbic acid was tested in an RIPT for skin irritation and allergic sensitization. A total of nine occlusive 24 h induction patches were applied to the upper backs of 98 panelists over a 3 week period. Reactions to these patches were scored 24 h after patch removal. Occlusive 24 h patches were applied to untreated sites in week 6 of the study, and reactions to these patches were scored 24 and 48 h later. Of 98 panelists, 10 had barely perceptible to mild reactions to at least one induction patch. There were two reactions to the challenge patch at the 24 h reading, and there were no reactions at the 48 h reading. Under the conditions of this RIPT, this formulation did not induce allergic sensitization.[168]

A white cream containing 0.15% potassium sorbate was tested for cumulative irritation in 12 subjects. Each day for 21 consecutive days, 23 h occlusive patches were applied to the backs of the subjects, and reactions to each patch were scored every 24 h. The total composite score for the cream was 83 of a maximum possible score of 756, and the total score with a base of 10 subjects was 69 of a maximum possible score of 630. There was a slight potential for very mild cumulative irritation under the conditions of this test; the cream is probably mildly irritating in normal use.[169]

The cumulative irritation potential of a moisturizer containing 0.15% potassium sorbate was evaluated with 23 or 47 h occlusive patches applied to the backs of 12 subjects. Patches were applied each day, with the exception of a holiday, for 20 consecutive days. Reactions were scored 1 h after patch removal. The composite total score for the 12 subjects was 169 of a possible maximum score of 720, and the total score with a base of 10 subjects was 140.83 of a possible maximum of 600. The moisturizer was probably a mild irritant in normal use; there was evidence of a slight potential for very mild cumulative irritation under the conditions of this test.[170]

A moisturizer containing 0.15% potassium sorbate was evaluated for cumulative irritation with 12 panelists. With the exception of a holiday, 23 or 48 h occlusive patches were applied to the backs of the subjects each day for 20 consecutive days. Reactions were scored 1 h after patch removal. The composite total irritation score for the 12 panelists was 52 of a possible maximum of

720, and the total score calculated for 10 panelists was 43.33 of a possible maximum of 600. There was essentially no evidence of cumulative irritation under the conditions of this test.[171]

A Shelanski-Jordan RIPT was conducted with a formulation containing 0.15% potassium sorbate. Occlusive induction patches were applied for 24 h to the backs of 209 to 210 subjects three times a week for a total of 10 applications. Reactions were scored at patch removal on a 0–4+ scale. A 48 h challenge patch was applied 10–14 days later, and this reaction was scored at patch removal. After another 7–10 days a second 48 h challenge patch was applied, and this reaction was scored 48 and 72 h after patch application. A single subject had a 2+ reaction to induction patches 9 and 10; these reactions appeared to be irritation due to occlusive patch testing. Another subject had a 2+ reaction at the 72 h reading of the second challenge; this reaction lacked signs of edema. No other reactions were observed. The formulation does not appear to be a strong irritant or a strong contact sensitizer.[172]

A bronzer containing 0.15% potassium srobate was evaluated for skin irritation and sensitization in a modified Draize-Shelanski RIPT. Occlusive induction patches were applied for 24 h to the upper backs or inner upper arms of 199–204 subjects three times a week for a total of 10 applications, and reactions were scored on a scale of 0–4+ at 24 or 48 h. To the same sites and to previously unpatched sites, 48 h occlusive challenge patches were applied 3 weeks later, and these reactions were scored 48 and 72 h after application. There were fourteen 1+ and three 2+ reactions to induction patches. There were six 1+ reactions to the challenge patch at the original site at the 48 h reading, two 1+ reactions to the challenge patch at the untreated site at the 48 h reading, five 1+ reactions to the challenge patch at the original site at the 72 h reading, and three 1+ reactions to the challenge patch at the previously untreated site at the 72 h reading. There were no other reactions. The 1+ and 2+ reactions were judged to be irritant in nature and were not considered clinically significant. The bronzer did not appear to be a primary irritant or an allergic contact sensitizer.[173]

A modified Draize-Shelanski RIPT was used to test the skin irritation and sensitization potential of a moisturizer containing 0.15% potassium sorbate. Occlusive induction patches were applied for 24 h three times a week for a total of 10 applications. These patches were applied to the upper backs or inner upper arms of 202–205 subjects, and reactions were scored on a scale of 0 to 4+ , 24 or 48 h later. After a 3 week rest period, 48 h occlusive challenge patches were applied to the original sites and to previously untreated sites. These reactions were scored 48 and 72 h after patch application. There were nine 1+ reactions and two 2+ reactions to induction patches; these reactions were judged irritant in nature and were not considered clinically significant. There was one 1+ reaction to the challenge patches applied to previously untreated sites at the 48 h reading, three 1+ reactions to the challenge patches applied to the original sites at the 72 h reading, and three 1+ reactions to the challenge patches applied to the previously untreated sites at the 72 h reading. The moisturizer appeared not to be a primary irritant or an allergic contact sensitizer.[174]

A facial scrub containing 0.1% potassium sorbate was diluted 1:100 in deionized water and was evaluated for skin irritation and sensitization in an

RIPT with 53 subjects. Eight semiocclusive induction patches, each 24 h in duration, were applied to the upper arm of each of the subjects over a 2 week period. Reactions were graded at patch removal. After a 2 week nontreatment period, a semiocclusive challenge patch was applied to a previously untreated site for 24 h. Reactions were graded at patch removal and 24, 48, and 72 h later. Seven minimal erythema reactions were observed during induction, and no reactions at any challenge reading. The facial scrub was a very mild cumulative irritant but was not a primary irritant. The formulation did not produce sensitization in any of the subjects tested.[175]

The skin irritation and sensitization of a facial scrub containing 0.1% potassium sorbate were evaluated in an RIPT using 53 panelists. The formulation was diluted 1:100 in deionized water. Four semiocclusive induction patches were applied to the upper arm of each subject for 24 h each week for 2 weeks. Reactions were scored at patch removal. A 24 h semiocclusive challenge patch was applied to a previously untreated site 3 weeks later. This reaction was evaluated at patch removal and 24, 48, and 72 h later. There were two minimal erythema reactions during induction and no other reactions. The facial scrub was a very mild cumulative irritant, but it was not a primary irritant. It did not produce sensitization in any of the subjects tested.[176]

An RIPT was conducted using 56 panelists and a facial scrub containing 0.1% potassium sorbate. The formulation was diluted 1:100 by weight with distilled water for the study. Eight 24 h semiocclusive induction patches were applied over a 2 week period to the lateral upper arm of each subject. Reactions were scored at patch removal. After an approximately 2 week rest period, a 24 h semiocclusive challenge patch was applied to a previously untreated site. Reactions to the challenge patch were graded at patch removal and 24 and 48 h later. Two slight, transient, questionable erythema reactions were observed during induction. No other reactions were observed during induction or challenge. The facial scrub did not induce dermal irritation or sensitization.[177]

The skin irritation and sensitization potential of a facial scrub containing 0.1% potassium sorbate was evaluated in an RIPT with 47 panelists. The formulation was diluted 1:100 in distilled water. Eight 24 h semiocclusive induction patches were applied to the lateral aspect of the upper arms of the subjects over a 2 week period, and reactions were scored on a scale of 0–5 at patch removal. After a 2 week rest period, a 24 h semiocclusive challenge patch was applied, and reactions were scored at patch removal and 24 and 48 h later. No reactions greater than 2 (moderate erythema) were observed during the induction period, and no reactions at challenge were indicative of sensitization.[178]

SUMMARY

Sorbic acid is a straight-chain monocarboxylic acid, also known as 2,4-hexadienoic acid. It is a white crystalline powder soluble in alcohol and ether but only slightly soluble in water. Potassium sorbate is the potassium salt of

sorbic acid and is a white crystalline powder or white granules or pellets freely soluble in alcohol and water.

Sorbic acid occurs naturally as the lactone, parasorbic acid, in berries of the mountain ash, *Sorbus aucuparia* L., Rosaceae. The sorbic acid used in cosmetics is synthesized by various commercial processes. Potassium sorbate is prepared by reacting sorbic acid with an equimolar portion of potassium hydroxide.

Solutions of sorbic acid are subject to autoxidation and atmospheric oxidation. Both the temperature and the type of container have also affected the breakdown of sorbic acid.

Sorbic acid and potassium sorbate are analyzed primarily by chromatographic techniques. Several analytic studies have been conducted to determine whether sorbic acid was contaminated with its isomer parasorbic acid, a suspected carcinogen. No traces of parasorbic acid were found (tests sensitive down to a concentration of 0.5 mg/kg).

Sorbic acid and potassium sorbate are used in cosmetics and toiletries as preservatives and antimicrobials generally at concentrations of $\leq 1\%$. According to the data voluntarily reported to the FDA through 1986, sorbic acid and potassium sorbate were used in 445 and 117 cosmetic formulations, respectively. These ingredients are primarily used in facial and eye makeup and skin care and hair preparations.

Sorbic acid and potassium sorbate are generally recognized as safe (GRAS) direct food additives. They are used as preservatives at low concentrations ($<0.01-1.40\%$) in many foods. Potassium sorbate is also a GRAS indirect food additive as it migrates to food from paper products used in packaging.

The Joint Food and Agricultural Organization–World Health Organization Expert Committee on food additives has estimated the acceptable daily intake of sorbic acid and its salts (expressed as sorbic acid) as 25 mg/kg body weight.

Sorbic acid and potassium sorbate are used as preservatives in a variety of pharmaceuticals. These chemicals also have various industrial uses.

Sorbic acid and potassium sorbate have a broad spectrum of fungistatic activity but are less active against bacteria. Their antimicrobial activity depends upon the amount of undissociated acid, which in turn is determined primarily by the dissociation constant and the pH of the system. Optimum effectiveness is attained at pH values up to 6.5. The mechanism by which sorbic acid inhibits microorganisms is not yet understood.

In biochemical studies, sorbic acid did not affect the protein content or the biosynthesis of RNA and DNA in mouse embryo fibroblast cells. Sorbic acid did not significantly affect biochemical parameters when administered orally to rats. Sorbic acid did inhibit both peroxidase and oxidase activity in cabbage and reduced the rate of aberrant mitosis caused by irradiation in onion root tips. Sorbic acid also affected the ultrastructural organization of yeast cells and effectively reduced the viability of the 14 human RNA and DNA enveloped viruses when combined with monolaurin.

The results of metabolic studies were that sorbic acid was qualitatively metabolized in the same manner as the saturated or singly unsaturated fatty acids of the same C-atom number. Under normal conditions, sorbic acid was almost completely oxidized to carbon dioxide and water.

Sorbic acid and potassium sorbate were practically nontoxic to rats and mice in acute oral toxicity studies. Intraperitoneal LD_{50} values in mice were 2800 and 2820 mg/kg for sorbic acid and 1300 mg/kg for potassium sorbate. Sorbic acid had a subcutaneous LD_{50} of 2820 mg/kg in mice. Formulations containing up to 5% sorbic acid administered orally at doses up to 7.0 g/kg were not toxic to rats.

In short-term to subchronic oral studies, sorbic acid did not produce significant adverse effects in rats, mice, or dogs at concentrations up to 10% (of the diet). Potassium sorbate was practically nontoxic in rats and dogs at concentrations up to 10 and 2%, respectively. Application to rabbit skin of formulations containing 0.5% sorbic acid or 0.15% potassium sorbate over short-term and subchronic periods, respectively, resulted in dermatitis.

Chronic oral studies in which sorbic acid was administered to mice and rats at concentrations up to 10% have established absolute no-effect levels of 1.5% in rats and 1.0% in mice. No significant toxic effects were noted in rats at a 5% concentration in the diet. Sorbic acid had no additive toxicity in rats when administered with benzoic acid. Adulteration of a 1.2% sorbic acid diet with 1000 ppm parasorbic acid produced not adverse effects in rats or mice administered these diets for 2 years and 80 weeks, respectively.

Sorbic acid (in petrolatum) and potassium sorbate (as aqueous solution) at concentrations of 1, 5, and 10% were practically nonirritating and nonirritating, respectively, to the rabbit eye. Formulations containing 0.1% sorbic acid or 0.15% potassium sorbate were nonirritating to the rabbit eye.

Sorbic acid (in petrolatum) and potassium sorbate (as aqueous solution) at concentrations of 1, 5, and 10% were slightly irritating and nonirritating, respectively, when evaluated using a modified Draize irritation test. In another Draize test, sorbic acid was classified a severe irritant after application of 1 mg under occlusive conditions. A 1% aqueous potassium sorbate solution was practically nonirritating to rabbit skin. No irritation or adverse effects were produced in rats by daily application, 6 days/week for 3 weeks, of 5% sorbic acid in a lanoline-petrolatum paste. A formulation containing 0.5% sorbic acid was not irritating to rabbit skin.

In a guinea pig sensitization test, sorbic acid produced four positive reactions to the first intradermal challenge although the reactions of all 20 guinea pigs were negative at the second epidermal challenge.

The results of studies of the potential formation of mutagenic or DNA-damaging reaction products in the presence of sorbic acid or potassium sorbate and sodium nitrite have varied. Sorbic acid and sodium nitrite, when reacted under acidic conditions, produced ethylnitrolic acid, considered by some to be mutagenic. Other reaction products, only partially identified, were both mutagenic and nonmutagenic. On the other hand, sorbic acid, in that it reacts readily with nitrite, has inhibited the formation of some carcinogenic nitrosamines from amines and nitrites.

Sorbic acid and potassium sorbate have been extensively tested for mutagenic effects using the Ames test, genetic recombination tests, reversion assays, *rec* assays, and tests for chromosomal aberrations, sister chromatid exchanges, and gene mutations. These tests have been conducted in various systems: *B. subtilis* strains 3308, 112, 566, and 168; *S. typhimurium* strains

TA-98, TA-100, TA-1535, TA-1537, and TA-1538; *S. cerevisiae* strain D4; silkworms; Chinese hamster cells; and rat bone marrow cells. The results have been both positive and negative.

A series of mutagenicity tests has also been used to evaluate the intestinal contents and urine of mice fed sorbic acid and potassium sorbate for periods of up to 15 months. The concentration of glutathione in the liver and the relative body weight–liver weight ratios were evaluated as well. Acidic components of the intestinal contents and the urine of those mice administered a diet containing 15% sorbic acid were mutagenic in *S. typhimurium* strain TA-98, but only with metabolic activation. The concentration of lipid peroxide in the livers increased almost linearly with the concentration of sorbic acid in the diet. Sorbic acid decreased hepatic glutathione concentrations and increased the relative body weight–liver weight ratios in these mice.

The oral administration of potassium sorbate as 0.1% of the diet or 0.3% of the drinking water for up to 100 weeks produced no neoplasms in rats. No carcinogenic effect was demonstrated by sorbic acid in rats or mice fed diets containing up to 10% sorbic acid for periods of 2 years and 80 weeks, respectively. A diet containing up to 15% sorbic acid has been reported to have a carcinogenic effect in the liver of mice after 88 weeks' administration.

No teratogenic effects have been observed in pregnant mice and rats administered potassium sorbate at doses of up to 460 and 340 mg/kg body weight, respectively.

In three repeat insult patch tests using a total of 478 subjects, sorbic acid had overall sensitization rates of 0, 0.33, and 0.8%. All the subjects sensitized were inducted with 20% sorbic acid and challenged with 5% sorbic acid. Formulations containing up to 0.5% sorbic acid or 0.15% potassium sorbate were not cumulative irritants or were very mild cumulative irritants. They were not primary irritants and were not sensitizers. A formulation containing 0.01% sorbic acid was not a photosensitizer.

CONCLUSION

On the basis of the data included in this report, the CIR Expert Panel concludes that sorbic acid and potassium sorbate are safe as cosmetic ingredients in the present practices of use and concentration.

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Amended Safety Assessment of Tall Oil Acid, Sodium Tallate, Potassium Tallate, and Ammonium Tallate

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Abstract

Tall oil acid is a mixture of oleic and linoleic acids (fatty acids) and rosin acids derived from tall oil, a by-product of pulp from resinous woods, used in cosmetic products as a surfactant at concentrations up to 8%. Ammonium, potassium, and sodium salts also are listed as cosmetic ingredients. In addition to the studies summarized in this report, extensive toxicity, genotoxicity, and carcinogenicity studies in animals are available for oleic, lauric, palmitic, myristic, and stearic fatty acids as published earlier by the Cosmetic Ingredient Review (CIR). These data may be extrapolated to tall oil acid and its salts. There are no reports of current uses or use concentration data for ammonium tallate, nor are use concentration data available for the other salts. The CIR Expert Panel found tall oil acid, ammonium tallate, potassium tallate, and sodium tallate to be safe cosmetic ingredients in the given practices of use and concentration.

Keywords

safety, cosmetics, Tall Oil Acid, tallates

The Cosmetic Ingredient Review (CIR) Expert Panel previously evaluated the safety of tall oil acid in cosmetics, finding it to be safe for use in cosmetic products.[1] The Expert Panel considered that the available data in that safety assessment were sufficient to also support the safety of the salts of tall oil acid that are used in cosmetics. This report, therefore, is an amended safety assessment of tall oil acid that includes sodium tallate, potassium tallate, and ammonium tallate as used in cosmetics. This safety assessment includes new data on tall oil acid, along with all available data addressing the safety of the sodium, potassium, and ammonium salts.

Because tall oil contains fatty acids, the CIR Expert Panel also considered relevant its earlier safety assessment of oleic, palmitic, myristic, and stearic acids and the finding that these fatty acids were safe for use in cosmetics.[2] In 2006, the Expert Panel considered all newly available data on these fatty acids and reaffirmed that conclusion.[3]

Chemistry

Tall Oil Acid

According to the *International Cosmetic Ingredient Dictionary and Handbook*, tall oil acid (CAS No. 61790-12-3) is the mixture of rosin acids and fatty acids recovered from the hydrolysis of tall oil (Table 1).[4]

Some technical and other names and trade names for tall oil acid given in the *International Cosmetic Ingredient Dictionary and Handbook* include the following[4]:

Technical and other names

- Acids, tall oil
- Fatty acids, tall oil

Trade names

- Actinol EPG
- Actinol FA-1
- Actinol FA-2
- Pamak 4

Sodium Tallate

According to the *International Cosmetic Ingredient Dictionary and Handbook*, sodium tallate (CAS No. 61790-45-2) is the sodium salt of tall oil acid (qv).[4]

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Table 1. Definitions and Functions of Tall Oil Acid and Its Salts as Given in the *International Cosmetic Ingredient Dictionary and Handbook*[4]

Ingredient	Definition	Function(s)
Tall oil acid	Mixture of rosin acids recovered from the hydrolysis of tall oil	Surfactants, cleansing agents Surfactants, emulsifying agents
Ammonium tallate	The ammonium salt of tall oil acid	Surfactants, cleansing agent
Potassium tallate	The potassium salt of tall oil acid	Surfactants, cleansing agents Surfactants, emulsifying agents
Sodium tallate	The sodium salt of tall oil acid	Surfactants, cleansing agents Surfactants, emulsifying agents

Potassium Tallate

According to the *International Cosmetic Ingredient Dictionary and Handbook*, potassium tallate (CAS No. 61790-44-1) is the potassium salt of tall oil acid (qv).[4] A synonym for potassium tallate is tall oil acid, potassium salt. A trade name mixture is Akypogene ZA 97 SP.

The Environmental Protection Agency reported the following synonyms for this chemical[5]:

- Fatty acids, tall-oil, potassium salts
- Tall oil, potassium salt
- Potassium soap of tall oil fatty acids (C18)
- Tall oil acids, potassium salt
- Tall oil fatty acid potassium soap

Ammonium Tallate

According to the *International Cosmetic Ingredient Dictionary and Handbook*, ammonium tallate (CAS No. 68132-50-3) is the ammonium salt of tall oil acid (qv).[4] Some technical and other names for ammonium tallate include fatty acids, tall oil, ammonium salts and tall oil fatty acids, and ammonium salts.

Physical and Chemical Properties and Composition

Tall oil acid, as used in cosmetic products, is a clear, pale-yellow liquid with a characteristic fatty odor and consists mainly of oleic acid (40%), linoleic acid (38%), other fatty acids (13%), and rosin acids (0.6%). Tall oil acid is soluble in most polar and nonpolar organic solvents, but it is insoluble in water.[6]

The chemical and physical properties of tall oil acid, sodium tallate, potassium tallate, and ammonium tallate are presented in Table 2.

Dybdahl[7] reported that the octanol/water partition coefficient (log P_{ow}) for fatty acids in tall oil acid ranged from 4.4 to 8.3 at pH 2 and from 3.6 to 7.4 at pH 7.5. A mixture of 7 materials with known log P_{ow} values was used for reference.[7]

According to Whitman,[8] tall oil acid is composed mainly of palmitic acid, stearic acid, oleic acid, and linoleic acid, which are all natural products derived from the pulping of pine trees. All of these fatty acids are labeled "generally recognized as safe" (GRAS) food additives by the Food and Drug Administration (FDA).[9]

Table 2. Physical and Chemical Properties and Chemical Class of Tall Oil Acid, Sodium Tallate, Potassium Tallate, and Ammonium Tallate

Ingredient and Properties	Value/Description
Tall oil acid	
Chemical class[4]	Fatty acids[4]
Description	Pale color, oily liquid[22]
Iodine value (Wijs)[31]	130
Saponification value[31]	200
Rosin acids (%)[31]	0.5
Unsaponifiables (%)[31]	0.5
Color (Gardner)[31]	1
Flash point, Cleveland Open Cup test[31]	400 °F
Specific gravity at 25°C	0.897
Viscosity (cps, at 25°C)[31]	20
Sodium tallate	
Chemical class[4]	Soaps[4]
Potassium tallate	
Chemical class[4]	Soaps[4]
Ammonium tallate	
Chemical class[4]	Soaps[4]

Taylor and King[10] reported that tall oil is a dark, odorous liquid.[10] Fatty acids, rosin acids, sterols, and other compounds mainly make up this resinous material. The chemical composition of tall oil varies with the age, species, and geographical location of the source coniferous trees. The resin acids are diterpene carboxylic acids based on an alkyl-substituted perhydrophenanthrene ring structure, and the fatty acids are predominantly 18-carbon, straight-chain mono-unsaturated or di-unsaturated fatty acids.

The Pine Chemicals Association[11] reported that the following chemicals are collectively known as tall oil fatty acids and tall oil fatty acid salts:

- CAS No. 61790-12-3, fatty acids, tall-oil (tall oil acid)
- CAS No. 65997-03-7, fatty acids, tall oil, low boiling
- CAS No. 68955-98-6, fatty acids, C16-C18 and C18 unsaturated, branched and linear
- CAS No. 68201-37-6, octadecanoic acid, branched and linear
- CAS No. 61790-44-1, fatty acids, tall oil, potassium salts (potassium tallate)

Table 3. Composition of Typical Tall Oil Fatty Acid[11]

Common Name	Chemical Structure	Percentage of Composition
Palmitic acid	$CH_3(CH_2)_{14}COOH$	1
Stearic acid	$CH_3(CH_2)_{16}COOH$	2
Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	48
Linoleic acid	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	35
Conjugated linoleic acid[a]	$CH_3(CH_2)_xCH{=}CHCH{=}CH{-}(CH_2)_yCOOH$	7
Other acids[b]	—	4
Unsaponifiable matter	—	2

[a] $x + y = 12$; x usually 4 or 5; y usually 7 or 8.
[b] Other acids include 5,9,12-octadecatrienoic acid; linoleic acid; 5,11,14-eicosatrenoic acid; cis,cis-5,9-octadecadienoic acid; eicosadienoic acid; elaidic acid; cis-11 octadecanoic acid; C-20, C-22, C-24 saturated fatty acids.

- CAS No. 61790-45-2, fatty acids, tall oil, sodium salts (sodium tallate)

Tall oil fatty acids and their derivatives are composed of a complex mixture and are often difficult to characterize. Their composition is variable. The melting point cannot be measured because these substances either will not give a sharp melting point when heated or will decompose before they melt. Tall oil fatty acid and all other nonsalts in this category are liquids at room temperature. Boiling points cannot be determined because these substances will decompose before they boil. Under ambient conditions, the vapor pressure of these chemicals is essentially zero, and experimental measurement is not possible. The partition coefficients can yield a range of values representing the various components rather than a single value representing the mixture.

The composition of a given tall oil fatty acid depends on the origin of the tall oil and the fractionation conditions used for its production. The composition of a typical tall oil fatty acid provided by Pine Chemicals Association[11] is shown in Table 3.

According to Waylan et al,[12] modified tall oil (from which tall oil acid is derived) has a high content of conjugated linoleic acid (67.4%) from further processing the fatty acid portion of tall oil.

Use

Cosmetics

Balsam and Sagarin described tall oil acid as a substitute for oleic acid or other fatty acids in formulating cosmetics.[13] According to these authors, tall oil acid is converted to a soap by reaction with bases and then used primarily as a conditioner or emulsifier in hair dyes and bleaches.

The Pine Chemicals Association stated that tall oil fatty acid salts are widely used as surfactants in liquid soaps.[11]

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, tall oil acid functions as a surfactant (cleansing agent and emulsifying agent) in cosmetics.[4] The tall oil acid salts also function as surfactants (cleansing agents and emulsifying agents) in cosmetics.

Manufacturers report current uses of cosmetic ingredients, as a function of product type, to the US FDA under the Voluntary Cosmetic Registration Program (VCRP). Use concentration data are obtained from a survey of the industry done by the industry trade association, formerly the Cosmetic, Toiletry, and Fragrance Association (CTFA) and now the Personal Care Products Council.

Table 4 presents the current product uses reported under the VCRP for tall oil acid, sodium tallate, and potassium tallate in cosmetics along with the total number of products in each category.[14] For example, of a total of 135 shaving cream products, only 1 use of tall oil acid was reported. Clearly, most shaving cream products do not contain tall oil acid. The industry survey done by CTFA reported use concentrations for tall oil acid from 0.6% to 8.0%.[15] No use concentration data were reported for the tall oil acid salts, and no product uses for ammonium tallate were reported under the VCRP.[14-16]

Noncosmetic

As included in the Code of Federal Regulations (CFR), the FDA has approved tall oil acid for use as an indirect food additive and defoaming agent used in the manufacture of paper and paperboard products and coatings of articles intended for use in packaging, transporting, or holding food (21CFR parts 176.200, 176.210, and 720.4).

The use of tall oil acid in preparations of edible oils and edible fat compositions has been patented.[17,18] Tall oil acid is used as a raw material for protective coatings, particularly in alkyd resins, soaps, detergents, and disinfectants.[19]

Pearl[20] stated that large quantities of tall oil acid are used as intermediate chemicals; they are further processed or modified chemically before being incorporated into a product or used in production.

Tall oil acid and its derivatives are used in the manufacturing of rubber, paper, soaps and detergents, printing inks, metalworking fluids, corrosion inhibitors, and plasticizers.[11,21,22]

General Biology

Absorption, Distribution, Metabolism, Excretion

No data are available on absorption, distribution, metabolism, and excretion.

Table 4. Current Uses and Concentrations of Tall Oil Acid, Sodium Tallate, and Potassium Tallate in Cosmetics

Product Category	Ingredient Uses (Total No. of Products in Category)[14]	Use Concentrations, %[15]
Tall oil acid		
Hair coloring preparations		
Hair dyes	NR (1600)[a]	0.6 (0.3 after dilution)
Personal hygiene products		
Other personal hygiene products	3 (390)	NR[a]
Shaving preparations		
Shaving cream	1(135)	NR[a]
Skin care preparations		
Skin cleansing creams, lotions, liquids, and pads	2 (1009)	8
Total uses/ranges for tall oil acid	6	8
Sodium tallate		
Personal hygiene products		
Other personal hygiene products	6 (390)	NR[a]
Total uses/ranges for sodium tallate	6	NR
Potassium tallate		
Personal hygiene products		
Other personal hygiene products	9 (390)	NR[a]
Total uses/ranges for potassium tallate	9	NR

NR, data not reported.
[a] In some cases, ingredient uses were not reported to FDA in the voluntary industry product survey program, but concentrations were provided. In other cases, the uses were reported, but no concentration was provided.

Animal Toxicology

Acute Oral Toxicity

Mallory[23] reported on an acute oral toxicity study in Sprague-Dawley rats (10 males, 10 females). Each animal received a single oral gavage dose of 10 000 mg/kg tall oil acid and were observed for 14 days. Parameters evaluated included clinical signs, mortality, body weight, and gross pathology. None of the animals died. One hour after dosing, piloerection was observed in 1 male, and abnormal stance was observed in 1 male and 1 female. By 4 hours, these effects had resolved. No body weight effects were observed. Gross necropsy revealed no treatment-related effects. The acute oral median lethal dose of tall oil acid was greater than 10 000 mg/kg.

Short-Term Oral Toxicity

An experiment was conducted to study the effect of tall oil acid distillate on the growth of rats. The distillate used in this study was described by the authors as containing 1.8% to 2.2% rosin and 2.8% to 3.2% unsaponifiable matter. It was composed of 42.8% linoleic acid, 38.8% oleic acid, and 17.4% other fatty acids. Male weanling Sprague-Dawley rats, 10 per group and weighing 40 to 60 g, were fed diets containing 15%, 30%, and 60% of the total calories as tall oil acid distillate for 4 weeks. Control groups received diets containing the same percentages of soybean oil. Feed consumption and body weight were measured at least every other day. The growth rate of animals fed a diet with 15% tall oil acid distillate did not differ significantly from that of the control group. Animals in the group receiving 30% of their calories from tall oil acid distillate had a significantly lower growth rate than did the controls, and their feed consumption was slightly more than half that of the control group. One animal in the 15% group died during the experiment. All 10 of the animals in the 60% group died in the first 4 days of the start of the experiment. The author concluded that there was "a growth-retarding or possibly a toxic factor in the tall oil fatty acid distillate."[24]

Subchronic Oral Toxicity Study

Fancher[25] reported on a 90-day subchronic oral toxicity of tall oil fatty acid in albino rats. Tall oil fatty acid was administered to Charles River rats (10 males, 10 females) in the diet at concentrations of 0%, 5%, 10%, or 25% for 90 days. The approximate doses were 0, 2500, 5000, or 12 500 mg/kg/d. Two control rats died during blood sampling. No other deaths occurred and no clinical signs were observed. Body weight and body weight gain were not affected by treatment, but food consumption was slightly decreased at 10% and 25%. No changes in hematology, clinical chemistry, or urinalysis parameters occurred at any dose. At gross pathology, no treatment-related effects were noted at any dose. No consistent organ weight changes and no histopathological effects were reported. Based on these data, the no observed effect level (NOEL) was 5% (approximately 2500 mg/kg/d).

Chronic Toxicity and Irritation

Data on chronic toxicity, ocular irritation, mucosal irritation, and dermal irritation were not available.

Reproductive and Developmental Toxicity

Tall oil acid had no effects when tested for reproductive and developmental toxicity in Sprague-Dawley rats in a full 2-generation study.[26] The test material was administered in the diet at concentrations of 0%, 5%, or 10% to 30 females per group and 15 males per group. The approximate doses were 0, 2500, and 5000 mg/kg/d. Males and females (F0) began treatment at 80 days of age and were mated at 100 days of age. Treatment of the F0 animals continued through the weaning of the first generation (F1). After weaning, the F1 males and females were maintained on the treatment diet. At 100 days of age, they were mated and allowed to deliver pups (F2).

There were no treatment-related effects on reproductive performance or on any parameter measured in either the F1 or F2 pups. No treatment-related changes in fertility, viability, lactation, or gestation indices were observed. Hematology, clinical chemistry, and urinalysis parameters were similarly unchanged, and there were no developmental effects in any F1 or F2 offspring. Tall oil acid did not alter or otherwise affect the reproduction or development of rats in this study at doses as high as 10% (approximately 5000 mg/kg/d).[26]

A 2-generation reproduction study was conducted in which tall oil acid was fed to Charles River CD Sprague-Dawley rats. The rats were classified into 5 groups each consisting of 15 males and 30 females. The experimental groups included negative control, 5% tall oil acid, 10% tall oil acid, 5% oleic acid, and 10% oleic acid. Tall oil acid used in this study was only described as a clear amber-colored liquid with a heavy odor similar to a vegetable oil. The rats (the F0 generation) were fed the test diets for approximately 3 weeks and were then put in mating cages with 1 male and 2 females per cage. The F1 litter was weaned onto the test chemical diets, and 20 female and 20 male rats were carried on to sexual maturity, having been fed the test diet for approximately 180 days, for each of the 5 test groups. These rats were then arranged in mating groups, and the following parameters were measured for the parents and offspring: mating behavior, number of pregnant dams, total number of pups (live born, stillborn, number discarded on day 4, and number alive on day 21), average number of pups per litter (born, day 4, and weaned), and the average weaning weight of the pups. The fertility, viability, lactation, and gestation indices were computed. Clinical chemistry determinations were made for 5 male and 5 female rats from each test group of the F1 generation. Rats from each test group of the F1 generation, 10 males and 10 females, were examined for any abnormalities occurring in hematologic and urinalysis values and organ weights. All rats, whether they died or were killed, were necropsied. No treatment-related effects were found. Several animals had lesions of chronic respiratory and renal diseases, which are endemic in this strain of rat.[27]

Genotoxicity

Godek[28] reported that tall oil fatty acid was not mutagenic in the Ames assay. It was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations of 100, 1000, and 10 000 μg per plate, with and without metabolic activation. Information regarding the controls was not provided. No increases in mutation frequency were reported at any concentration, with or without metabolic activation. Tall oil acid was not mutagenic in this assay, and there are no in vivo genotoxicity data using mammalian cells.

Carcinogenicity

No carcinogenicity studies using these cosmetic ingredients were available.

Clinical Assessment of Safety

Dermal Irritation

CTFA[29] reported that tall oil acid in a liquid soap was tested for dermal irritancy potential. The soap contained 12% tall oil acid and was tested at a concentration of 25% in water for a total tested concentration of 3% tall oil acid. The controlled use study was performed according to the CTFA testing guidelines.[30] This type of study is expected to detect adverse reactions under the conditions of expected normal use. The hands and fingers of 54 subjects were examined every week during the 4 weeks of in-use study. No positive reactions occurred during the test, and the soap was nonirritating.

A prophetic patch test also was conducted with a liquid soap containing 12% tall oil acid. The formulation was tested undiluted. The 100 subjects received 2 patches 10 to 14 days apart; both open and closed patches were used. None of the subjects had positive reactions at any of the patch sites. The soap formulation was nonirritating.[29]

Dermal Sensitization and Photosensitization

A liquid soap containing 12% tall oil acid was tested in a repeat-insult patch test. The soap formulation was tested undiluted. A total of 11 patches were applied to the skin of 50 panelists. It was not stated how long the patches stayed in contact with the skin or at what interval the patches were applied. The subjects were exposed to ultraviolet light, of an unspecified wavelength, at patch numbers 1, 4, 7, 10, and 11. No positive reactions were observed at open or closed patch sites. The soap formulation was determined to be nonsensitizing and nonphotosensitizing.[29]

Summary

Tall oil acid is a mixture of oleic and linoleic fatty acids and rosin acids derived from the hydrolysis of tall oil, a by-product of pulp from resinous woods (mainly pine). Safety assessments of the oleic and linoleic acids previously were reported. The salts of tall oil acid also were included in this

safety assessment, including sodium tallate, potassium tallate, and ammonium tallate.

Tall oil acid was reported in 2006 to be used in a small number of formulations at concentrations ranging from 0.6% to 8%. Similar numbers of uses were reported for sodium and potassium tallate, although no use concentration data were available. Ammonium tallate was not reported to be used.

When fed to rats as 15% of total caloric intake, tall oil acid was nontoxic. At 30% and 60% of total caloric intake, tall oil acid had a growth-retarding or toxic effect. Growth was reduced in rats fed tall oil acid at 6% of their diet by mass, equal to 15% of the total calories. The subchronic oral NOEL was 5%.

No treatment-related effects were observed in rats used in a 2-generation feeding study. The rats were fed diets containing 5% and 10% tall oil acid.

Tall oil acid was determined to be nonmutagenic in the Ames assay when tested at concentrations of 100, 1000, and 10 000 μg/plate.

Liquid soap formulations containing up to 12% tall oil acid did not cause dermal irritation, sensitization, or photosensitization in human subjects in a repeat insult patch test.

Discussion

The CIR Expert Panel recognized that there are limited animal and human toxicity data and dermal irritation/sensitization studies for tall oil acid. Tall oil acid is, however, known to be composed of fatty acids for which safety test data were available.

When considered with the subchronic and chronic oral toxicity, reproductive and developmental toxicity, genotoxicity, carcinogenicity, and photosensitization studies available for oleic acid, lauric acid, palmitic acid, myristic acid, and stearic acid, the available data for tall oil acid itself are a sufficient basis for reaching a conclusion regarding tall oil acid. It is the experience of the panel in its review of fatty acids of varying carbon chain lengths that there is little difference in toxicity.

The panel also considered that there is little difference between members of this family of salts of tall oil acid. The salts are expected to be dissociated in any product formulation independent of whether the salt is sodium, potassium, or ammonium. Accordingly, the available data for tall oil acid are considered supportive of the safety of the entire group as used in cosmetics.

The CIR Expert Panel recognizes that there are data gaps regarding use and concentration of these ingredients. However, the overall information available on the types of products in which these ingredients are used and at what concentrations indicates a pattern of use, which was considered by the Expert Panel in assessing safety.

Conclusion

The CIR Expert Panel concluded that tall oil acid, sodium tallate, potassium tallate, and ammonium tallate are safe as cosmetic ingredients in the practices of use and concentration as described in this safety assessment. In the case that ingredients in this group not in current use are used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

Authors' Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th Street, Suite 412, Washington, DC 20036, USA.

Declaration of Conflicting Interests

No potential conflict of interest relevant to this article was reported. F. Alan Andersen and Valerie Robinson are employed by the Cosmetic Ingredient Review.

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