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Final Report on the Safety Assessment of Arachidonic Acid

ABSTRACT

Arachidonic Acid is an essential, polyunsaturated, fatty acid that is used as a surfactant–cleansing agent and a surfactant–emulsifying agent in cosmetic formulations. Arachidonic Acid is well absorbed from the gastrointestinal tract and the circulatory system; it distributes rapidly into the lipid compartment of the body and is rapidly converted to phospholipid by the liver.

Arachidonic Acid may alter the cutaneous immune response; in one study, the effect was more pronounced at lower test concentrations than at higher. Application of Arachidonic Acid to mouse skin produced edema and inflammation, with high dosages possibly causing ulceration of the skin. Arachidonic Acid has mutagenic potential. In a 24 h single insult patch test, a formulation containing 0.04% Arachidonic Acid was not a skin irritant.

The safety of use of this ingredient in cosmetic products has not been documented and substantiated. It cannot be concluded that Arachidonic Acid is safe for use in cosmetic products until the needed additional safety test data have been obtained and evaluated. If the requested skin absorption data indicate that absorption occurs, immunomodulatory data, carcinogenicity and photocarcinogenicity data, human irritation, sensitization, and photosensitization data may also be required.

INTRODUCTION

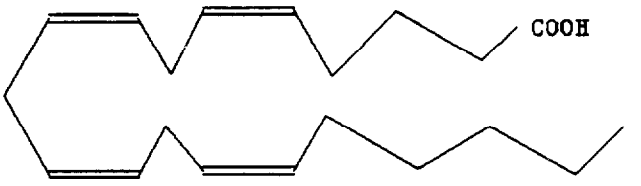
ARACHIDONIC ACID IS A POLYUNSATURATED FATTY acid with a hydrocarbon chain consisting of 20 carbons, four nonconjugated double bonds, and a terminal carboxyl group. It is used as a surfactant–cleansing agent, which includes the soap form of Arachidonic Acid, and as a surfactant–emulsifying agent (Nikitakis, 1988).

The data base for Arachidonic Acid is large. Additional data not included in this review are contained in the Appendix to this report.

CHEMISTRY

Definition and Structure

Arachidonic Acid (CAS No. 506-32-1) is the polyunsaturated fatty acid (Estrin et al., 1982) that conforms to the formula (Windholz, 1983):



Fatty acids whose structures contain multiple double bonds, such as Arachidonic Acid, are kinked and relatively rigid in comparison to saturated fatty acids (Lehninger, 1982).

Arachidonic Acid is also known as 5,8,11,14-Eicosatetraenoic Acid (Estrin et al., 1982; Windholz, 1983), (all-Z)-5,8,11,14-Eicosatetraenoic Acid (Sweet, 1987; Osol, 1980), and Eicosa-5:8:11:14-tetraenoic Acid (West and Todd, 1961). Collectively, Arachidonic Acid, linoleic acid, and linolenic acid have been called Vitamin F (Rossoff, 1974); these acids are known as the essential fatty acids.

Properties

Arachidonic Acid is a liquid at room temperature (Windholz, 1983; Hawley, 1971); unsaturated fatty acids are oily liquids at body temperature (Lehninger, 1982). Arachidonic Acid has a melting point of −49.5°C (Weast, 1982; Hawley, 1971) and decomposes before reaching a boiling point (Weast, 1982). Arachidonic Acid absorbs at 257 nm (ϵ 12,303), 268 nm (ϵ 16,218), and 315 nm (ϵ 6607) (Grasselli, 1973). Physical and chemical properties of Arachidonic Acid are summarized in Table 1.

Table 1. Properties of Arachidonic Acid

Property	*Value*	*Reference*
Physical appearance	Liquid	Windholz, 1983; Hawley, 1971
Molecular formula	$C_{20}H_{32}O_2$	Estrin et al., 1982
Structural formula	$CH_3(CH_2)_4(CH{=}CHCH_2)_4CH_2CH_2COOH$	Estrin et al., 1982
Molecular weight	304.5	
Melting point	−49.5°C	Lehninger, 1982; Weast, 1982; Osol, 1980; Hawley; 1971
Boiling point	Decomposes 245°C	Weast, 1982 Grant, 1972
UV absorption	257 nm (ϵ 12,303) 268 nm (ϵ 16,218) 315 nm (ϵ 6607)	 Grasselli, 1973
Iodine value	333.50	Windholz, 1983; Hawley, 1971
Neutralization value	184.20	Windholz, 1983
Refractive Index for D line of Na spectrum (20°C)	1.4824	Windholz, 1983; Weast, 1982
Solubility	Soluble in alcohol, ether, and water	Weast, 1982
Other	Nontoxic; combustible	Hawley, 1971

Biological Sources

Some of the best natural sources of Arachidonic Acid include liver, fish oils, bovine adrenal gland, butterfat, and lard (Rossoff, 1974). Arachidonic Acid occurs in the brain, glandular organs, and depot fats of animals; it occurs in small amounts in human depot fats (Windholz, 1983; Osol, 1980). Arachidonic Acid is also found in lecithin (phosphatidylcholine) (Hawley, 1971) and is a constituent of animal phosphatides (Windholz, 1983); it is a major component (15%) of the mixed fatty acids of adrenal gland phosphatides (Swern, 1979).

Manufacture and Production

Arachidonic Acid has been synthesized from acetylenic intermediates (Ege et al., 1961; Rachlin et al., 1961). Ege et al. (1961) described the synthesis of Arachidonic Acid from 1-heptyne and Rachlin et al. (1961) described the synthesis of Arachidonic Acid from 2-propargyloxytetrahydropyran.

Pure Arachidonic Acid, in the form of its methyl ester, can be isolated in limited amounts from animal lipids; however, this method is not practical for producing large amounts of Arachidonic Acid (Rachlin et al., 1961).

Analytical Methods

Arachidonic Acid can be determined via gas chromatography–mass spectrometry (GC-MS) (Bennett et al., 1987), gas–liquid chromatography (GLC) (Chandler and Giri, 1981), gas–liquid radiochromatography (Swell and Law, 1967), radio gas chromatography (Nakazawa et al., 1976), high-performance liquid chromatography (HPLC) (Audera et al., 1988; Voelkel et al., 1980), radioimmunoassay (Voelkel et al., 1980), radio-HPLC (Robinson et al., 1989), thin layer chromatography (TLC) (Wang et al., 1989), TLC/autoradiography (Shirazi et al., 1989), and by an isomerization procedure (Swern, 1979).

A capillary GLC method has been developed that provides an accurate, precise, and reliable means for the quantitative determination of Arachidonic Acid in suspensions of washed erythrocytic membranes (Taylor et al., 1987).

USE

Cosmetic

The product formulation data submitted to the Food and Drug Administration (FDA) in 1992 stated that Arachidonic Acid was contained in 29 cosmetic product formulations (Table 2). Arachidonic Acid was used in face, body, and hand skin care preparations (excluding shaving preparations), moisturizing skin care preparations, night skin care preparations, wrinkle smoothers (removers), and other skin care preparations. The greatest reported use of Arachidonic Acid was in other skin care preparations: 11 formulations.

Concentration of use values are no longer reported to the FDA by the cosmetics industry (Federal Register, 1992). However, product formulation data submitted to the FDA stated that Arachidonic Acid was used in skin care preparations at a concentration of <5% (FDA, 1989).

TABLE 2. PRODUCT FORMULATION DATA FOR ARACHIDONIC ACID[a] (FDA, 1992)

Product category	Total no. of formulations in category	Total no. containing ingredient
Face, body, and hand skin care preparations (excluding shaving preparation)	832	5
Moisturizing skin care preparations	747	5
Night skin care preparations	219	4
Wrinkle smoothers (removers)	38	4
Other skin care preparations	349	11
1992 Totals		29

[a]CIR requests that the cosmetic industry provide current formulation data on each product category.

Noncosmetic

Arachidonic Acid is used in medicine and biochemical research (Hawley, 1971). It has been recommended for use with other fatty acids in treatment of infant eczema and dermatitis (Osol, 1980). Arachidonic Acid is used, often with linoleic and linolenic acids, in the prevention and treatment of eczema and dermatitis in animals, particularly dogs, cats, and swine (Windholz, 1983; Rossoff, 1974).

BIOLOGY

Detailed biology information on Arachidonic Acid is contained in the Appendix to this report.

General

Arachidonic Acid is a polyunsaturated fatty acid that can be synthesized metabolically from linoleic acid (Lehninger, 1982); however, linoleic acid cannot be synthesized by mammals and must be obtained from plant sources. Fatty acids required in the diet are called essential fatty acids (EFAs).

The conversion of linoleic acid into Arachidonic Acid involves the desaturation of linoleic acid, as linoleyl-CoA, to γ-linolenic acid (γ-linolenyl-CoA), which is then elongated by the addition of a 2-carbon unit from malonyl-CoA to form dihomo-γ-linolenyl-CoA. Dihomo-γ-linolenyl-CoA is then desaturated at carbon 5 and 6 to form Arachidonic Acid, as arachidonyl-CoA.

Arachidonic Acid is a normal constituent of cells and is mainly found in membrane-bound phospholipids (Samuelsson, 1987). The concentration of Arachidonic Acid in most cells is normally less than 10^{-6}M (Morrison, 1986). Arachidonic Acid is released from membrane phospholipids primarily by the action of phospholipase A_2 and phospholipase C (Zoja et al., 1989).

Absorption, Distribution, Metabolism, Excretion

Dermal absorption studies for Arachidonic Acid have not been reported.

Arachidonic Acid is well absorbed from the gastrointestinal tract (Coots, 1965; Chow and Hollander, 1978; Ramesha et al., 1985) and the circulatory system (de

Tomas and Mercuri, 1971; Puri et al., 1975; Zijlstra and Vincent, 1985). Arachidonic Acid distributes rapidly into the lipid compartment of the body (Zijlstra and Vincent, 1985) and is rapidly converted to phospholipid by the liver (de Tomas and Mercuri, 1971). Direct release is catalyzed by phospholipase A_2. Phospholipase C first forms a diglyceride that yields free Arachidonic Acid by hydrolyzation. Cyclooxygenase and lipoxygenase facilitate hydrogen removal, double-bond rearrangement, and inclusion of oxygen to yield several unstable fatty acids (McGiff, 1987). The rate-limiting step of Arachidonic Acid metabolite formation is the release of free Arachidonic Acid from the phospholipid (Malmsten, 1986).

Arachidonic Acid can be metabolized by three different pathways (McGiff, 1987). If oxygenation occurs via the cyclooxygenase pathway, prostaglandins E_2, $F_{2\alpha}$, I_2, and D_2, (PGE_2, $PGF_{2\alpha}$, PGI_2, and PGD_2), and thromboxane A_2 (TxA_2) are formed (Zoja et al., 1989). If oxygenation occurs through the lipoxygenase pathway, hydroxy fatty acids and leukotrienes are formed.

The third pathway for the metabolism of Arachidonic Acid in animal tissues is by the cytochrome P450 system (Schwartzman et al., 1987), which is called the epoxygenase pathway (Basu and Karmazyn, 1987). Cytochrome P450-dependent monoxygenase converts Arachidonic Acid to monohydroxyeicosatetraenoic acids, epoxyicosatetrienoic acid, and the ω,ω-1, and ω-2 hydroxylation products (Schwartzman et al., 1987). The formation of these metabolites is strictly dependent on molecular oxygen and NADPH (McGiff, 1987; Schwartzman et al., 1987).

Iron, or heme, is an essential cofactor for the cyclooxygenase, lipoxygenase, and peroxidase enzymes involved in the formation of platelet endoperoxides and thromboxanes (Rao et al., 1978). For the oxidation of Arachidonic Acid, iron must be in the ferrous form, and the reaction between Arachidonic Acid and iron is dependent on the presence of oxygen.

Arachidonic Acid and Inflammation

Arachidonic Acid metabolites are involved in the inflammatory process. Metabolism of Arachidonic Acid via the cyclooxygenase pathway produces the metabolites PGI_2 and PGE_2, which can be vasodilators (Zoja et al., 1989), and the prostaglandin endoperoxidases, G_2 and H_2, and TxA_2, which induce rapid irreversible aggregation of human platelets and are inhibitors of smooth muscle contraction (Malmsten, 1986). TxA_2 is a potent vasoconstrictor (Zoja et al., 1989).

Metabolism of Arachidonic Acid occurs by the lipoxygenase pathway in cells involved in host inflammatory responses; these cells include human neutrophil polymorphonuclear leukocytes, eosinophils, monocytes, alveolar macrophages, and mast cells (Lee et al., 1984). Hydroxyeicosatetraenoic acids (HETEs) and leukotriene B_4 are products of the lipoxygenase pathway of Arachidonic Acid metabolism that have chemotactic and chemokinetic activity; leukotriene B_4 is the most active compound derived from Arachidonic Acid for chemotactic effects on polymorphonuclear leukocytes (Malmsten, 1986).

In vivo, the inflammatory response appears to begin with adherence of leukocytes to the endothelium of microvessels near inflammatory areas. *In vitro*, it seems to correspond to leukocyte aggregation and adherence. Leukotrienes C_4 and D_4, also products of the lipoxygenase pathway, induce a contractile response to decrease the surface area of isolated glomeruli and cause shape changes in cultured mesangial cells (Zoja et al., 1989). Leukotrienes C_4, D_4, and E_4 do not induce chemotaxis, enzyme

release, or leukocyte aggregation, but do have a potent effect on smooth muscle in the peripheral airways and the ability to increase permeability in venules to macromolecules (Malmsten, 1986).

Arachidonic Acid and Major Diseases

A chronic cellular imbalance between Arachidonic Acid, γ-linolenic acid, and eicosapentaenoic acid, and of their respective eicosanoid derivatives, may have major health implications (Booyens and van der Merwe, 1985). Such an imbalance may be involved in diseases such as atherosclerotic heart disease, arterial hypertension, hypercholesterolemia, chronic inflammatory and autoimmune disorders, allergic eczema, and other atopic disorders.

In part, these diseases arise from a chronic excess of Arachidonic Acid with a relative deficiency of γ-linolenic and eicosapentaenoic acid in the membrane phospholipids of many cells (Booyens and van der Merwe, 1985).

General Immunologic and Pharmacologic Effects

Male DBA/2 mice were used in a study to determine the potential role of Arachidonic Acid and its metabolites in ultraviolet (UV) light-induced cutaneous immune suppression (Rheins et al., 1987). Several groups of mice were fed a diet containing 0.15 mg/kg indomethacin on days 1–5. Some of these and other groups of mice, which were fed normal diet, received dermal applications of 100 μl of Arachidonic Acid (2.0 g/dl) in dimethyl sulfoxide:water (DMSO:H_2O) or were exposed to UVB (FS 40 lamps, 11 mJ/cm^2, 8.2 mJ UVB, 2.8 mJ UVA) on shaved backs on days 2–5. On day 6, all rats were fed control feed and sensitized with applications of 25 μl of 0.5% 2,4-dinitro-1-fluorobenzene (DNFB) on their backs. On day 13, baseline measurements of pinnal thickness were taken and the mice were then challenged with 20 μl of 0.2% DNFB. Pinnal thickness was measured 24 h later to determine the reaction to DNFB. Negative controls were challenged with DNFB but were not previously sensitized. A group of diluent controls was also used.

Mice treated with Arachidonic Acid or UVB only did differ significantly from negative control animals in their response to DNFB. The mice treated with Arachidonic Acid and indomethacin had a significantly increased degree of pinnal swelling when compared to mice given Arachidonic Acid only. The response to the DNFB challenge for the mice treated with Arachidonic Acid or UVB and indomethacin was not significantly different from mice treated with diluent or indomethacin only. Arachidonic Acid caused an increase in the number of epidermal pigment cells, melanocyte hyperplasia, and induced melanogenesis; indomethacin, 0.15 mg/kg, abrogated Arachidonic Acid's immunosuppressive effects. The authors proposed that Arachidonic Acid altered the cutaneous immune response, possibly by prostaglandin production.

Arachidonic Acid was applied to mouse skin to determine its effect on epidermal Langerhans cells and cutaneous immune reactivity (Rheins and Nordlund, 1986). A dose of 0.05 or 2% Arachidonic Acid was applied either to the back or the pinnae of the mice on days 1–7; controls were treated with diluent, DMSO:water.

Upon microscopic examination on day 8, it seemed that Arachidonic Acid had a biphasic effect on Langerhans cells. A dose of 0.05% Arachidonic Acid increased identifiable ATPase- and immune-associated (Ia)-positive Langerhans cells; 2% Arachidonic Acid decreased the identifiable cells. A distal effect on Langerhans cell populations was not observed at either dose.

Mice dosed on the back, both dose groups, were sensitized with 0.5% DNFB on days 8 and 10; on day 15, the untreated pinna was challenged with 0.2% DNFB. A 62% increase in pinnal edema was observed in the group dosed with 0.05% Arachidonic Acid; a 24% increase in edema was observed for DMSO-treated controls. Mice that had a reduction in identifiable ATPase- and Ia-positive Langerhans cells after being dosed with 2% Arachidonic Acid had only a 21% increase in pinnal edema; DMSO-treated controls had a 34% increase in edema. When the mice dosed on the pinna with 2% Arachidonic Acid were sensitized and challenged as above, the Arachidonic Acid-treated pinnae with fewer ATPase/Ia-positive cells had a 20% increase in edema after 24 h; the DMSO-treated controls had a 37% increase in edema.

In a study examining the effects of chronic intracutaneous administration of Arachidonic Acid into the posterior aspect of both pinnae of female albino guinea pigs, only minor dermal changes due to Arachidonic Acid administration were found (Ruzicka and Burg, 1987). Positive results were obtained by injection of some metabolites of Arachidonic Acid. These results suggested that apart from hyperproliferative and inflammatory dermatoses, lipoxygenase-derived Arachidonic Acid metabolites may be involved in the pathophysiology of vasculitis and dermatoses characterized by tissue infiltrate of eosinophils.

A study was conducted to examine whether sodium Arachidonate could trigger the reaction between normal human lymphocytes and rat isolated atria, therefore replacing the initial triggering step provided by lectin stimulation of the lymphocyte's membrane (Borda et al., 1984). Human lymphocytes incubated with sodium Arachidonate *in vitro* had positive inotropic and chronotropic effects. Stimulation of lymphocytes with phytohemagglutinin resulted in a prompt release of Arachidonic Acid to the medium.

Exogenous Arachidonic Acid was added to phagocytic cells to determine the effects of Arachidonic Acid on various human polymorphonuclear and mononuclear leukocyte functions (Henricks et al., 1984). The effects of Arachidonic Acid on the phagocytic cell function were specific for neutrophils. Incubation with Arachidonic Acid resulted in large amounts of oxygen consumption, superoxide production, and chemiluminescence generation by neutrophils; these results were not observed for monocytes. Neutrophils aggregated to a much greater degree than monocytes upon Arachidonic Acid incubation. Neutrophil chemotaxis towards an attractant was lost and phagocytosis of bacteria decreased when incubated with Arachidonic Acid. At 0°C, Arachidonic Acid incubation did not affect phagocytosis of staphylococci. Mononuclear phagocytes were not affected at any temperature.

The oxidative degradation of unsaturated fatty acids was followed by the determination of the amount of malonyldialdehyde, a metabolite formed during lipid peroxidation by oxygen metabolites. A dose-dependent response occurred when polymorphonuclear leukocytes were incubated with Arachidonic Acid; a greater amount of malonyldialdehyde was formed with a greater dose of Arachidonic Acid.

Incubation of neutrophils with indomethacin prior to Arachidonic Acid incubation reduced the effects of Arachidonic Acid on the stimulation of neutrophil metabolism. Indomethacin partially abolished the decreasing effect of Arachidonic Acid on phagocytosis and it inhibited the chemiluminescence response; indomethacin did not inhibit oxygen consumption and glucose metabolism after Arachidonic Acid stimulation. An oxygen species scavenger interfered with Arachidonic Acid's effect on phagocytosis.

Arachidonic Acid metabolism in the skin of normal female Hartley guinea pigs, guinea pigs with contact dermatitis induced by 1-chloro-2,4-dinitrobenzene (DNCB) and guinea pigs with irritant dermatitis induced by a nicotinic acid derivative was

compared (Ruzicka and Printz, 1982). The main metabolite of exogenous Arachidonic Acid in normal skin was HETE. In the presence of a cofactor, reduced glutathione, high cyclooxygenase activity was observed, with PGD_2 being the main product. There was a marked inhibition of the lipoxygenase pathway and the production of PGD_2 from Arachidonic Acid with contact dermatitis. Dermal application of DNCB to unsensitized animals resulted in increased PGE_2 production. With irritant dermatitis, there was an inhibition of the cyclooxygenase pathway in the epidermis; however, there was a stimulation of this pathway in the dermis, with PGE_2 being the main metabolite. PGH_2 metabolism was unchanged; the lipoxygenase pathway was slightly stimulated.

ANIMAL TOXICOLOGY

Acute Toxicity

Acute toxicity data are summarized in Table 3. Details of the studies are contained in the Appendix.

Dermal Irritation

Arachidonic Acid in acetone solution, 25 μl of a 4% w/v solution, was applied daily, 5 days/wk for 3 wks, to the inner face of the right pinna of male OF1 mice (Bouclier et al., 1989). The left pinna served as a control and was treated with vehicle only. Animals were killed on days 1, 3, 5, 12, and 19. The thickness of both pinnae was measured 4 h after Arachidonic Acid application.

A marked increase in swelling of the treated pinnae was observed on day 1, swelling was reduced on day 2, and on days 3+, thickness of treated pinnae increased until reaching a plateau on day 10. A reduction of pinnal thickness associated with skin atrophy was observed on the untreated pinna.

At microscopic examination, extracellular dermal edema was observed on day 1. During week 1, vascular congestion, erythrocyte extravasation, and polymorphonuclear (PMN) infiltrates were observed in the dermis along with slight hyperplasia in the epidermis with necrosis and PMN infiltrates. Inflammation with PMN and mononuclear cell infiltrates was seen in the dermis, and some marked hyperkeratosis and a marked hyperplasia in the epidermis, during the second week. During the third week, regression of inflammation and marked epidermal hyperplasia were observed.

Application of Arachidonic Acid to mouse skin produced edema and inflammation (Fischer et al., 1989). High doses of Arachidonic Acid can cause ulceration of the skin.

Teratogenic/Reproductive Effects

Arachidonic Acid was administered subcutaneously to pregnant female Sprague-Dawley rats that were made diabetic by intravenous (i.v.) injections of 40 mg/kg streptozotocin on day 6 of gestation (Goldman et al., 1985). One group of pregnant diabetic rats was given 200 mg/kg/day Arachidonic Acid on days 9–12 of gestation; another group of pregnant diabetic rats was given 200 mg/kg Arachidonic Acid twice daily on days 5–10 of gestation.

Arachidonic Acid had no significant effect on maternal weight gain or blood glucose level. The weight gain per embryo in the diabetic rats was not significantly

Table 3. Acute Toxicity of Arachidonic Acid (I.V. Administration)

Species and sex	*Methods*	*Lethal dose$_x$*	*Comments and results*	*References*
Male CD-1 mice		LD_{80} = 75 mg/kg	Arachidonic Acid, by i.v. injection, caused a thrombotic/ischemic death.	Myers et al., 1988
Male Swiss mice	A fast, <2 sec, injection of Arachidonic Acid was given.	LD_{50} = 39.2 mg/kg (in 1 h)	Arachidonic acid, i.v. injection, had a dose-related toxic effect.	de Clerck et al., 1985
Male Swiss mice	Mice were given ≥50 mg/kg Arachidonic Acid.		The mortality rate, 75%, was highly significant. A proportional reduction in survival time was observed.	de Clerck et al., 1985
Male Swiss mice	Arachidonic Acid, 45-50 mg/kg, was given to 339 mice.		The mortality rate was 90%. Medial survival time was 95 sec.	de Clerck et al., 1985
Male Swiss mice	A slow, 10 sec, dose of 50 mg/kg Arachidonic Acid was given.		No mortality was observed. Short-lasting respiratory distress was observed.	de Clerck et al., 1985
Male Swiss mice	Arachidonic Acid was aged for 24 h at 4°C in ethanolic suspension.		Toxic effects due to Arachidonic Acid were decreased.	de Clerck et al., 1985
Male Swiss mice	The effects of sodium Arachidonate were compared to Arachidonic Acid.		75 mg/kg: Arachidonic Acid killed 6/6 mice; sodium Arachidonate killed 3/5 mice 50 mg/kg: Arachidonic Acid killed 4/6 mice; sodium Arachidonate did not kill any mice.	de Clerck et al., 1985
Male and female CD-1 mice	Mice were dosed with Arachidonate.	LD_{50} = 33 mg/kg (males) LD_{50} = 46 mg/kg (females)	Male mice are more susceptible to Arachidonate-induced sudden death than female mice.	Myers et al., 1983
Male and female CD-1 mice	Mice were dosed with 100 mg/kg Arachidonate.		The mortality was 100% for males and females. Sudden death was probably due mainly to pulmonary thrombosis and ensuing hypoxia. Massive platelet aggregation occurred in the pulmonary vessels.	Myers et al., 1983

Table 3. Acute Toxicity of Arachidonic Acid (I.V. Administration) (*Continued*)

Species and sex	*Methods*	*Lethal dose$_x$*	*Comments and results*	*References*
Male and female CD-1 mice	Various doses of Arachidonate were administered to 50-day old mice over a 10 sec period.		Mortality rates: 12.5 mg/kg: 10% in males and 2% in females 25 mg/kg: 38% in males and 16% in females 50 mg/kg: 67% in males and 44% in females 100 mg/kg: 100% in males and females. At 25 and 50 mg/kg, the mortality rate was significantly greater in males than in females.	Myers et al., 1982
Male and female CD-1 mice	Immature mice of different ages were dosed with 50 mg/kg Arachidonate over 10 sec.		Mortality rates: 23 day old: 56% in males and 47% in females 29 day old: 62% in males and 48% in females 35 day old: 80% in males and 57% in females. At 35 days of age and maturity, the mortality rate with 50 mg/kg Arachidonic Acid was significantly greater in males than in females.	Myers et al., 1982
Male and female CD-1 mice	Mice were gonadectomized at 23 days of age and dosed with Arachidonate over 10 sec at 50 days of age.		There was no significant difference in the mortality rate between males and females or in within-sex comparisons of intact and gonadectomized mice.	Myers et al., 1982
Male and female CD-1 mice	Mice were pretreated with estradiol and dosed with sodium Arachidonate over 10 sec.		The mortality rate in immature intact males and immature intact and gonadectomized females pretreated with estradiol was less than the mortality rate in the intact control males and gonadectomized females.	Myers et al., 1982

Male and female CD-1 mice	Mice were pretreated with testosterone and dosed with sodium Arachidonate over 10 sec.	Testosterone had no effect on mortality in intact or gonadectomized mice.	Myers et al., 1982
Male CD-1 mice	A dose of 50 or 75 mg/kg sodium Arachidonate was given over 10 sec.	50 mg/kg: mortality rate of 71% 75 mg/kg: mortality rate of 80% Platelet aggregates were observed in 4 mice at microscopic examination of the lungs.	Rabbani et al., 1981
Male and female CF1 mice	A dose of 50mg/kg sodium Arachidonate was given over 10 sec. Some mice were pretreated with cortisone acetate for 4 days or indomethacin 2 h before dosing.	The mortality rate was 56.7% for males and 36.7% for females. Cortisone and indomethacin pretreatment significantly reduced mortality.	Pehnos et al., 1979
Male and female CF1 mice	Adrenalectomized mice were dosed with 50 mg/kg Arachidonate. Some mice were pretreated with cortisone.	A 100% mortality rate was observed for both sexes. Cortisone pretreatment significantly reduced the lethal effects of Arachidonate and eliminated sex-dependent survival.	Penhos et al., 1979
Male and female CF1 mice	Intact and gonadectomized mice were dosed with 26 mg/kg Arachidonate.	A significant diffference in mortality rate was observed between male and female mice. Gonadectomy did not significantly protect males or females from Arachidonate-induced death.	Penhos et al., 1979
Male and female CF1 mice	Adrenalectomized mice were dosed with 26 mg/kg Arachidonate. Some mice were also gonadectomized.	The mortality rate was 60% and 45% for adrenalectomized males and females, respectively. Castration did not significantly affect Arachidonate-induced mortality.	Penhos et al., 1979

Table 3. Acute Toxicity of Arachidonic Acid (I.V. Administration) (*Continued*)

Species and sex	*Methods*	*Lethal dose$_x$*	*Comments and results*	*References*
Male Fullinsdorf albino, SPF, mice	Ten mice were dosed with 90 mg/kg sodium Arachidonate.		90-100% mortality in 1–2 min.	Strub and Muller, 1979
Male CF1 mice	Mice were dosed with 50 or 100 mg/kg sodium Arachidonate.		50 mg/kg: mortality rate of 0–10%; respiratory distress, which lasted for an average of 7.5 min. 100 mg/kg: mortality rate of 100% in 1–3 min. Platelet aggregates were observed in the lungs.	Kohler et al., 1976
Sprague-Dawley (CD) rats (gender not specified)	Rats were doses with 10, 20, 50, or 100 mg/kg sodium Arachidonate.		10 mg/kg: 0 deaths; 4.4 min respiratory distress 20 mg/kg: 0 deaths; 6.7 min respiratory distress 50 mg/kg: 0 deaths; 11 min respiratory distress 100 mg/kg: 100% mortality rate in 1–3 min.	Kohler et al., 1976
Male or female rabbits (strain not specified)	45 rabbits were doses with 1.5 mg/kg sodium Arachidonate over 3 sec. Some rabbits were given i.v. infusions of PGE_2, PGD_2, and PGI_2 for 8 min. Arachidonate was injected 3 min after the start of infusion. Some rabbits were dosed with indomethacin 20 h prior to Arachidonate administration		The mortality rate was 75.6%; 35/45 rabbits died in 1–4 min. Of the prostaglandins infused, only PGI_2 reduced mortality in a dose-related manner. Mortality was attributed to occlusive thrombi formation in the pulmonary microvascular bed. Indomethacin abolished the toxic effects of Arachidonate, with 0% mortality observed.	Bayer et al., 1979
New Zealand White rabbits (gender not specified)	Rabbits were dosed with 0.5–0.75, 1.0, or 10.0 mg/kg sodium Arachidonate.		0.5–0.75 mg/kg: 0 deaths; no obvious respiratory effects 1.0 mg/kg: 100% mortality in 1–3 min 10.0 mg/kg: 100% mortality in 1–2 min	Kohler et al., 1976

New Zealand White rabbits (gender not specified)	Rabbits were dosed with 0.5 , 0.7, 1.0, 1.4, or 6.0 mg/kg sodium Arachidonate. Five rabbits that survived a dose of <1.4 mg/kg were later challenged with 1.4 mg/kg Arachidonate.	$LD_{50} \approx 1.0$ mg/kg	0.5 mg/kg: 1/5 died in 2 min; no gross toxic effects were observed in 4 surviving rabbits 0.7 mg/kg; rapid respiration observed in 3 rabbits 1.0 mg/kg: 2/4 rabbits died rapidly; rapid respiration and gasping observed in 2 surviving rabbits 45 sec after dosing 1.4 mg/kg: 15/15 rabbits died in 15 sec-3 min 6.0 mg/kg: 1 rabbit died in 2 min 5 challenged rabbits: 5/5 died in 2 min All rabbits that died had platelet aggregates in the pulmonary microcirculation.	Silver et al., 1974

changed by Arachidonic Acid; weight gain per embryo was significantly decreased in diabetic rats compared to nondiabetic controls rats.

Conceptus complexes composed of the embryo, visceral yolk sac, amniotic sac, and ectoplacental cone were obtained from female pregnant CD rats on day 10 of gestation and cultured in male rat serum (Pinter et al., 1986). Some cultures had 1, 10, 20, or 80 μg/ml sodium Arachidonate added to the male rat serum.

After 48 h of incubation, no significant changes in the conceptuses due to 1 or 10 μg/ml Arachidonic Acid were observed. A significant increase in conceptus diameter and crown–rump length was observed due to the addition of 20 or 80 μg/ml Arachidonic Acid. No embryonic malformations were observed at any concentration. Arachidonic Acid was not teratogenic when added to control medium and, in fact, improvement of conceptus development was observed.

Prevention Teratogenic Effects

Arachidonic Acid had a significant protective effect against the teratogenic action of hyperglycemia (Goldman et al., 1985). The addition of Arachidonic Acid to hyperglycemic serum medium reduced the number of hyperglycemic-induced conceptus malformations such as an open neural tube, advanced neuropil formation in the neuroepithelium, significant reduction of the rough endoplasmic reticulum, decreased size and number of lipid droplets, and increased number of lysosome-like structures in the visceral endodermal yolk sac cells (Pinter et al., 1986). Addition of relatively high concentrations of Arachidonic Acid to hyperglycemic medium prevented malformations and many of the normally observed abnormalities in fatty acid composition in conceptuses cultured in hyperglycemic conditions (Pinter et al., 1988).

Subcutaneous administration of Arachidonic Acid to pregnant diabetic rats significantly reduced neutral tube fusion defects, cleft palate, and micrognathia (Goldman et al., 1985).

Phenytoin disrupts normal embryonic development and produces teratogenic effects by interrupting the Arachidonic Acid cascade (Kay et al., 1988). Administration of exogenous Arachidonic Acid can reverse the effect of phenytoin. The occurrence of anomalies such as small, misshapen facial arches, small or misshapen head folds, and unfused neural tubes was significantly reduced by Arachidonic Acid administration.

Exogenous administration of Arachidonic Acid has, *in vivo*, significantly reduced cortisone-induced cleft palate in fetuses (Piddington et al., 1983). *In vitro*, a dose-dependent reversal by Arachidonic Acid of cortisol inhibition of epithelial breakdown in single palatal shelves has been demonstrated.

Antimasculization of embryos was induced by a number of compounds (Gupta and Goldman, 1986). Arachidonic Acid dose-dependently reversed antimasculization produced by estradiol-17β, cyproterone acetate, cortisone, and phenytoin.

Indomethacin administration is able to stop the reversal of teratogenic effects by Arachidonic Acid (Gupta and Goldman, 1986; Piddington et al., 1983).

MUTAGENICITY

Arachidonic Acid mutagenicity studies are summarized in Table 4.

Chinese hamster embryonic lung fibroblasts, V79 cells, were used in assessing the mutagenic activity of Arachidonic Acid, based on the induction of 6-thioguanine resistance (TG^r) (Sevanian and Peterson, 1989). Incubation with 10 μM Arachidonic

TABLE 4. ARACHIDONIC ACID MUTAGENICITY STUDIES

Test	*Organism and strain*	*Methods*	*Results and comments*	*Reference*
6TG^r induction	Chinese hamster embryonic lung fibroblasts, V79 cells	Cells were incubated with ≥10 μM Arachidonic Acid for 24 h. BPdiol and nordihydroguaiaretic acid were added to some colonies. Positive and negative controls were used.	Eicosanoid concentration was significantly increased. During the first 3 h of incubation, a rapid initial uptake of Arachidonic Acid was independent of cell division; there was another distinct phase in which Arachidonic Acid incorporation appeared to be a function of cell division. After 24 h, the proportion of Arachidonic Acid in several lipid pools was increased. After 24 h treatment with ≥20 μM Arachidonic Acid, the frequency of TG^r colonies was significantly increased. Arachidonic Acid facilitated BPdiol mutagenic activity. Cell treatment with BPdiol for 2 h produced a small, dose-dependent decrease in the surviving fraction of cells; no induction of TG^r mutants. BPdiol addition to cells pretreated with Arachidonic Acid for 24 h resulted in a significant, dose-dependent increase in the frequency of TG^r colonies; facilitation of BPdiol-induced TG^r colony formation had maximum effects after pretreatment with 20–40 μM Arachidonic Acid. Nordihydroguaiaretic acid inhibited Arachidonic Acid's mutagenic activity.	Sevanian and Peterson, 1989
Sister chromatid exchange	CHO cells	Cells were incubated with Arachidonic Acid 30 min prior to the addition of 10^7 leukocytes. PMA was added to some cultures. Indomethacin, nordihydroguaiaretic acid, and piroxicam were also added.	80 μM Arachidonic Acid significantly increased the number of phagocyte-induced SCEs. PMA-stimulated phagocytes induced a significant increase in the number of SCEs. Phagocyte stimulation by Arachidonic Acid and PMA resulted in the greatest number of SCEs. Indomethacin, nordihydroguaiaretic acid, and piroxicam significantly reduced the number of Arachidonic Acid-induced SCEs.	Weitberg, 1988

Table 4. Arachidonic Acid Mutagenicity Studies (*Continued*)

Test	*Organism and strain*	*Methods*	*Results and comments*	*Reference*
Chromosomal aberration	Human lymphocytes	Cultures were treated with 0.05 ml of 1×10^{-5} M Arachidonic Acid or 0.1 ml 1×10^{-6} M BP. The effect of Arachidonic Acid and BP combined was also examined.	Arachidonic Acid significantly increased the number of chromosomal aberrations; BP also increased the number of aberrations. Arachidonic Acid and BP combined did not significantly increase the number of aberrations as compared to either compound alone.	Das et al., 1987
Sister chromatid exchange	CHO cells	CHO cell cultures were treated with Arachidonic Acid, concentration not given. The effects of the addition of an oxygen-radical generating system, inhibitors of Arachidonic Acid metabolism, and vitamin E were also examined.	Arachidonic Acid significantly increased the number of SCEs as compared to controls, but produced less than that observed with an oxygen radical generating system. The addition of Arachidonic Acid to hypoxanthine and xanthine oxidase produced a marked increase in the number of SCEs. Inhibitors of Arachidonic Acid metabolism and steroidal and nonsteroidal inhibitors of the enzymatic oxidation of Arachidonic Acid significantly decreased the number of oxygen radical-induced SCEs. Preincubation of CHO cells with 10^{-5} M vitamin E prior to the addition of Arachidonic Acid metabolism inhibitors produced no oxygen radical-induced genetic toxicity. Cells were also protected, but to a lesser extent, by vitamin E preincubation without the addition of Arachidonic Acid metabolism inhibitors.	Weitberg, 1987
Sister chromatid exchange	AHH-inducible human hematoma cell line, C-HC-4; AHH-non-inducible rat ascites hematoma cell line, AH66-B; rat esophageal neoplasm cell line, R1; Chinese hamster cell line, Don-6	The effect of Arachidonic Acid alone and Arachidonic Acid's effect on BP's and DMBA's induction of SCEs was examined. 0.05 or 0.1 mM Arachidonic Acid was used. Indomethacin was added to some cultures.	Arachidonic Acid did not significantly change the baseline SCE frequency of any of the cell lines tested. SCE induction by BP or DMBA was significantly potentiated dose-dependently by Arachidonic Acid in C-HC-4 cells. SCE induction due to Arachidonic Acid was seen to a lesser, sometimes insignificant, extent in the other cell lines. Indomethacin completely inhibited Arachidonic Acid's potentiating effect on SCE production by BP and DMBA in C-HC-4 cells.	Abe, 1986

Table 4. Arachidonic Acid Mutagenicity Studies (*Continued*)

Sister chromatid exchange	CHO cells	Cells were incubated with 320 μM Arachidonic Acid. Cultures cells were also incubated with Arachidonic Acid- and PMA-stimulated white blood cells.	Arachidonic Acid produced a significant increase in the number of SCEs. A significant increase in SCEs was seen after incubation with Arachidonic Acid-stimulated white blood cells. The greatest increase in SCEs was observed when CHO cells were incubated with PMA-stimulated white blood cells in the presence of Arachidonic Acid.	Weitberg and Calabresi, 1986
	Human urothelial cells	Cell cultures were treated with 100 μM Arachidonic Acid. Synthase-mediated metabolism of benzidine was assessed in intact tissue. 100 μM indomethacin was added to some cultures.	Radioimmunoassayable PGE_2 was increased 11-fold in response to Arachidonic Acid. 3-(glutathion-S-yl)-benzidine and [^{3}H]benzidine binding to DNA were observed. Arachidonic Acid increased the thioether synthesis sixfold and DNA binding fourfold; these increases were completely inhibited by indomethacin.	Zenser et al., 1986
MAL Growth	MAL from adult female RIII mice	Mammary gland organ cultures were maintained for 8 days in IPAH medium. On day 9, the cultures were exposed to 10 μg/ml Arachidonic Acid in medium that contained insulin as the only hormone. Controls were exposed to ethanol. Total duration in culture was 23 days. Some cultures were also exposed to 0.358 μg/ml indomethacin.	A significant increase in MALs was observed in the Arachidonic Acid-treated cultures. Cultures exposed to Arachidonic Acid and indomethacin had a significantly lower number of MALs than cultures exposed to only Arachidonic Acid.	Telang et al., 1984
[^{3}H]Thymidine incorporation	Mammary gland organ culture	Cultures were continuously exposed to 10 μg/ml Arachidonic Acid during an 8 day culture period in IPAH medium. Controls were exposed to ethanol. Test and control cultures were then exposed to 5 μCi/ml of ^{3}H-thymidine for 24 h.	Arachidonic Acid produced approximately 1.0–2.5-fold greater incorporation of ^{3}H-thymidine/mg cellular DNA than did the controls.	Telang et al., 1984

Acid for 24 h produced significantly increased concentrations of eicosanoids. During the first 3 h of incubation, a rapid initial uptake of Arachidonic Acid, independent of cell division, was observed. Another distinct phase followed in which Arachidonic Acid incorporation appeared to be a function of cell division. After 24 h, fatty acid composition analysis reported increased proportions of Arachidonic Acid in several lipid pools; V79 cell treatment with 10 μM Arachidonic Acid for 24 h approximately tripled the proportion of Arachidonic Acid in cell lipids and increased its proportions in phosphatidylcholine. The frequency of TG^r colonies was significantly increased after 24 h treatment with ≥20 μM Arachidonic Acid.

Arachidonic Acid facilitated 7,8-dihydrobenzo[a]pyrene (BPdiol) mutagenic activity. Treatment of V79 cells with BPdiol for 2 h produced a small, dose-dependent decrease in the surviving fraction of cells; there was no induction of TG^r mutants. The addition of BPdiol to cells pretreated for 24 h with 10 μM Arachidonic Acid resulted in a significant and dose-dependent increase in the frequency of TG^r colonies. Facilitation of BPdiol-induced TG^r colony formation by Arachidonic Acid was concentration dependent, with maximum effects resulting after 24 h pretreatment with 20–40 μM Arachidonic Acid. Nordihydroguaiaretic acid inhibited the mutagenic activity of Arachidonic Acid. Negative and positive controls for the study were the vehicle (ethanol) and N-methyl-N-nitroso-N-nitroguanidine, respectively.

The mutagenic activity of Arachidonic Acid was examined by counting the number of sister chromatid exchanges (SCEs) it induced in Chinese hamster ovary (CHO) cells (Weitberg, 1988). CHO cells were incubated with Arachidonic Acid 30 min prior to the addition of 10^7 leukocytes. A concentration of 80 μM Arachidonic Acid significantly increased the number of phagocyte-induced SCEs compared to the controls.

The effect of Arachidonic Acid and 12-O-tetradecanoyl-phorbol-13-acetate (PMA) on the number of SCEs was also examined (Weitberg, 1988). PMA-stimulated phagocytes induced a significant increase in the number of SCEs compared to the controls. Phagocyte stimulation with both Arachidonic Acid and PMA resulted in the production of the greatest number of SCEs. Indomethacin, nordihydroguaiaretic acid, and piroxicam, inhibitors of Arachidonic Acid metabolism, significantly reduced the number of SCEs induced by Arachidonic Acid.

The number of chromosomal aberrations was determined after human lymphocyte cultures were treated with 0.05 ml of 1×10^{-5} M Arachidonic Acid (Das et al., 1987). Arachidonic Acid significantly increased the number of chromosomal aberrations as compared to the controls.

The effect of the addition of 0.1 ml of 1×10^{-6} M benzo[a]pyrene (BP) was also examined; BP increased the number of chromosomal aberrations. Addition of 0.05 ml of 1×10^{-5} M Arachidonic Acid and BP did not produce a significant increased in the number of abberations as compared to the addition of Arachidonic Acid or BP alone.

Arachidonic Acid, concentration not given, significantly increased the number of SCEs in CHO cells compared to controls, but produced less than the number observed with an oxygen-radical generating system (Weitberg, 1987). The addition of Arachidonic Acid to hypoxanthine and xanthine oxidase, an oxygen-radical generator system, produced a marked increase in the number of SCEs. Inhibitors of Arachidonic Acid metabolism significantly decreased the number of oxygen radical-induced SCEs. Steroidal and nonsteroidal inhibitors of the enzymatic oxidation of Arachidonic Acid also significantly reduced the number of oxygen-radical induced SCEs. Preincubation of CHO cells with 10^{-5} M vitamin E prior to the addition of inhibitors of Arachidonic Acid metabolism produced no oxygen radical-induced genetic toxicity. Cells were also

protected, but to a lesser extent, by pre-incubation with vitamin E without the addition of the inhibitors of Arachidonic Acid metabolism.

The effect on SCE induction by Arachidonic Acid alone and the effect of Arachidonic Acid's presence on SCE induction due to BP or 7,12-dimethylbenz[a]anthracene (DMBA) was investigated (Abe, 1986). Doses of 0.05 or 0.1 mM Arachidonic Acid did not significantly change the baseline SCE frequency of the aryl hydrocarbon hydroxylase (AHH)-inducible human hematoma cell line C-HC-4, the AHH-noninducible rat ascites hematoma cell line AH66-B, the rat esophageal neoplasm cell line R1, or the Chinese hamster cell line Don-6.

SCE induction by BP or DMBA was significantly potentiated in the presence of Arachidonic Acid in the C-HC-4 cells; induction enhancement was dependent upon the concentration of Arachidonic Acid. SCE enhancement due to the presence of Arachidonic Acid was also seen in the other three cell lines to a lesser, and sometimes insignificant, extent. Indomethacin completely inhibited the potentiating effect of Arachidonic Acid on SCE production by BP and DMBA in the C-HC-4 cell line.

Cultured CHO cells were incubated with 320 μM Arachidonic Acid and the number of SCEs was counted (Weitberg and Calabresi, 1986). A significant increase in SCEs was induced by Arachidonic Acid. Cultured CHO cells were also incubated with Arachidonic Acid- and PMA-stimulated leukocytes. A significant increase in SCEs by Arachidonic Acid-stimulated leukocytes was observed compared to the control. The greatest increase in SCEs was observed when PMA-stimulated leukocytes were incubated with CHO cells in the presence of Arachidonic Acid.

Arachidonic Acid metabolism was studied in human urothelial cells to assess its role in bladder cancer (Zenser et al., 1986). Urothelial cells increased radioimmunoassayable PGE_2 11-fold in response to 100 μM Arachidonic Acid. The PGH synthase-mediated metabolism of the bladder carcinogen benzidine was assessed in intact tissue. In intact tissue, 3-(glutathion-S-yl)-benzidine, a thioether product of peroxidatically-activated benzidine, and ^{3}H-benzidine binding to DNA were observed. Arachidonic Acid increased thioether synthesis sixfold and DNA binding fourfold; both of these were completely inhibited by 100 μM indomethacin.

Telang et al. (1984) used an adult mouse mammary gland organ culture system assay to examine the effects of Arachidonic Acid on the survival and growth of mammary alveolar lesions (MALs) from adult female RIII mice. Mammary gland organ cultures were maintained for 8 days in medium supplemented with insulin, prolactin, aldosterone, and hydrocortisone (IPAH medium) and then, on day 9, exposed to 10 μg/ml Arachidonic Acid in media containing insulin as the only hormone; control cultures were exposed to ethanol. The total duration in culture was 23 days.

A significant increase in MAL number was observed in the cultures exposed to Arachidonic Acid as compared to the controls. Cultures exposed to 10 μg/ml Arachidonic Acid plus 0.358 μg/ml indomethacin had significantly less MALs compared to cultures exposed to Arachidonic Acid only.

Mammary gland organ cultures were continuously exposed to 10 μg/ml Arachidonic Acid during an 8 day cultivation period in IPAH medium in a study to determine the effect of Arachidonic Acid on incorporation of ^{3}H-thymidine (Telang et al., 1984). Control cultures were exposed to ethanol. After treatment, both the test and control cultures were exposed to 5 μCi/ml of ^{3}H-thymidine for 24 h. In three separate runs, Arachidonic Acid produced ≈1.0 to 2.5-fold greater incorporation of ^{3}H-thymidine/mg cellular DNA than did the controls.

CARCINOGENICITY

A brief summary of the metabolic biochemistry of Arachidonic Acid with emphasis on the Arachidonic Acid cascade provides a basis for evaluating its role in tumor promotion. The essential fatty acids, linoleic and linolenic, must be supplied by the diet; they serve as the metabolic precursors of Arachidonic Acid, an essential fatty acid. Arachidonic Acid is the precursor of several hormones known as the eicosanoids, specifically the prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT). The eicosanoids are highly effective cellular control elements. Phospholipids, particularly phosphatidyl choline, serve as the reservoir for Arachidonic Acid. Arachidonic Acid is liberated by the action of phospholipase, allowing for the synthesis of eicosanoids by the cyclooxygenase and the lipoxygenase pathways. The cyclooxygenase pathway is responsible for the synthesis of several prostanoids, including PGE_2.

Using the phorbol ester skin tumor production model system in mice, the following observations were made that established an essential role for the Arachidonic Acid cascade in skin tumor promotion. Rohrschneider and Boutwell (1972) observed that the turnover rate of phosphatidyl choline in mouse skin was increased fourfold over the basal level within 2 h after application of 18 nmol of the tumor-promoting phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) to the skin, thereby liberating increased amounts of Arachidonic Acid. Ashendel and Boutwell (1979) reported that, as a consequence of the increased availability of Arachidonic Acid and the activation of cyclooxygenase caused by TPA, PGE_2 levels in mouse epidermis were elevated 10-fold within 6 h following application of 17 nmol of TPA to skin. Furstenberger and Marks (1980) confirmed and extended this observation, reporting that the PGE_2 level in the epidermis was elevated 6-fold within 90 min of treatment with 10 nmol of TPA. Furthermore, the application of cyclooxygenase inhibitors to mouse skin in conjunction with TPA inhibited the formation of skin tumors and the inhibition was reversed by PGE_2 (Verma et al., 1980). Nakadate et al. (1982) found that several inhibitors of phospholipase A, the enzyme responsible for liberating Arachidonic Acid from phosphatidyl choline, blocked a specific response to TPA in mouse that is dependent on the Arachidonic Acid cascade. A study using SENCAR mice performed by Fischer et al. (1982) confirmed and extended the findings of Nakadate et al. (1982) to the promotion of neoplasms. They reported that the inhibition of the initial step of the Arachidonic Acid cascade (i.e., phospholipase A_2) provided the most effective inhibition of promotion. This observation by Nakadate et al. and Fischer et al. (1982) may be interpreted to mean that lipoxygenase products as well as the cyclooxygenase product, PGE_2, are critical components of the mechanisms of carcinogenesis. PGE_2 alone could not promote skin tumors (Fischer, 1987; Furstenberger et al., 1987) nor could Arachidonic Acid (Fischer et al., 1989).

Agents other than TPA as well as complete carcinogens for various organs provide evidence that enhanced activity of the Arachidonic Acid cascade is a component of the mechanisms involved in tumor promotion. Thapsigargin, a non-TPA type tumor promoter, was found to stimulate Arachidonic Acid metabolism in rat peritoneal mast cells (Ohuchi et al., 1989). Brune et al. (1978) correlated initiating and promoting activities of polycyclic aromatic hydrocarbons and of diterpene (e.g., phorbol) esters in mouse skin with their prostaglandins releasing potency *in vitro*. Releasing activity correlated with promoting activity.

Additional evidence of a role for PG in carcinogenesis and therefore indirectly for the Arachidonic Acid cascade is based on the inhibitory effect of cyclooxygenase

inhibitors on carcinogenesis in various organs. Corwall et al. (1983) found that ibuprofen given orally to hamsters inhibited hydrocarbon-induced cheek pouch cancer. Indomethacin inhibited-rat mammary gland tumorigenesis (Carter et al., 1983). McCormick et al. (1985) confirmed that observation. A dose-related inhibition of colon cancer incidence induced by azoxymethane in rats attributable to piroxicam has been reported by Pollard and Lockert (1983) and by Reddy et al. (1987). Giving humans indomethacin resulted in a small but definite *in vitro* reduction of a biochemical marker of the tumor promoter TPA (Loprinzi et al., 1985).

A study was conducted to determine the role of Arachidonic Acid in the stimulation of superoxide anion radical production by peritoneal macrophages stimulated with PMA (TPA); results of studies by Kensler and Trush (1984), Goldstein et al. (1981), and Slaga et al. (1981) suggested that free radicals and active species of oxygen may be involved in neoplasm promotion in mouse skin (Czerniecki and Witz, 1989). CD-1, C57BL/6, and SENCAR female mice were used. Inhibitors of Arachidonic Acid metabolism inhibited O_2^- production. Arachidonic Acid metabolism stimulated by neoplasm promoters, such as TPA, could potentiate the production of superoxide anion radicals by murine peritoneal macrophages.

Exogenous Arachidonic Acid added to inflammatory mouse peritoneal macrophages stimulated O_2^- production; however, Arachidonic Acid was much weaker in stimulating O_2^- production than TPA, which is a potent stimulator of the respiratory burst. Low concentrations of Arachidonic Acid potentiated by twofold the production of superoxide anion radicals by inflammatory macrophages treated with low concentrations of TPA.

CLINICAL ASSESSMENT OF SAFETY

Irritation

The irritation potential of a product containing 0.04% Arachidonic Acid was evaluated in a 24 h single insult patch test (CTFA, 1985a). The test article was applied, as received, under occlusive patch to 21 subjects; volume of test article applied, number of subjects per sex, and site of application were not given. Twenty-one subjects had patches with control material; the control material was not identified. The primary irritation index (PII) of the test material was 0.03/4. No significant difference in irritancy was observed between the test and control materials.

A second 24 h single insult patch test was performed using the same procedure as above with a product containing 0.04% Arachidonic Acid (CTFA, 1985b). There were 17 subjects in both the test and control groups. The PII of the test material was 0/4. No significant difference in irritancy was observed between the test and control materials.

SUMMARY

Arachidonic Acid is an essential, polyunsaturated, fatty acid that is used as a surfactant–cleansing agent and a surfactant–emulsifying agent in cosmetic formulations. Arachidonic Acid is a liquid at room temperature, is soluble in alcohol, ether, and water, and absorbs in the ultraviolet B (UVB) range. In 1992, it was reported to the FDA that Arachidonic Acid was used in 29 cosmetic formulations.

Arachidonic Acid is well absorbed from the gastrointestinal tract and the circulatory system, it distributes rapidly into the lipid compartment of the body, and is rapidly converted to phospholipid by the liver. Arachidonic Acid can be metabolized by three different pathways: the cyclooxygenase, lipoxygenase, and cytochrome P450 systems.

Arachidonic Acid metabolites are involved in the inflammatory process. A chronic cellular imbalance of Arachidonic, γ-linolenic, and eicosapentaenoic acids, and of their respective eicosanoid derivatives, may have major health implications. Arachidonic Acid may alter the cutaneous immune response.

In a study in which Arachidonic Acid was applied to the pinnae of mice, an increase in pinnal thickness was observed. Microscopic effects were also observed throughout the study. Application of Arachidonic Acid to mouse skin produced edema and inflammation, with high doses possibly causing ulceration of the skin.

Arachidonic Acid did not produce teratogenic effects. Exogenous Arachidonic Acid appeared to help prevent the teratogenic effects caused by hyperglycemia and phenytoin. Subcutaneous administration to pregnant diabetic rats significantly reduced neural tube fusion defects, cleft palate, and micrognathia. Arachidonic Acid has also dose-dependently reversed antimasculization caused by a number of compounds. However, indomethacin has been found to stop the reversal of teratogenic effects by Arachidonic Acid.

Arachidonic Acid has mutagenic potential. Arachidonic Acid has increased the frequency of TG^r colonies, phagocyte-induced SCEs, chromosomal aberrations, thioether synthesis, MAL number, and the incorporation of [^{3}H]thymidine/mg cellular DNA.

In 24 h single insult patch tests, a formulation containing 0.04% Arachidonic Acid was not an irritant.

DISCUSSION

The CIR Expert Panel recognizes that dermal absorption data are lacking in this report and believes that such data are necessary before a determination of safety can be made. Based on the results of dermal absorption studies, there may be a need for additional data. The studies by Rheins and Nordlund (1986) and Rheins et al. (1987) indicate that Arachidonic Acid may be involved in UV light-induced cutaneous immune suppression. Therefore, immunomodulatory data may be requested (dependent on the results of the dermal absorption studies). In addition to immunomodulatory data, carcinogenicity, photocarcinogenicity, and human irritation, sensitization, and photosensitization data may also be requested.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Arachidonic Acid are insufficient to determine whether the ingredient, under each relevant condition of use, is either safe or unsafe. The Panel released a "Notice of Insufficient Data Report" on February 12, 1992 outlining the data needed to assess the safety of Arachidonic Acid. The types of data required included:

1. Dermal absorption data

Based on the results of the absorption studies, the Panel indicated there may be a need for the following data:

2. Immunomodulatory data
3. Carcinogenicity and photocarcinogenicity data
4. Human irritation, sensitization, and photosensitization data

No offer to supply the dermal absorption data was received. In accordance with Section 45 of the CIR Procedures, the Expert Panel will issue a Final Safety Evaluation Report—Insufficient Data. When the requested new data are available the Panel will reconsider the Final Report in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

CONCLUSION

The safety of this ingredient has not been documented and substantiated for cosmetic product use. The CIR Expert Panel cannot conclude whether Arachidonic Acid is safe for use in cosmetic products until the appropriate safety data have been obtained and evaluated.

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