FINAL REPORT OF THE SAFETY ASSESSMENT FOR AVOCADO OIL

Toxicological test data from human and animal studies using Avocado Oil, as well as cosmetic formulations containing Avocado Oil are reviewed. Skin irritation and sensitization results, as well as general toxicity test data in animals and humans, are presented in support of the conclusion that Avocado Oil, as currently used in cosmetic formulations, is safe.

CHEMICAL AND PHYSICAL PROPERTIES

General

The edible portion of the avocado fruit, *Persea americana,* is rich in oleic acid with lesser amounts of palmitic, linoleic, and palmitoleic acids; it contains only trace amounts of stearic, capric, lauric, myristic, and arachidic acids. The oil, a mixture of these and other fatty substances, obtained by expressing the dehydrated sliced fruit, is a clear, yellow-green liquid. Though it can be decolorized, the oil is used mostly in its natural color. It has a pleasant taste and odor, and does not readily become rancid. It is discolored by heating (Yazicioglu, 1951; Estrin, Ed., 1974; Biale and Young, 1971; Franzke and Henning, 1956).

Not all the varieties of avocado contain the same proportion of fat; in fact, the same variety grown in different areas does not contain the same proportion of fat. The West Indian fruit has from 4 to 7%, the Guatemalan fruit 10 to 13%, and the Mexican (Fuerte), 10 to 15% in Mexico and 15 to 32% in California. (An additional variable is the degree of ripeness of the fruit when dried and expressed.) The riper fruit contains the largest amount of fat (Biale and Young, 1971; Appleman and Noda, 1941). For these reasons the quantities of oil in the fruit flesh may vary from 8 to 32% (Biale and Young, 1971). This being the case, it is not possible to be precise about the proportions of the fatty acids present, or about any of the other chemical properties.

Lipids

The various classes of lipids in the oil have been analyzed by gas chromatography and thin-layer chromatography. As shown in Table 1, the fatty acid composition of the lipids closely follows that of triglycerides, which comprise the major part of the lipid (86%) (Biale and Young, 1971). All of the lipid fractions are low in both stearic acid and diglyceride I; the monoglyceride fractions are more saturated than the triglyceride; diglyceride II is similar to the triglyceride fraction. Glycolipids I and II and the phospholipid fraction are very different from all the other fractions and are comprised of higher proportions of unsaturated acids; the phospholipid fraction has more stearic acid than any of the other fractions, and about 9% of the hydrolyzed fatty acids contains one or more unidentified acids (Biale and Young, 1971).

Properties

The principal constituents of the nonsaponifiable fraction are squalene and saturated hydrocarbons (C_{12}, C_{20}, C_{22}), a complex mixture of aliphatic alcohols, sitosterol, methylsterols, and cholesterol (Estrin, Ed., 1974; Führer, 1953; Itoh *et al.*, 1976; Paquot and Tassel, 1966).

TABLE 1. Fatty Acid Composition of Avocado Oil (Biale and Young, 1971)

	Palmitic %	Palmitoleic %	Stearic %	Oleic %	Linoleic %	Linolenic %	Arachidic %	Unknown %
Free fatty Acid	20.3	9.7	0.4	43.7	22.5	3.0	...	0.4
Triglyceride	25.4	7.0	0.5	54.3	12.3	...	0.5	...
DiglycerideI	15.0	9.5	...	45.0	28.0	3.0	...	...
DiglycerideII	18.4	3.9	0.7	64.8	12.2	...	...	...
Monoglyceride	17.1	7.2	2.7	43.2	24.3	1.0	0.9	3.6
GlycolipidI	6.7	2.5	1.6	13.1	76.1	...	...	...
GlycolipidII	3.8	2.2	1.2	12.8	74.1	6.0	...	...
Phospholipid	16.9	4.4	3.3	20.5	36.1	9.8	...	9.0

Analytical Methods

These are described in Itoh *et al.* (1976) and Paquot and Tassel (1966). The techniques involve gas chromatography and mass spectroscopy.

USE

As shown in Table 2, a total of 240 products contain Avocado Oil in concentrations of $\leq$ 0.1 to 50%. The oil is used in many different products: bath oils, eye makeup, lipstick, makeup bases, cleansing cream, hair conditioners, suntan lotions, skin care, moisturizers, and others. Contact with body orifices is unavoidable because numerous products are used around the mouth and eyes, or as bath additives. Some of the products, e.g., the lipstick formulations, may be used more than once a day (FDA, 1976).

BIOLOGICAL PROPERTIES

General Effects

The oil has few active biological principles. Poor in vitamin content, it has no hormonal properties and apparently no medicinal value other than its

TABLE 2. Product Formulation Data (FDA, 1976)

Ingredient	Cosmetic Product Type	Concentration (%)	Number of Product Formulations
Avocado Oil	Bath oils, tablets, and salts	>0.1 to 1	1
	Other bath preparations	>1 to 5	1
	Eyeliner	>0.1 to 1	3
	Eye shadow	>0.1 to 1	1
	Eye lotion	≤0.1	1
	Eye makeup remover	>0.1 to 1	1
	Other eye makeup preparation	≤0.1	1
	Hair conditioners	>1 to 5	3
		>0.1 to 1	4
	Permanent waves	≤0.1	2
	Shampoos (noncoloring)	>1 to 5	1
		≤0.1	2
	Wave sets	≤0.1	1
	Foundations	>1 to 5	2
	Lipstick	>25 to 50	6
		>10 to 25	42
		> 5 to 10	106
		> 1 to 5	2
		≤0.1	1
	Makeup bases	>0.1 to 1	2
	Rouges	>1 to 5	1
	Other makeup preparations	>1 to 5	6
	Cleansing (cold creams, cleansing lotions, liquids, and pads)	>5 to 10	1
		>1 to 5	4
		>0.1 to 1	4
	Face, body, and hands (excluding shaving preparations)	>1 to 5	6
		>0.1 to 1	5
	Moisturizing	>25 to 50	1
		> 5 to 10	2
		> 1 to 5	4
		>0.1 to 1	7
	Night	>1 to 5	4
		>0.1 to 1	3
	Paste masks (mud packs)	>1 to 5	3
	Wrinkle smoothing (removers)	>0.1 to 1	2
	Other skin care preparations	>10 to 25	1
		>0.1 to 1	1
	Suntan gels, creams, and liquids	>1 to 5	2

possible use in treating fatty acid deficiencies (Bacharach and Smith, 1938). The properties of Avocado Oil resemble those of olive oil, though unlike olive oil it is absorbed quickly through the intact skin (Valette and Sobrin, 1963).

The vitamin content of Avocado Oil has long been debated; however, there is now little doubt that the oil contains, at most, only traces of beta-carotene and vitamin D progenitors and little or no vitamin E. Even the avocado fruit, which is rich in B vitamins, is poor in vitamins A, C, K, and folic acid (Biale and Young, 1971; Franzke and Henning, 1956; Führer, 1953; Bacharach and Smith, 1938; Robbins and Bilger, 1934).

Animal Studies

Acute Toxicity

Oral No data are available on the acute oral toxicity to animals of Avocado Oil alone. Data are available only on formulations containing 0.1 to 10.7% Avocado Oil.

Estimates of the oral LD50s of two different shampoos containing 0.1% Avocado Oil were reported (Table 3). In rats, shampoo A administered by stomach tube had LD50s of 10.4 and 8.26 ml/kg, respectively, in males and females. The oil content of these doses is 0.0104 and 0.00826 ml/kg. Under similar conditions, test material shampoo B, showed LD50s of 8.26 and 7.65 ml/kg in male and female rats, respectively. In these tests depression, labored respiration, ataxia, diarrhea, and excessive urination occurred 1 to 4 hours after administration. Survivors recovered completely after 2 to 7 days. At necropsy, animals that died showed generalized congestion of internal organs and gastrointestinal inflammation. At the highest doses there were adhesions of the internal organs (CTFA, 1978)[1].

In dogs, shampoo A administered in gelatin capsules at dose levels of 1, 5, and 10 ml/kg was found to be emetic to the females at 5.0 and 10.0 ml/kg. There was 0.1% Avocado Oil in these doses. No other symptoms or gross histopathological changes occurred after a dose of 10 ml/kg (CTFA, 1978)[1].

In oral toxicity studies in Sprague-Dawley male rats performed during 1972 and 1973, seven skin products containing 2 to 5% Avocado Oil were estimated to have LD50s greater than 50 ml/kg; in addition, one face toner containing 0.5% Avocado Oil was reported to have an LD50 "slightly less than 21.5 ml/kg" ($\geqslant$1 ml/kg of oil). Diarrhea was observed in all animals receiving 21.5 ml/kg or greater during the first or second day after dosing. Two of five animals receiving 21.5 ml/kg survived the 14-day observation period. Gross necropsy of the rats that died showed congestion of the lungs, kidneys and/or adrenals with irritation and distention of the stomach and intestines. It is doubtful if the small amount of Avocado Oil, a normal constituent of a common food, was the causative agent for the foregoing adverse effects. Similarly, in the years of 1974 and 1977, five lipstick products containing 9.6 to 10.7% Avocado Oil were found to have LD50s in the Sprague-Dawley rat

[1]Available upon request. Administrator, Cosmetic Ingredient Review, Suite 212, 1133 15th St., NW, Washington, DC 20005.

greater than 40 g/kg of product or 4 g/kg of oil. Except for diarrhea with one lipstick formulation, no symptoms or pathologic effects in these studies were described in the summary provided. At autopsy the organs were normal in all animals as shown by gross examination of the lungs, liver, heart, kidneys, gastrointestinal tract and spleen (CTFA, 1978)[1].

In view of the small amounts of Avocado Oil in the toxic doses of formulations, it is unlikely that the Avocado Oil contributes to the acute oral toxicity of these formulations. For perspective, a 60 kg person eating one California avocado ingests about 625 mg/kg oil.

Dermal Three shampoos were tested for systemic toxicity by 24-hour occluded percutaneous applications in rabbits, and their percutaneous median lethal doses (PC LD50) were estimated. The results, which are given in Table 3 show that two of the formulations were more toxic percutaneously to rabbits than they were orally to rats (rabbit PC LD50 5 to 6 ml/kg, rat oral LD50 7.6 to 10.4 ml/kg) (CTFA, 1978)[1].

Deaths of the rabbits occurred within 2 to 10 days at the highest doses of 10 ml/kg, and within 8 to 12 days at 4.64 ml/kg, the lowest dose that caused any deaths. Similar symptoms were exhibited approximately 24 hours after the initial application of all three test materials. These were depression, labored respiration, weakness and unsteadiness. Anorexia and weight loss occurred in animals most severely affected. Autopsy findings in animals that died included congestion of lungs and kidneys, spotty livers and cardiac abnormalities.

The skin areas to which these test materials were applied showed moderate erythema, edema and discoloration after one to two days, and necrotic areas after the first week, followed by some sloughing, scar formation and thickening of the underlying skin during the second week.

TABLE 3. Acute Toxicity of Shampoos Containing Avocado Oil (CTFA, 1978)[1]

Product	Avocado Oil (%)	Rabbit PC LD50 ml/kg	Rat Oral LD50 ml/kg
Shampoo A	0.1	5-6	8.26-10.4
Shampoo B	0.1	5.62	7.65-8.26
Shampoo C	0.05	10->10	•••

Intradermal A study by Norred (1954) reported on the irritant effect of Avocado Oil injected intracutaneously in rabbits. After injecting 0.3 ml of Avocado Oil at several points on the abdomen, the researchers determined the degree of irritation by observing the intensity of blue color at the injected area following the intraventricular injection of trypan blue. Under these conditions only a "faint but discernible" blue color was observed one to three hours after

the injection of Avocado Oil in 12 of the 21 abdominal areas tested in six rabbits. Sesame oil, used as a reference, produced no detectable blue color. It was concluded that Avocado Oil was not sufficiently irritant to prohibit its parenteral use.

Primary Skin Irritation The skin irritating potential of several shampoo products containing Avocado Oil was tested by occlusive skin application. These tests involved a 24-hour exposure period followed by observation for an additional two to six days. Gross signs of irritation were scored according to Draize. One undiluted formulation and another diluted 1:10 with water gave scores of 6.0 and 5.2, respectively, of a maximum possible score of 8.0. These and another formulation when diluted 1:20 or 1:40 had scores ranging from 1.6 to 2.6. The data are shown in Table 4. It is apparent that these shampoos in undiluted form are quite irritating. This effect is undoubtedly due to an ingredient, or ingredients, other than the 0.5% concentration of Avocado Oil (CTFA, 1978)[1].

TABLE 4. Rabbit Skin Primary Irritation Index (PII) Scores of Shampoos Containing Avocado Oil (CTFA, 1978)[1]

Product	Dilution	PII
Shampoo D	Undiluted	6.0
Shampoo D	1:40	2.6
Shampoo D[2]	1:20	1.8
Shampoo D[2]	1:40	2.1
Shampoo D (USA)	1:10	5.2
Shampoo E	1:40	1.6

[1]Available upon request. Administrator, Cosmetic Ingredient Review, Suite 212, 113 15th St., NW, Washington, DC 20005.
[2]Identical to Shampoo D in formulation but manufactured outside of the U.S.A.

Eye Irritancy The same shampoo products tested for skin irritancy (above) were tested for acute eye irritation in rabbits and monkeys. In these tests 0.1 ml of the undiluted material was introduced into one eye. Four seconds after instillation the eye was irrigated with warm water in one group of animals; in another group the eye was not irrigated. The Draize method was used to evaluate the level of irritancy and the results are displayed in Table 5 (CTFA, 1978e and 1978f).

The maximum possible score at any given time after instillation of the test material is 110, which is the sum of the separate maximum scores for the cornea, iris and conjunctiva. In each of these tests the scores were highest at the first reading, one hour after instillation and diminished rapidly at subsequent daily readings. When the eyes were irrigated four seconds after instillation, the scores ranged from 0 to 1 in 4 to 7 days. When the eyes were not irrigated, the total scores after seven days were 13 and 10 in the rabbits and 0 in the monkeys at three days. The results of these tests suggest that the rabbit's eye is more sensitive to these shampoo products than is the eye of the monkey.

TABLE 5. Eye Irritation Scores of Shampoos Containing Avocado Oil (CTFA, 1978)[1]

Product	Animal (no.)	Irrigated	Score at 1 hr
Shampoo D[2]	rabbit (5)	yes	12, 14[3]
Shampoo D[2]	rabbit (5)	no	43
Shampoo E	rabbit (5)	yes	22
Shampoo E	rabbit (5)	no	38
Shampoo D	monkey (10)	yes	2
Shampoo D	monkey (10)	no	10

[1]Available upon request. Administrator, Cosmetic Ingredient Review, Suite 212, 1133 15th St., NW, Washington, DC 20005.

[2]Identical to Shampoo D in formulation but manufactured outside of the U.S.A.

[3]Results of two tests.

A similar test was done on five rabbits with a skin care preparation night cream containing 1.5% Avocado Oil. The cream was instilled in the eye without subsequent irrigation and the total score was 13 at 1 hour, 1 at 3 days and 0 at 7 days. These results showed this product to be less irritating to the rabbit eye than the shampoos tested above even though it contained 3 times as much Avocado Oil, indicating that components other than Avocado Oil were the irritants (CTFA, 1978)[1].

Another series of eye irritation tests was conducted with six skin formulations. These contained Avocado Oil at concentrations of 0.5 to 3.0%. They were tested in three groups of rabbits: in the first group the eyes were not washed, in the second group the eyes were washed immediately after instillation, and in the third group the eyes were washed 10 seconds after instillation. The animals were examined at 24, 48 and 72 hours after exposure and the effects scored according to the Draize method. One group of rabbits with unwashed eyes had scores of 3.33 and 2.0 at 24 and 48 hours, respectively, due to "very slight irritation" of the conjunctiva. All other scores were zero (CTFA, 1978)[1].

Subchronic Toxicity The subchronic toxicity of Avocado Oil that had been heated to 120°C for two hours was investigated by using three groups of nine rats each. They were injected subcutaneously with 0.05 ml or 0.25 ml of Avocado Oil, or 0.25 ml of sesame oil (as control) daily for 30 days. No evidence of toxicity was observed, nor was there statistical difference in weight gain between the three groups of rats. No gross pathological effects were seen, nor any histopathologic changes in the liver, kidneys, spleen, and skin at autopsy of all the rats after the 30-day treatment. Though the weights of the rats used in this study were not indicated, it may be estimated that the dose of 0.25 ml represents about 1 ml/kg of Avocado Oil (Norred, 1954).

Chronic Toxicity There are no data from animal studies on the chronic toxicity of Avocado Oil nor on its reproductive, teratogenic, mutagenic, carcinogenic, or other special toxic effects.

Clinical Assessment of Safety

Several studies have been completed on product formulations containing Avocado Oil. One study, discussed in point 4 of this section, has been conducted on the primary irritation and sensitization properties of Avocado Oil (100%) for humans.

1. A skin care preparation/night cream containing 1.5% Avocado Oil was applied undiluted under occlusive patches to the lateral aspect of the upper arm of 25 subjects (age, race and sex not given) for 18 hours. Four daily applications were made: readings at 24 hours after each patch was applied showed six subjects with slight erythema after the second application, 11 with slight to severe erythema following the third, and 11 with slight to moderate erythema after the fourth. All readings following the first patch were negative (CTFA, 1978)[1].
2. A shampoo containing 0.05% Avocado Oil diluted 1:100 in water was applied to the medial surface of the upper arm of 50 human subjects (males and females, race not stated) under occlusive patches for eight 12-hour periods. Following a two-week rest a patch was applied for 24 hours. Results during the induction tests showed 19 responses of slight erythema and one with severe erythema. The greatest effect was noted on the eighth day when four subjects had slight erythema and one had severe erythema. Reactions after removal of the challenge patch test at 24 hours included three subjects with slight erythema and one with moderate erythema. At 24 hours after removal, one subject showed slight erythema and at 48 hours following removal, one subject had slight erythema (CTFA, 1978)[1].
3. Four percent Avocado Oil in hand cream used in a Draize repeated insult test on 51 black subjects was found to be essentially nonirritating and nonsensitizing (Hill Top Research, 1976)[1].
4. One hundred white females were subjected to prophetic patch tests using 100% Avocado Oil. Patches were removed after 48 hours and the application sites observed immediately for skin reaction. Subjects were re-examined at 24 hours. The test was repeated 14 days later on the same sites. There was no evidence of primary irritation after the initial 48-hour application and no evidence of sensitization after the repeated test (CTFA, 1978)[1].
5. A series of skin formulations were patch tested on 100 white females as described in item 4 above. The scores, presented in Table 6 give no evidence of irritation or sensitization (CTFA, 1978)[1].
6. Human patch testing of five lipstick formulations are described below. Neither race nor skin color of the volunteers was reported (CTFA, 1978)[1].
   a. Lipstick A containing 9.6% Avocado Oil was applied under an occlusive patch to the backs of 57 females and one male for 72 hours. The examining physician concluded there was "little or no primary irritation potential."
   b. Lipstick B containing 10% Avocado Oil was tested on 92 females and 18 males by a Draize-Shelanski test. The physician concluded the formulation had "little or no primary irritation potential," "does not cause sensitivity," and "appears safe."

c. Lipstick C containing 9.8% Avocado Oil and Lipstick D containing 10.2% Avocado Oil were applied under occlusive patches to the upper backs of 86 to 90 females and 7 to 13 males for one 72-hour period, followed by eight alternate 24-hour periods. After a rest of 13 days, challenge patches were applied for 48 hours. The physician concluded for both formulations that the "tests indicated little or no primary irritation," and "the product appears safe, does not cause sensitivity."
d. Formulation Lipstick E containing 10.7% Avocado Oil was tested on 47 females and five males using the same procedure and with the same conclusions as in 6(c) above.

7. Five percent Avocado Oil in peach kernel oil was tested by occlusive patch tests on 98 volunteers using a procedure similar to that described by A.A. Fisher (1973). The results were "negative." It was also reported that Avocado Oil is used in approximately 45 products including makeup foundations, moisturizing lotions and creams, cleansing creams, and balancing and toning lotions. When all of these were patch tested on humans, they showed the "absence of either primary skin irritation or measurable allergic potential." Ten years of public use of cosmetics containing Avocado Oil "has provided no evidence of incompatability with the skin" (CTFA, 1978)[1].
8. There are no data on the photosensitizing potential of Avocado Oil.

SUMMARY

About 240 cosmetic formulations contain Avocado Oil in concentrations ranging from ≤0.1 to 50%. More than 150 of these are lipstick formulations, many of which contain 10% or more, and some up to 50% of Avocado Oil.

Avocado Oil has no hormonal properties, and the presence of small amounts of certain vitamins is of no physiologic significance in the use of cosmetic products containing the oil.

The results of one test using Avocado Oil (100%) and numerous formulations containing it on humans and animals show that it has little skin irritating or sensitizing quality. It is more readily absorbed through the skin than is olive oil.

Doses of approximately 1 ml/kg of Avocado Oil injected subcutaneously in rats daily for 30 days had no adverse effects, and cosmetic formulations containing approximately 10% Avocado Oil were reported to have LD50s greater than 40 ml/kg in rats. Some of the formulations, e.g., shampoos, tested on skin and eyes, showed irritation in both animals and humans, but there was no evidence of sensitization.

The safety assessment of this ingredient rests on the information at hand and on its extensive usage in various concentrations in a variety of cosmetic formulations. Additional biological assessment of Avocado Oil might reasonably be expected to incorporate studies of its potential for contact sensitization and photosensitization utilizing Draize repeated insult patch testing and maximization tests at usage concentrations and at 100%.

TABLE 6. Scores From Human Patch Tests of Cosmetic Formulations Containing Avocado Oil (CTFA, 1978)[1]

Avocado Oil (%)	Formulation Tested	48-Hr. Irritation				14-Day Sensitization	
		Immed.	15 min.	24 hrs.	72 hrs.	Immed.	72 hrs.
2.0	Sample 1	0	0	0	-	-	-
	Sample 1	0	0	0	-	-	-
	Sample 1	0	0	0	-	-	-
	Sample 1	0	-	-	0	0	0
	Sample 1	0	-	-	0	0	0
2.8	Sample 2	0	-	-	0	0	0
5.0	Sample 3	0	-	-	0	0	0
3.0	Sample 4	0	-	-	0	0	0
	Sample 4	0	-	-	0	0	0
0.5	Sample 5	0	-	-	0	0	0
2.0	Sample 6	0	-	-	0	0	0
2.8	Sample 7	0	-	-	0	0	0
2.4	Sample 8	0	-	-	0	0	0

CONCLUSIONS

The Panel believes that the current information available regarding Avocado Oil is relevant and sufficient to indicate that the ingredient is safe for use as presently incorporated into cosmetic formulations.

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