

Chemical Information Review Document
for
Evening Primrose Oil (*Oenothera biennis* L.)
[CAS No. 90028-66-3]

**Supporting Nomination for Toxicological Evaluation by the
National Toxicology Program**

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National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S Department of Health and Human Services
Research Triangle Park, NC
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Abstract

Evening primrose oil (EPO) has been used to treat a variety of ailments including PMS, atopic eczema, psoriasis, multiple sclerosis, cancer, coronary heart disease, diabetic neuropathy, autoimmune conditions, and gastrointestinal symptoms. It is classified as a "dietary supplement" under the Dietary Supplement Health and Education Act of 1994. Overall, the data on the efficacy of EPO for treating the various diseases indicated is limited and EPO appears to have little toxicological effect in humans. Some side effects from the use of EPO have been reported including occasional headache, abdominal pain, nausea, loose stools and seizures. The mouse LD₅₀ reported in a single acute toxicity study was 3.12×10^4 µg/kg. Subchronic and chronic studies indicated that EPO produced few toxicological effects. EPO, given in the diet starting three weeks after dimethylbenz(a)anthracene treatment, had no effect on mammary tumor incidence in female Sprague-Dawley rats but did reduce the number of tumors per rat and increased the tumor latency period. EPO also inhibited the promotion stages of skin papilloma development. *In vivo* studies showed that EPO blocked benzo(a)pyrene (BaP)-induced micronuclei in murine bone marrow cells and BaP binding to murine skin cell DNA. It is cytotoxic to a variety of cell types (e.g., human and mouse leukemic cell lines and Ehrlich ascites tumor cells). In one study, EPO enhanced male reproductive function in ICR mice (e.g., number of successful copulations during a three-hour period, the number of sperm-positive females) but had no effect on female reproduction in Wistar rats. Results from studies of EPO's carcinogenic effects were conflicting; EPO increased the mean tumor mass in C57 mice transplanted with BL6 melanoma cells but had no effect on either tumor growth in mice that had murine sarcoma allografts or tumor incidence in CD-1 mice. Additional activities of EPO included immunomodulatory, cardiovascular, enzyme, endocrine, and antibacterial, antifungal, and antioxidant effects.

Executive Summary

Basis for Nomination

Evening primrose oil (*Oenothera biennis*, *O. biennis*, EPO) was nominated for toxicological characterization by the National Institute for Environmental Health Sciences because of its widespread use as a dietary supplement, particularly for immune conditions, and the lack of adequate toxicological data. There is also concern regarding potential adverse effects among populations that use EPO regularly for the treatment of other disorders such as bronchitis, premenstrual syndrome (PMS), menopause, diabetes, and rheumatoid arthritis.

Nontoxicological Data

Evening primrose is a biennial weed of the Onagraceae family, native to North America and found in parts of Asia and Europe. It is grown commercially in more than 30 countries. EPO consists of a variety of essential fatty acids, including γ -linolenic acid (GLA), linoleic acid (LA), oleic acid, palmitic acid, and stearic acid. It has been used to treat a variety of ailments including PMS, atopic eczema, psoriasis, multiple sclerosis, cancer, coronary heart disease, diabetic neuropathy, autoimmune conditions, and gastrointestinal symptoms. Constituents of EPO are typically identified using gas chromatography-mass spectrometry or high-performance liquid chromatography techniques. EPO is commercially available in a variety of forms (e.g., capsules) via the Internet, natural food stores, drug stores, chemical companies, and other retail stores. EPO is marketed in the United States as a dietary supplement and is classified as a "dietary supplement" under the Dietary Supplement Health and Education Act of 1994. It is produced by cold-pressing with screw presses or by extracting with hexane; the crude product is then refined. Annual global production of EPO was estimated to be between 1000 and 4000 metric tons in 2000. In the United Kingdom, EPO has been licensed for the treatment of mastalgia, PMS, and prostatitis. EPO also is approved for atopic dermatitis and eczema in several countries but not the United States.

Human Data

Several reviews of the efficacy of EPO have been published. These reviews suggest that, except atopic dermatitis, there is not significantly strong evidence for the use of EPO for most of the indicated ailments. Side effects that have been reported are occasional headache, abdominal pain, nausea, and loose stools. Seizures have also been reported in some persons taking EPO. There were 193 adverse reactions to EPO reported from 1968-1997. The severe cases included convulsions, aggravated convulsions, face edema, and asthma. Many EPO interactions with drugs, herbs/supplements, and lab tests are theoretical. A previously published theory that EPO may increase the risk of seizure when used simultaneously with anesthetics or antipsychotics and may lower seizure threshold when used with anticonvulsant agents or stimulants was re-examined. The results suggested that the association of EPO with seizures was spurious.

Toxicological Data

No initiation/promotion, genotoxicity, cogenotoxicity, or immunotoxicity studies were available.

Chemical Disposition, Metabolism, and Toxicokinetics

Rat tissues are more active in conversion of LA and α -linolenic acid to longer-chain polyunsaturated fatty acids (PUFAs) than humans. In experimental animals (rats, mice, guinea pigs, and cats) EPO increases GLA and dihomo-GLA in erythrocytes and tissue phospholipids or triacylglycerols.

MCF7 cells differentially metabolized EPO and GLA. GLA supplementation increased GLA levels in cells after 72 hours. Comparatively, supplementation with EPO increased LA, but not GLA, levels.

Acute Toxicity

A single study in mice (sex and strain not provided) reported an LD₅₀ of 3.12×10⁴ µg/kg.

Short-Term and Subchronic Exposure

Male rats maintained on a diet supplemented with 11% EPO for 6 weeks showed no difference in body weights when compared to control and other treated groups.

Chronic Exposure

Overall, the chronic exposure studies in rats all showed dietary or gavage administration of EPO did not affect body weight gain or food consumption. One study noted gavage administration of EPO to rats increased potassium levels in female rats at 25 and 51 weeks and reduced liver weights in male treated rats. Testicular shrinkage or softening also was noted.

Synergistic/Antagonistic Effects

Synergistic/Antagonistic Effects of EPO

EPO has been reported to antagonize the effects associated with various chemicals. For example, EPO altered mercuric chloride-induced autoimmune glomerulonephritis in female Brown-Norway rats. EPO also attenuated many of the alterations in lipid content and fluidity produced by ethanol. An animal model of fetal alcohol syndrome showed that concomitant administration of EPO with ethanol improved both male and female offspring learning ability and activity. EPO also reversed *in vivo* effects of cyclosporine A on tissue lipid composition in specific tissues and the effects of carbon tetrachloride on blood sugar levels, glucose-6-phosphatase and glycogen phosphorylase activities, and liver glycogen content.

Anticarcinogenicity

Numerous studies have shown that EPO has anticarcinogenic effects. For example, several studies showed that EPO can affect mammary tumor progression. Dietary supplementation with EPO after a single dose of dimethylbenz(a)anthracene resulted in a tumor incidence rate similar to levels observed in corn oil fed animals. Additionally, the number of tumors per tumor bearing rat and the total number of tumors were decreased and the tumor latency period was increased in EPO-treated rats. In an initiation and promotion model of skin carcinogenesis, EPO effectively inhibited the promotion stages of skin papilloma development. A concurrent increase in skin lipid peroxidation during the promotion stage and modulation of fatty acid levels during the initiation and promotion stages also were noted.

Antigenotoxicity

In vivo studies showed that EPO blocked benzo(a)pyrene (BaP) induced changes in murine bone marrow cells. EPO also significantly inhibited BaP binding to murine skin cell DNA.

Cytotoxicity

EPO is cytotoxic to a variety of cell types. A phenolic fraction purified from defatted seeds of *O. biennis*, which contained gallic acid, induced selective apoptosis in human and mouse leukemic cell lines and inhibited [³H]-thymidine incorporation in CaCO₂ and WEHI164 cells. Studies in Ehrlich ascites tumor cells showed that evening primrose extract induced apoptosis, decreased intracellular polyamine levels, increased the formation of reactive oxygen species, induced a dose-dependent increase in the number of cells in the G1 phase, inhibited DNA synthesis, induced an increase in the loss of mitochondrial membrane potential, and increased release of cytochrome c.

Reproductive and Teratological Effects

Oral administration to male ICR mice for 28 days increased testis weight, the number of complete penile insertions during a three-hour period, the number of sperm-positive females, and serum testosterone

levels in one study. Comparatively, dietary supplementation had no effect on parturition, birth weight, postnatal growth rate, maternal weight during pregnancy, and fetal or placenta prostaglandin E₂ levels.

The developmental effects of EPO in zinc deficient animals are inconsistent. Concomitant administration of EPO to weanling and pregnant rats fed a zinc-deficient diet did not improve zinc deficiency-induced fetal toxicity, teratogenicity, or growth retardation in one study. In another study, EPO decreased maternal fluid loss and litter size, and increased neonate survival in control and some zinc deficient animals.

Carcinogenicity

Dietary supplementation with EPO, given to mice transplanted with BL6 murine melanoma cells, increased mean tumor mass when compared to other diets. However, these results contrast other results obtained in mice with murine sarcoma allograft, where EPO had no effect. Additionally, long-term studies in rats showed that EPO had no effect on tumor incidence up to 104 weeks after initiation of the study. Additional dietary components (e.g., d- α -tocopherol) could modulate the effects produced by EPO on melanoma tumor growth.

Genotoxicity

No data were available.

Other Data

EPO has a variety of additional activities, including immunomodulatory, cardiovascular, enzyme, endocrine, and antibacterial, antifungal, and antioxidant activities.

Structure-Activity Relationships

No data were available that were directly applicable.

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1.0 Basis for Nomination

Evening primrose oil (*Oenothera biennis*, *O. biennis*, EPO) was nominated for toxicological characterization by the National Institute for Environmental Health Sciences because of its widespread use as a dietary supplement, particularly for immune conditions, lack of adequate toxicological data, and concern regarding potential adverse effects among populations that use EPO regularly in the treatment of various disorders including bronchitis, premenstrual syndrome (PMS), menopause, diabetes, and rheumatoid arthritis.

2.0 Introduction

Evening primrose (*Oenothera biennis*, *O. biennis*) is a biennial weed of the Onagraceae family, native to North America and found in parts of Asia and Europe. It has large fragrant yellow blooms that open all evening. It thrives under adverse climactic conditions, in dunes, sandy soils, roadways, meadows, ground wastes, or open areas. Although the entire plant is edible; the flowers are added in salads, leaves eaten like greens, and the roots boiled like potatoes, it is primarily a minor oilseed crop used to produce the dietary supplement EPO (ACS, 2009; Altnature Herbals, undated; Budavari, 1996; herbs2000.com, 2009; Natural Standard, 2008).

O. biennis is grown commercially in more than 30 countries. In North America, most commercially grown *O. biennis* is in Canada (Nova Scotia and Ontario) and the eastern United States in areas of high rainfall and moderate temperature. Production of EPO from *O. biennis* largely occurs in China (>90% of the annual supply) either by cold-pressing with screw presses or by extracting with hexane. The crude EPO extract contains high levels of antioxidants, including tocopherols, which are removed during refinement (Eskin, 2008; Lapinskas, 2000). Therefore vitamin E and/or other antioxidants may be added to the final product before packaging to improve oxidative stability (Khan and Shahidi, 2001).



Source: [Altnature Herbals \(undated\)](#)

2.1 Chemical Identification and Analysis

2.1.1 Evening Primrose Oil

EPO (CASRN: 90028-66-3) consists of a variety of fatty acids, including the non-essential ω -6 polyunsaturated fatty acid γ -linolenic acid (GLA), the essential ω -6 polyunsaturated fatty acid linoleic acid (LA), oleic acid, palmitic acid, and stearic acid. Its major constituents have been identified as LA (~75%) and GLA (~9%) (Christie, 1988 [PMID:3235598], 1991). Other common names and trade names for preparations of EPO include:

Evening Primrose (*Oenothera biennis*) oil [seed]; *Oenothera biennis* Oil; Echte Nachtkerze; fever plant; γ -linolenic acid; herbe aux anes; Huile D'Onagre; ksempe natlys;

King's Cureall; la belle de nuit; linoleic acid; nachtkerzenol; night willow-herb; *Oenothera biennis* L; *Oenothera communis* Leveill; *Oenothera graveolens* Gilib; omega-6 essential fatty acid; scabish; Spach; stella di sera; sun drop; Teunisbloem; Tree Primrose; Sundrop; Evening Star; Field Primrose; Scurvish; Bronchicum[®] Tropfen (thyme and primrose); Bronchipret[®] TP FCT (thyme and primrose)

Sources: Budavari (1996); ChemIDplus (undated-a); evening-primrose-oil.com (2003); [Global Herbal Supplies](http://GlobalHerbalSupplies.com) (2008); herbs2000.com (2009); Natural Standard (2008)

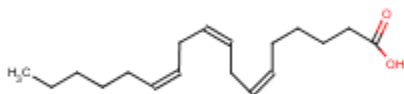
EPO and Triacylglycerols

Most of the fatty acids in EPO and other dietary lipids, such as borage oil, are in triacylglycerols (triglycerides; triacylglycerides; TAGs). The TAG structure is expected to affect absorption, metabolism, and biological activity. Many studies have reported that the fatty acid at the stereochemical number (sn)-2 position is more favorably absorbed (Mottram et al., 1997). In general, ingested TAG is converted to two fatty acids, from the sn-1 and sn-3 positions, and a 2-monoacylglycerol in the small intestine, which are then absorbed into the circulation (Murota and Storch, 2005).

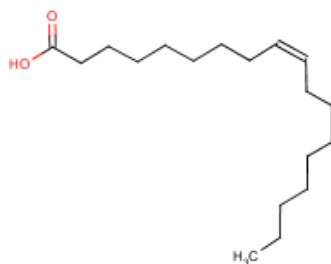
Some studies have suggested that GLA from EPO has greater biological activity than that from borage oil, which contains twice as much GLA (Redden et al., 1998). Only 7% of the GLA in EPO, compared to 36% in borage oil, is present in TAGs containing two or three GLA moieties. *In vitro* studies indicate that hydrolysis of TAGs with one versus two or more GLA moieties is much faster (80% of the GLA-containing TAGs in EPO versus 20% in borage oil). Hydrolysis was even more favorable when the GLA-containing TAG also contained two LA moieties. The presence of other acyl groups from long-chain monounsaturated and saturated fatty acids retarded the release of GLA from the sn-1 and sn-3 positions of the glycerol backbone (Huang et al., 1995).

2.1.2 Major Constituents

γ -Linolenic Acid (GLA)
[506-26-3]



Linoleic Acid (LA)
[60-33-3]



Identification

γ -Linolenic Acid (C₁₈H₃₀O₂; mol. wt. = 278.4296) is also called:

6,9,12-Octadecatrienoic acid, (Z,Z,Z)-
Acide gamolenique [French]
Acido gamolenico [Spanish]

Acidum gamolenicum [Latin]
Gamma-Linolenic acid

PubChem CID: [5280933](#)

InChI: 1/C18H30O2/c1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18(19)20/h6-7,9-10,12-13H,2-5,8,11,14-17H2,1H3,(H,19,20)/b7-6-,10-9-,13-12-

Smiles: CCCCC\C=C/C\C=C/C\C=C/C\CCCC(=O)O

Linoleic Acid (C₁₈H₃₂O₂; mol. wt. = 280.44548) is also called:

9,12-Octadecadienoic acid, (Z,Z)-
9-cis,12-cis-Linoleic acid
9Z,12Z-Linoleic acid
all-cis-9,12-Octadecadienoic acid
alpha-Linoleic acid
cis,cis-9,12-Octadecadienoic acid
cis,cis-Linoleic acid
cis-9,cis-12-Octadecadienoic acid
cis-delta9,12-Octadecadienoic acid
Emersol 310
Emersol 315
Extra Linoleic 90
Grape seed oil
Linolic acid
Polylin 515
Polylin No. 515
Telfairic acid

PubChem CID: [5280450](#)

InChI: 1/C18H32O2/c1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18(19)20/h6-7,9-10H,2-5,8,11-17H2,1H3,(H,19,20)/b7-6-,10-9-

Smiles: C(C\C=C/C\C=C/CCCC)CCCCC(O)=O

Sources: ChemIDplus (undated—b-c); PubChem (undated)

GLA Background

GLA plays a role in maintaining membrane integrity and fluidity (Natural Standard, 2008). Other physiological roles attributed to GLA include control of skin impermeability to water and the permeability potential of other membranes, and regulation of cholesterol synthesis and transport (Horrobin, 1992). Recent reviews provide detailed information on the biochemistry and physiological effects of GLA (e.g., Das, 2006a [PMID:[16892270](#)]).

GLA is available over the counter and has been used to treat numerous conditions (e.g., skin diseases, diabetic complications, auto-immune disorders, chronic fatigue syndrome, and depression). Based on results from well-designed clinical trials, there is evidence that it is useful for the treatment of rheumatoid arthritis, acute respiratory distress syndrome, and diabetic neuropathy. It also has been used together with tamoxifen in the treatment of breast cancer (Horrobin, 1992; Natural Standard, 2008). The Δ 6-desaturase activity required for conversion of LA to GLA may be reduced in inflammatory diseases and stress as well as in aged humans and

animals. Theoretically, dietary supplementation with EPO or other GLA-containing oils should be beneficial for conditions associated with $\Delta 6$ -desaturase depletion (Horrobin, 1992).

The metabolism of essential fatty acids in humans and other animals has been described extensively in numerous reviews and articles (e.g., Das, 2006b [PMID:17168663]; Horrobin, 1992; Mir, 2008). Basically, the process is a series of desaturation and elongation reactions starting from α -linolenic acid and LA. LA is converted to GLA by $\Delta 6$ -desaturase, which is the rate-limiting step. GLA is then elongated by elongase to dihomo-GLA (DGLA) which then may be converted to the 1 series prostaglandins or the 3 series leukotrienes by cyclooxygenases (COX1 and COX2); to 15-hydroxy DGLA by 15-lipoxygenase; or to arachidonic acid by $\Delta 5$ -desaturase, another rate limiting step. Arachidonic acid may be further converted to the pro-inflammatory 2 series prostaglandins by COX1 and 2, or 4 series leukotrienes by 5-lipoxygenase (Mir, 2008).

GLA is generally considered nontoxic. No significant side effects were noted in numerous clinical trials conducted and it has been shown to be well tolerated for up to 18 months in humans (Natural Standard, 2008). Long-term (1-2 years) animal studies in dogs, mice, rats, and rabbits support these results. At doses ranging from 400 to 800 mg/kg/day, no toxic effects were attributed to GLA administration (Horrobin, 1992).

Anticarcinogenic properties have been demonstrated for GLA (Das, 2006b [PMID:17168663]). In a two-stage model of skin cancer using benzo[a]pyrene (BaP) as the initiator and croton oil/tetradecanoyl phorbol-13-acetate as the promoter, GLA significantly inhibited papilloma formation during the initiation phase (Ramesh and Das, 1996 [PMID:8620442]).

2.1.3 Analysis

A variety of methods, including high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), thin layer chromatography (TLC), and gas chromatography have been used to separate and identify components of EPO. A sample of publications is provided below that describe the use of these methods and the results obtained.

Silver ion HPLC and gas chromatography have been used to separate and identify components of commercial EPO. Analysis of fatty acid composition indicated the presence of palmitic acid (6.7 mol %), stearic acid (1.6 mol %); oleic acid (7.8 mol %), LA (74.9 mol %), and GLA (8.9 mol %) (Christie, 1991). A variety of TAG molecular species that differed in saturation were present in EPO (Christie, 1988 [PMID:3235598], 1991).

Commercial EPO samples (n=22; 16 raffinated or partially raffinated, 5 cold-pressed, and one extracted with carbon dioxide [CO₂]) were prepared by solid-phase extraction and components were separated by HPLC with photodiode array detector (PAD). Peak identity and purity was confirmed by electrospray ionization mass spectrometry and the photodiode array spectra. Caffeoyl derivatives of betulinic, morolic, and oleanolic acid were identified in seven of the 16 raffinated oil samples (average ester content was 4.7 mg/100 g). The total average ester content was 58 mg/100 g in the cold-pressed extracts and 27 mg/100 g in the CO₂ extracted oils (Knorr and Hamburger, 2004 [PMID:15161190]).

Acylglycerol components of several vegetable oils, including EPO, were identified by HPLC/atmospheric pressure chemical ionization mass spectrometry. EPO showed large amounts (not specified) of LA and linolenic acid-containing TAG. The method did not allow for identification of the form of linolenic acid (i.e., α or γ form); however, fatty acid methyl ester analyses indicated that there was little α -linolenic acid in the sample (Mottram et al., 1997).

Comparison of components of a virgin organic cold-pressed, non-refined EPO preparation to a commercially available refined EPO preparation was conducted using [^{13}C] NMR spectroscopy. The spectra indicated the presence of at least one compound in the cold-pressed, non-refined sample that was not in the commercial preparation. Based on comparison to the literature, the substance was proposed to be lup-20(29)en-28-oic-3 β -yl caffeate (Puri, 2004 [PMID:14729014]).

Seed samples (collected from five cultivars and eight wild evening primrose plants) were ground, cryomilled, mixed with 5 g kieselgur (an adsorbent; [The Free Dictionary \[2009\]](#)), packed into 22 mL extraction cartridges, and extracted twice with 80% (v/v) ethanol using a pressurized liquid extraction method. The extracts of each extraction cycle were evaporated to dryness and analyzed by HPLC with PAD. The total amount of triterpenoid esters in the samples tested ranged from 1.5 to 2.8 mg/g (Zaugg et al., 2006 [PMID:16939318]).

Evening primrose meal crude extracts were processed with 56% (v/v) acetone and separated into six fractions using a Sephadex LH-20 column. Analysis of the fractions using a silica gel TLC and preparative HPLC indicated the presence of three compounds. Structural analysis by NMR and electron impact mass spectrometry identified the compounds as (+)-catechin, (-)-epicatechin, and gallic acid. TLC analysis identified catechins in each fraction, crude extract, and the meal at 35.4, 10.1, and 1.62% (w/w) and gallic acid at 6.33, 0.413, and 0.066% (w/w). These compounds amounted to approximately 10.5% and 1.7% of the dry mass of crude extract and meal, respectively (Wettasinghe et al., 2002 [PMID:11853516]).

2.2 Physical-Chemical Properties

EPO is a clear, golden yellow oil that has a density of 0.9283/cm³ (Budavari, 1996).

Property	Information	Reference(s)
<i>γ-Linolenic Acid</i>		
Physical State	not available	
Odor	not available	
Boiling Point (°C)	379.5 \pm 11 @ 760 Torr	Registry (2009a)*
Melting Point (°C)	not available	
Flash Point (°C)	276.4 \pm 14.4	Registry (2009a)*
Vapor Pressure (Torr)	8.26 \times 10 ⁻⁷ @ 25 °C	Registry (2009a)*
Density (g/cm ³)	0.924 \pm 0.06 @ 20 °C	Registry (2009a)*
Water Solubility	not available	
Octanol-water partition coefficient (log K _{ow})	not available	
LogP	6.568 \pm 0.336 @ 25 °C	Registry (2009a)*
Bioconcentration Factor	57684.77 - 10.59 @ pH 1-10, respectively @ 25 °C	Registry (2009a)*
<i>Linoleic Acid</i>		
Physical State	colorless oil	Budavari (1996)
Odor	not available	

Property	Information	Reference(s)
Boiling Point (°C)	202 @ 14 Torr, 230 @ 16 Torr 210, 365.2 (141 - 205 @ 0.17 - 1 Torr)	Budavari (1996) Registry (2009b)
Melting Point (°C)	-12 -11 - 54	Budavari (1996) Registry (2009b)
Flash Point (°C)	273.0 ± 14.4	Registry (2009b)*
Vapor Pressure (Torr)	3.54 × 10 ⁻⁶ @ 25 °C	Registry (2009b)
Density (g/cm ³)	0.9038, 0.9007 1.03 (0.9016 - 0.9122 @ 20 or 25 °C)	Budavari (1996) Registry (2009b)
Water Solubility (mg/L)	0.139 mg/L @ 25 °C (estimated)	HSDB (2008)
Octanol-water partition coefficient (log K _{ow})	7.05	HSDB (2008)
LogP	7.180 ± 0.256 @ 25 °C	Registry (2009b)*
Bioconcentration Factor	168085.28 - 30.95 @ pH 1-10, respectively @ 25 °C	Registry (2009b)*

*calculated properties using Advanced Chemistry Development (ACD/Labs) Software V8.14 (©1994-2008 ACD/Labs)

2.3 Commercial Availability

EPO is commercially available in capsules and gel-caps, in liquid form, and as a powder for use in tea preparation. It is available in many health food and other retail stores (ACS, 2009; herbs2000.com, 2009). Chemical companies from the United States (e.g., Sinova Inc. and Charkit Chemical Corporation) and China (e.g., Dayang Chemicals Co., Ltd; Shandong Yaroma Perfumery and Chemicals Co., Ltd; yick-vic; and Commingchem Co., Ltd.) are listed as suppliers of EPO (ChemExper, 2008). Evening primrose products are available as evening primrose with fish oil capsules, dried evening primrose, evening primrose essential oil, and evening primrose capsules. EPO products include Efamol, Efamast, Epogram, Bronchicum[®] Tropfen, and Bronchipret[®] TP FCT (Budavari, 1996; Global Herbal Supplies, 2008; Natural Standard, 2008).

Cold-pressed EPO products (e.g., Nature's Way EFA Gold EPO; Freedom from Menopause EPO; NOW's EPO; and EPO 9% GLA certified organic cold pressed) are available online (Bio-Alternatives, 2008; Google, 2009).

Additives and Potential Impurities in EPO

Oils with a high content of PUFAs are subject to air oxidation. Direct oxygen attack produces hydroperoxides, which decompose over time to aldehydes and secondary oxidation products. The decomposition products may polymerize in highly oxidizing environments. The oxidation process is autocatalytic, increasing its rate exponentially. Since refinement of EPO removes the high level of antioxidants, like γ -tocopherol, that are present in the crude extracts, tocopherols or artificial antioxidants are sometimes added to the refined EPO to improve oxidative stability (Eskin, 2008; Khan and Shahidi, 2001; Lapinskas, 2000). Studies have reported that freshly opened EPO contained hydroperoxides and that the total tocopherol content was inversely related to hydroperoxide content. Hydroperoxide content increased with age while tocopherol decreased (Nourooz-Zadeh, 1998).

EPO was among the dietary supplements that the United Kingdom Ministry of Agriculture, Fisheries, and Food analyzed for metal content (MAFF, 1998). Polish field-grown evening primrose showed low to very low uptake of heavy metals from soils into the seed oil (Markiewicz et al., 1994).

A commercial product manufactured in the United Kingdom that contains cold-pressed EPO and is nonraffinated contains free-radical-scavenging *trans*-caffeoyl esters of pentacyclic triterpenols that may have health benefits (Puri, 2004 [PMID:14729014]). EPO also contains a great variety of triterpenic alcohols in its nonsaponifiable fraction (1.88%), the major one being lupeol (32.0%) (Ntsourankoua and Artaud, 1997). Trace amounts of some of the compounds that have been extracted with polar solvents from evening primrose seed or from the seed cake/meal that is left after extraction of the crude oil could also be present in the refined oil. Such compounds include saponins and steroids, and polyphenols such as gallic acid (Karamac et al., 2006; Xiao et al., 2007).

3.0 Production Processes

Agriculture

Evening primrose is an established minor oilseed crop with seed yields that are generally 0.3-0.8 metric tons (t)/hectare. New varieties have been bred that produce nonsplitting seed pods to increase seed yields (BioMatNet, 2007). The prices for Chinese-produced EPO are low and tend to discourage extensive cultivation of evening primrose in other countries (Lapinskas, 2000). Other *Oenothera* species that have been cultivated for medicinal oilseed include *O. glazioviana* Micheli (also called *O. lamarckiana*) and *O. parviflora* Micheli (Small and Catling, 1999).

Extraction

EPO is produced in China by cold-pressing with screw presses or by extracting with hexane. The crude EPO is then refined. Residual oil may be solvent-extracted from the press cake, but the resulting product requires more extensive refining. The introduction of new extraction and refining equipment in collaboration with Western companies was expected to improve the quality of Chinese EPO (Lapinskas, 2000). A review of concentration and purification processes (e.g., crystallization, urea fractionation, and enzymic enhancement) is available (Gunstone, 1997).

4.0 Production and Import Volumes

Combined U.S. and Canadian EPO seed production was about 300–400 t in 1988. Estimated combined production was less than 200 t in 1999. Annual global production was estimated to be 1000–1500 t in 2000 by one source and about 4000 t by another. The latter source stated that global annual production of evening primrose seeds had increased 20-fold from 1979 to 1999. In 1999, Chinese seed production was 16,000–19,000 t (Deng et al., 2001; Lapinskas, 2000; McKenney and Auld, undated; Small and Catling, 1999).

EPO has consistently been one of the top 20 herbal dietary supplements sold in food, drug, and mass market retail outlets; it typically has been ranked from 11 to 13 among all herbal supplements. Total sales of EPO ranged from \$8,422,798 – \$9,005,161 between 1998 and 1999, and \$3,901,131 – \$6,088,103 between 2001 and 2008. Sales of EPO have generally decreased from 2001 to 2008; yearly decreases (vs. the previous year) have ranged from 3.6% to 14%. [Note: Prior to 2001, it appears that Wal-Mart sales were included in the total sales of herbal supplement but starting in 2001 this data was excluded] (American Botanical Council, 2008; Blumenthal, 2000, 2002, 2005; Blumenthal et al., 2006; Cavaliere et al., 2009) Total estimated dollar sales of EPO from natural and health food stores in 2008 show that EPO was the 20th top

selling botanical dietary supplement; total sales were \$4,547,996, which represented a 5.75% decline when compared to the previous year (Cavaliere et al., 2009). [Note: Cavaliere and colleagues (2009) note that different definitions and coding techniques are used to classify and evaluate the sales data. Therefore, sales information from food, drug, and mass market retail outlets may not be directly comparable to sales information from natural and health food stores.]

5.0 Uses

EPO has been used for a wide range of conditions including PMS, mastalgia, hot flashes, atopic eczema, psoriasis, acne, ulceration, osteoporosis, Raynaud's syndrome, multiple sclerosis, Sjogren's syndrome, cancer, hypercholesterolemia, coronary heart disease, child dyspraxia, recurrent claudication, alcoholism, Alzheimer's disease, schizophrenia, chronic fatigue syndrome, asthma, diabetic neuropathy, neurodermatitis, attention deficit hyperactivity disorder, myalgia, dysmenorrhea, depression, allergies, whooping cough, inflammation, asthma, autoimmune conditions, obesity, and gastrointestinal symptoms. It is also used for pre-eclampsia prevention during pregnancy, shortening and stimulating labor, and for preventing early delivery. It is used as a dietary source of essential fatty acids and in the production of soaps and ingredients in cosmetics (ACS, 2009; Budavari, 1996; evening-primrose-oil.com, 2003; herbs2000.com, 2009; Natural Medicines Comprehensive Database, 2008).

Different ranges of EPO doses have been used in trials and/or for treatment of various disease states. For treatment of mastalgia or PMS, 3-4 and 2-4 g/day were recommended. For rheumatoid arthritis, doses have ranged from 540 mg/day to 2.8 g/day. Daily doses of 4-8 g/day and 2-4 g/day were used for the treatment of atopic eczema in adults and children, respectively. For the treatment of acute bronchitis, different dosing regimens also have been evaluated using a primrose-thyme combination. In one evaluation, a single tablet of Bronchipret[®] TP FCT was administered three times a day for 11 days. Two other products, Bronchicum[®] Elixir S and Bronchicum[®] Tropfen, also have been evaluated (Artz, 2007; Natural Medicines Comprehensive Database, 2008; Natural Standard, 2008).

Native American tribes reportedly used EPO seeds as food; the entire plant as a poultice applied to bruises; the root extract to control hemorrhoids; and the leaves to treat superficial wounds, gastrointestinal symptoms, and sore throats (Natural Standard, 2008).

6.0 Environmental Occurrence and Persistence

O. biennis is widely naturalized in North America, where it is a native species, and other temperate and subtropic regions. In North America, it is found in the Canadian provinces (more frequently found in the eastern provinces) and in Texas, New England, North Central, and Southeastern states (USDA ARS NGRP, 2009). Another U.S. Department of Agriculture source showed a North American distribution map with occurrence in all states except Alaska, Hawaii, Idaho, Wyoming, Utah, Colorado, and Arizona (USDA NRCS, undated). Evening primrose is frequently found as a weed along roads, waste places, and abandoned land, and growing in high sandy and gravelly soils. The predominant wild plant in western North America is *O. biennis* var. *conescens* (Small and Catling, 1999).

7.0 Human Exposure

The general population is exposed to EPO from its use to treat numerous ailments such as those listed in Section 5.0 above (Natural Standard, 2008). The 2002 National Health Interview Survey extrapolated interview results indicated 4.7% of the U.S. population used EPO (Gardiner et al., 2007 [PMID:17405675]). Some U.S. groups surveyed reported either no use or limited use (<10% of respondents) of EPO. These groups included: cosmetic surgery patients and the general public in Los Angeles, CA; adolescents; college students; migrant farm workers; ambulatory patients who received prescriptions at a primary care practice in Boston, MA; peri- or postmenopausal women attending a health conference in San Francisco, CA; New Mexican complementary therapy user with rheumatoid arthritis (GLA given as a variety of forms including EPO); and patients interviewed before surgery (Graham et al., undated; Heller et al., 2006 [PMID:16462323]; Herman et al., 2004; Norred, 2002 [PMID:11969062]; Poss et al., 2005 [PMID:15859058]; Wilson et al., 2006 [PMID:16549299]). A study of peri- and postmenopausal women at the University of Illinois at Chicago clinics showed that evening primrose was used by <15% of the respondents (Mahady et al., 2003 [PMID:12544679]).

Since 1985, EPO has been recommended as a remedy for long prodromal labor and post-dates pregnancy. Since 1993, its use as a "natural" way to promote cervical ripening has been advocated by the free-standing birth center in Delaware (Dove and Johnson, 1999 [PMID:10380450]). A national survey of certified nurse-midwives on the use of herbal preparations showed that of those that recommend its use to stimulate labor, 60% (54/90) used evening primrose oil (McFarlin et al., 1999 [PMID:10380441]).

Cancer patients have been known to use complementary alternative medicines. In a study of 164 patients, 33 (19.9%) reported taking EPO; patients with breast cancer used it for mastalgia or hormonal disturbances (Werneke et al., 2004). Comparatively, another study indicated that EPO was not among the top-ten supplements used by another population of cancer patients (Gupta et al., 2005 [PMID:15856334]).

EPO was not on the top-ten list of most popular U.S. herbal supplements from 1997-2000, although its use is apparently growing as evidenced by a trend noted in New Mexican elderly patients (IARC, 2002; Wold et al., 2005 [PMID:15635346]). It ranked ninth in popularity in a 1998 Canadian survey, and a more recent survey listed EPO as second in popularity among herbal supplements among women in Ontario (IARC, 2002; Pakzad et al., 2007 [PMID:17985680]).

8.0 Regulatory Status

In the United States, EPO is regulated as a "dietary supplement" under the Dietary Supplement Health and Education Act of 1994 (DSHEA). Under the DSHEA, the manufacturer is responsible for ensuring that its dietary supplement products are safe before they are marketed. Unlike drug products that must be proven safe and effective for their intended use before marketing, there are no provisions in the law for U.S. Food and Drug Administration (FDA) to "approve" dietary supplements for safety or effectiveness before they reach the consumer. Once the product is marketed, FDA has the responsibility for showing that a dietary supplement is "unsafe," before it can take action to restrict the product's use or removal from the marketplace (FDA, 2009). In 1990, the FDA banned EPO from importation (import alert No. 66-04),

claiming that the oil was an "unapproved food additive" and therefore unsafe until otherwise proven. The ban was removed in December 1994 due to the DSHEA, which stated that dietary supplements were not food additives (Blumenthal, 1995).

In the United Kingdom, EPO has been licensed for the treatment of mastalgia, PMS, and prostatitis. It is considered first-line therapy in other European countries. Additionally, EPO is approved for atopic dermatitis and eczema in several countries (e.g., Germany and the United Kingdom) but not the United States (Janke, 2004; Natural Standard, 2008). EPO is approved as an over-the-counter product for essential fatty acid-deficiency disorders in Canada, classified as a natural product in Sweden, and permitted for use as food in Germany (Artz, 2007).

9.0 Toxicological Data

9.1 General Toxicology

Several reviews of studies of the efficacy of EPO have been published (e.g., Artz, 2007; Barre, 2001; Horrobin, 1992). Key information provided in the Natural Standard Monograph for EPO has been briefly summarized and included here (Natural Standard, 2008). No attempt was made to incorporate all of the information from the monograph into this report; rather, the reader is referred to the monograph (as well as the other mentioned reviews) for details. In the following sections, new studies not cited in the monograph have been included.

The Natural Standard Monograph summarizes results from the numerous studies of EPO for the treatment of various ailments. Disorders include atopic dermatitis, diabetes, osteoporosis, menopause, schizophrenia, and breast cancer, cysts, and pain. Evidence for most uses, excluding atopic dermatitis, is not rated as strong. Either unclear scientific evidence for the use or fair scientific evidence against the use of EPO for treating specific ailments was reported as shown below:

- Unclear scientific evidence for use: acute bronchitis, breast cancer/cysts/pain (mastalgia), chronic fatigue syndrome/postviral infection symptoms, diabetes, diabetic neuropathy, ichthyosis vulgaris, multiple sclerosis, obesity/weight loss, osteoporosis, pre-eclampsia/high blood pressure of pregnancy, Raynaud's phenomenon, and rheumatoid arthritis
- Fair scientific evidence against use: asthma, attention deficit hyperactivity disorder, cardiovascular health, menopause, PMS, psoriasis, and schizophrenia (Natural Standard, 2008).

The above ratings, however, are not consistent within reviews. For example, the clinical evidence regarding treatment of dermal conditions with EPO was reported as "mixed" by Artz (2007) and stated as "possibly ineffective" by [Natural Medicines Comprehensive Database \(2008\)](#).

9.1.1 Human Data

EPO products used in clinical trials included Efamol[®] (4.32 g LA and 0.54 g GLA), Efamol Marine[®] (8% EPO containing 73% LA and 9% GLA; 20% fish oil); Naudicelle[®], Epogam[®], and Efamast[®]. Although contact dermatitis from the use of EPO is possible, allergic reactions or hypersensitivity to EPO has not been commonly described. Side effects that have been reported are occasional headache, abdominal pain, nausea, and loose stools. Seizures have also been

reported in persons taking EPO (Natural Standard, 2008). The adverse effects are rare when taken at recommended doses. There were only 193 adverse reactions to EPO reported from 1968-1997. The severe cases that were reported were convulsions, aggravated convulsions, face edema, and asthma (Artz, 2007).

Absorption, Distribution, Metabolism, and Excretion

When six healthy volunteers (3 males and 3 females, aged 21-25 years) were given EPO (Epogam; 480 mg/day GLA, 6 capsules in the morning and 6 capsules 12 hours later), the mean serum level of GLA was significantly increased (11.4 versus 4.8 µg/mL). Small changes were reported for seven other fatty acids, including the GLA metabolites DGLA and arachidonic acid. GLA exhibited an absorption-elimination pattern. The mean peak plasma concentration was 22.6 µg/mL after the morning dose and 20.7 µg/mL after the evening dose, and the area under the concentration-time curve at 24 hours was significantly increased over baseline values (274.1 versus 114.5 µg/mL×hour). Additionally, gastric absorption of GLA was much slower in the morning than in the evening; the mean time to reach the maximum concentration of GLA was 4.4 hours in the morning and 2.7 hours in the evening (Martens-Lobenhoffer and Meyer, 1998 [PMID:9707349]). [Note: This publication was mentioned in Artz (2007) and briefly in the Natural Standard (2008).]

Other human studies have shown increases in serum GLA, its metabolite DGLA, or both after dosing with EPO (e.g., Horrobin et al., 1991 [PMID:1871175]; Ishikawa et al., 1989 [PMID:2540757]). Pre-emulsification of the oil may improve GLA absorption (Garaiova et al., 2007). Breast milk and formula have been supplemented with GLA-containing oils such as EPO to improve the PUFA intake of infants, since total fat content and concentrations of essential fatty acids decline with prolonged breast feeding (Cant et al., 1991 [PMID:1668100] [Efamol[®]]; Woltil et al., 1999 [PMID:10359022] [EPO and fish oils]).

Short-Term or Subchronic Exposure Studies

Dermatitis (erythema and vesicles on the fingers and erythema with edema on the eyelids and cheeks) was reported in a 52-year-old woman who had no history of atopy and worked in a motorcar spare parts shop. The doctor and patient believed the dermatitis to be induced by metals, oils, and greases in her work environment, but when a patch test for primin resulted in positive readings, the patient recalled bringing home and growing a primrose (*Primula obconica*) two months earlier. Further patch testing with primrose leaf and flower confirmed the cause (Kiec-Swierczynska et al., 2006 [PMID:16881603]). [Note: This publication was briefly mentioned in the Natural Standard (2008).]

In hyperkinetic children given a normal diet with EPO (0.5 g, containing 0.04 g GLA) for six weeks, no significant change in the concentration of plasma triglycerides, phospholipids, or cholesterol was reported. Concentrations of GLA, however, were increased. Additionally, no apparent differences in behavior were observed by parents or teachers (Gibson, 1985).

Chronic Exposure Studies

In a two-year double-blind, multicenter study, 26 subjects experiencing painful neuropathy were administered EPO (480 mg/day GLA) for 12 months. Compared to placebo, no significant effects were observed in standard tests of severity of diabetic peripheral and autonomic

neuropathy: vibration perception thresholds at the hallux, valsalva ratio, R-R interval variation on deep breathing, heart rate response to standing, and degree of postural hypotension (Purewal et al., 1997 abstr.).

Synergistic/Antagonistic Effects

A summary of the interactions of EPO with other drugs, herbs/supplements, and lab tests can be found in the Natural Standard Monograph. Many interactions are theoretical. Of particular interest is the suggestion that EPO may increase the risk of seizure when used simultaneously with anesthetics or antipsychotics (e.g., chlorpromazine [Thorazine[®]] and trifluoperazine [Stelazine[®]]) and may lower seizure threshold when used with anticonvulsant agents or stimulants. This theory is based on case reports of seizures in patients with or without known seizure disorders (Natural Standard, 2008). [These case reports were published by Holman and Bell (1983) and Vaddadi (1981 [PMID:6269135]).]

In a recent publication, the two case reports noted above were re-examined and it was suggested that formularies remove seizures/epilepsy as a listed side effect of EPO and that these also be removed as a contraindication to taking EPO. The reasons provided were that in the 1981 study, the three patients were simultaneously taking EPO with phenothiazines, which are well-known for lowering the threshold for seizures. Additionally, it was noted that none of the patients suffered from clinical seizures while taking EPO and that all three patients were re-diagnosed as suffering from temporal lobe epilepsy and not schizophrenia. In the 1983 study, of the 2 of 23 patients with schizophrenia who developed seizures while taking EPO, one was also taking fluphenazine and the other was also taking fluphenazine, which was then replaced with chlorpromazine. Both chemicals, being phenothiazines, are well-known causes of seizures. Furthermore, it was noted that a strong relationship between schizophrenia and epilepsy may exist (Puri, 2007 [PMID:17764919]).

Anticarcinogenicity

In 21 patients with untreatable cancer (i.e., hepatocellular carcinoma, malignant mesothelioma, cerebral astrocytoma/ependymoma, renal clear cell carcinoma, breast adenocarcinoma, esophageal adenocarcinoma, bronchogenic carcinoma, or gastric adenocarcinoma), oral administration of EPO (Efamol G; 500 mg EPO, comprising about 45 mg GLA; 18-36 capsules/day for various periods) in the diet produced significant subjective improvements in most patients. Distinct objective improvements (e.g., weight gain and measurable reduction of tumor mass) were also observed. For example, a 59-year-old man with malignant mesothelioma of the biphasic type had a progressive pleural effusion which required frequent drainage of up to 4.5 L at 7- to 10-day intervals. Thirty days after receiving EPO, 1.2 L was drained and no further drainage was required. In another notable case, a 28-year-old male with primary liver cancer receiving EPO exhibited a reduction in the size of his liver (212 mm in May 1983 to 130 mm in October 1984) and the tumor (158 mm in August 1983 and 112 mm in October 1984) (van der Merwe et al., 1987).

In contrast to the above study, in 26 South African patients with a histological diagnosis of primary liver cancer, EPO (Efamol; 500 mg EPO; 36 capsules/day) resulted in no statistically significant effect on survival time or liver size, although the survival curve and the objective and subjective measurements of those taking EPO were always better than those taking placebo.

There was, however, a statistically significant decrease of γ -glutamyl transferase values to normal in seven patients. It was noted that the low doses of GLA used compared to the large size of tumor may have caused the lack of positive effect on survival times (van der Merwe et al., 1990 [PMID:2169638]). A study in which 25 patients with terminal gastrointestinal tract cancer received GLA in its natural form (i.e. EPO containing 10% GLA) also showed no statistically significant differences between the treatment group and control group, but in general overall survival was significantly better (e.g., gastric cancer groups: 9.1 months treated versus 2.2 months controls and colorectal cancer group: 13.3 months treated versus 4.1 months controls) (Manolakis et al., 1995). Additionally, EPO did not significantly reduce the size of the tumors (52% versus 42% controls) in 21 patients with clinically diagnosed fibroadenomas (Kollias et al., 2000 [PMID:14731582]).

Reproductive and Teratological Effects

In 54 low-risk nulliparous women, oral administration of EPO (500 mg three times per day for 1 week beginning at gestational week 37 and then 500 mg once per day until labor) for the purpose of advancing cervical ripening did not shorten gestation period or decrease the overall length of labor. Instead, labor lasted 3 hours (mean) longer in women taking EPO than those who did not (nearly 7 hours longer in the fourth quartile). Additionally, EPO increased certain active phase labor abnormalities—incidence of prolonged rupture of membranes, protracted active phase, oxytocin augmentation, arrest of descent, cesarean delivery, and vacuum extraction. No significant differences on age, Apgar score, or days of gestation were observed between treatment and control groups. Birth weight was slightly higher in infants in the EPO group compared to those in the control group (Dove and Johnson, 1999 [PMID:10380450]).

In a recent case study, a woman had self-medicated with raspberry leaf tea and EPO (500 mg, 13 capsules total) vaginally and orally for the purpose of cervical ripening one week before delivery. At 17 hours of age, the newborn infant developed diffuse ecchymoses and petechiae on her trunk, extremities, and face. At 5 days of age, purpura had cleared, and the infant was doing well (Wedig and Whitsett, 2008).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Rat tissues are more active in conversion of LA and α -linolenic acid to longer-chain PUFAs than humans. The endogenous formation of GLA from LA via Δ 6-desaturation is inhibited by α -linolenic acid, arachadonic acid, eicosapentaenoic acid, and docosahexaenoic acid. They also inhibit Δ 5-desaturation of DGLA to arachadonic acid (Bézar et al., 1994 [PMID:7840871]). Animal studies (rats, mice, guinea pigs, and cats) have shown that EPO increases GLA and DGLA in erythrocytes and tissue phospholipids or TAGs (Høy et al., 1983 [PMID:6318009]; Hrelia et al., 1991 [PMID:1808628]; Huang et al., 1985 [PMID:2857052]; Raederstorff and Moser, 1992 [PMID:1336802]; Sinclair et al., 1979 [PMID:513981]). Supplementing mice diets with borage oil or borage oil and fish oil mixtures gave a more favorable prostaglandin ratio (PG₁/PG₂) in their macrophages than EPO supplementation (Fan and Chapkin, 1992).

MCF7 cells differentially metabolized EPO and GLA. Supplementation of cells with GLA led to a fivefold increase in GLA levels after 72 hours. Comparatively, supplementation with EPO led to a fourfold increase in LA, but no change in GLA levels (Tyers et al., 1997 abstr.).

9.1.3 Acute Exposure

A single study in mice (sex and strain not provided) reported an LD₅₀ of 3.12×10⁴ µg/kg (RTECS, 2008).

9.1.4 Short-Term and Subchronic Exposure

Male Wistar rats (2 months old, 214 g) were maintained on a diet supplemented with 11% EPO (SP Farma, Sao Paulo, Brazil; 85% n-6 fatty acid) for 6 weeks. Additional test groups included rats maintained on a normal diet and rats treated with imipramine. The authors noted that there was no difference in body weights between the groups (Borsonelo et al., 2007 [PMID:17275891]).

Male Sprague-Dawley rats (200-230 g) were administered EPO (1 mL/kg; Scotia Pharmaceuticals, Surrey, UK) by daily gavage for 20 weeks. When compared to control animals, there was no difference in body weight or weight gain between the groups (Meehan et al., 1995 [PMID:8749808]).

Male F344/DuCrj rats (24 weeks old) were fed a diet containing 10% EPO (Summit Oil Co., Ciba, Japan) for 15 weeks. Animals had free access to food and water during the experiment. Clinical observations compared to animals on other fatty acid-enriched diets showed that body weight gain, final body weights, and food intake were similar among rats on all fatty acid enriched diets evaluated (Fukushima et al., 2001 [PMID:11337981]).

9.1.5 Chronic Exposure

Male and female Sprague-Dawley rats (100 each sex, 5-6 weeks old) were administered Efamol (8.5-9.0% GLA and 70-73% LA) by gavage (0-2.5 mL/kg/day). Rats were euthanized after 53 weeks of dosing, and external and internal examinations conducted. No significant differences were noted in food consumption or body weight between treated and control animals. Hematology and urinalysis results indicated that there were no dose-dependent changes. Female rats treated with 2.5 mL/kg/day Efamol showed increased potassium levels at 25 and 51 weeks. Necropsy and histopathology evaluation showed no differences between groups in a majority of the tissues evaluated. Reduced liver weights were observed in male treated rats (doses not provided), but no significant histopathological changes were noted. Testicular shrinkage or softening was noted in 24% of 2.5 mL Efamol/kg/day treated rats (versus 8% control animals). Abdominal fat necrosis in six treated rats (doses not provided) was not attributed to Efamol (Everett et al., 1988a).

9.1.6 Synergistic/Antagonistic Effects

Synergistic/Antagonistic Effects of EPO and GLA

EPO altered mercuric chloride (MC)-induced autoimmune glomerulonephritis in female Brown-Norway rats (145-170 g, 14-15 weeks old). Rats received MC (1 mL/kg in water) three times per week and Efamol (9.0% GLA and 72% LA) as part of the diet (80 days prior through the 43rd day of the experiment). When compared to MC-treated only rats, EPO significantly decreased body weight, urinary protein levels, and thromboxane B2 and 6-keto-prostaglandin F1a excretion. Comparatively, prostaglandin E2 excretion was significantly increased and creatinine excretion was unaffected (Papanikolaou et al., 1987).

Several studies have evaluated the effects of EPO on alcohol-induced alterations *in vivo*. Chronic ethanol administration to adult and pup rats was shown to alter lipid concentration in a variety of tissue preparations (e.g., neuronal membranes, cortical synaptosomes, erythrocytes, and liver). Concomitant administration of EPO attenuated many of the alterations in lipid content produced by ethanol (Corbett et al., 1991 [PMID:1760057], 1992 [PMID:1316121]; Duffy et al., 1992a [PMID:1330473]). An animal model of fetal alcohol syndrome showed that concomitant administration of EPO with ethanol improved both male and female offspring learning ability and activity compared to controls (Duffy et al., 1992b [PMID:1317288]).

Male Sprague-Dawley rats (200-230 g) were administered EPO (1 mL/kg; Scotia Pharmaceuticals, Surrey, UK) by daily gavage. After 2 weeks, ethanol was added to the diet of half the animals until a total concentration of 10% (v/v) was reached. Animals were maintained on the diet for 19 additional weeks. Synaptosomes were prepared from cerebral cortex tissues isolated from 8 or 10 rats. EPO alone significantly decreased the fluidity of the inner core region of the synaptosomal membranes (as measured by fluorescence polarization) when compared to controls. In animals administered ethanol and EPO, a decrease in fluidity of the inner leaflet membrane was noted. Membrane sensitivity to ethanol was determined by *in vitro* addition of ethanol to the prepared synaptosomes. Studies showed that animals treated with EPO and ethanol were less sensitive to an alcohol challenge than controls or animals treated with ethanol only (Meehan et al., 1995 [PMID:8749808]).

EPO reversed effects of cyclosporine A (CS) on tissue lipid composition in specific tissues. Oral daily administration of CS (10 mg/kg) to borderline hypertensive rats for 4 weeks altered tissue lipid composition in numerous tissues including, liver, brain, and renal tissues. Diet supplementation with EPO (dose not provided in abstract) reversed altered lipid composition in these tissues (Mills et al., 1994 [PMID:8139392]).

EPO has been shown to antagonize a variety of effects produced by other toxicological and pharmacological agents. Using the Forced Swim Test, Borsonelo and colleagues (2007 [PMID:17275891]) showed that dietary supplementation with 11% primrose oil (SP Farma, Sao Paulo, Brazil) reversed the antidepressant effects produced by imipramine in two-month-old Wistar rats. EPO emulsion reduced the level of malondialdehyde levels in cochlear tissue and the auditory threshold shift when compared to kanamycin-treated animals (Cheng et al., 2000). EPO administered daily (1 mL/100 g) for three weeks antagonized the effects of carbon tetrachloride (CCl₄) in female Wistar rats (e.g., increased blood sugar levels and glucose-6-phosphatase and glycogen phosphorylase activities and decreased liver glycogen content) (Coprean et al., 1994).

In multidrug-resistant K562/ADM leukemic cells GLA differentially modulates cellular responses to anticancer drugs. At a concentration of 10 µg/mL, GLA enhanced anti-tumor growth activities of doxorubicin, etoposide, and vincristine. Comparatively, GLA either had no effect or antagonized anti-tumor growth activities of cisplatin, mitomycin, and fluorouracil (Kong et al., 2009 [PMID:19268700]).

Anticarcinogenicity

Several studies showed that EPO can affect mammary tumor progression (e.g., Cameron et al., 1989; Ghayur and Horrobin, 1981; Muñoz et al., 1999 [PMID:10198915]). In one study, a single intragastric dose of dimethylbenz(a)anthracene (DMBA) was administered to virgin female Sprague-Dawley rats (50 days old). The rats were then placed on different diets, including 20% corn oil and 20% EPO (Efamol Research, Inc., Nova Scotia, Canada), three weeks after DMBA exposure and were maintained on the diet for an additional 16 weeks. No significant effect on final body weight or food consumption was correlated to consumption of EPO. The tumor incidence in animals fed EPO was similar to corn oil fed animals (84% [21/25] versus 80% [20/25]); however, the number of tumors per tumor bearing rat and the total number of tumors were decreased and the tumor latency period was increased in EPO rats (Bunce and Abou-El-Ela, 1990 [PMID:2177199]).

Female Sprague-Dawley rats (30 days old) were administered one of three oils (including EPO; Oeparol, Agropharm, Poland, 0.4 mL) by intragastric intubation. At 50 days old, the animals were subdivided and one group received DMBA (65 mg/kg body weight). Animals were euthanized 20 weeks after DMBA administration. Animals fed EPO had the lowest tumor incidence rate (28%) at necropsy. However, the mean tumor weight (5.95±4.62 g) and the tumor weight ranges (0.84–16.44 g) were greater than observed for the other two diets (Jelinska et al., 2003 [PMID:12697300]).

Using an initiation and promotion model of skin carcinogenesis, the effects of EPO were evaluated. Ten random bred Swiss albino mice (6-7 weeks old) were administered a single dose of BaP followed by multiple applications of croton oil, as the initiator and promoter, respectively. EPO (10 mg) was applied to the skin during the initiation and promotion stages. EPO was only effective in inhibition promotion stages of skin papilloma development. A concurrent increase in skin lipid peroxidation during the promotion stage and modulation of fatty acid levels during the initiation and promotion stages also were noted (Ramesh and Das, 1998 [PMID:9844986]). As noted earlier in Section 2.1.2 under GLA background, anticarcinogenic properties also were demonstrated for GLA in a two-stage model of skin cancer (Das, 2006b [PMID:17168663]; Ramesh and Das, 1996 [PMID:8620442]).

Aspinall et al. (1988) showed that prophylactic or therapeutic treatment of mice bearing PLC/PRF/5 human hepatocellular carcinoma xenografts, which produced hepatitis B surface antigens, with Efamol G did not affect the time to tumor appearance, tumor growth rate, or serum levels of hepatitis B surface antigen or alpha-fetoprotein. The authors noted that average percentage tumor weight to total body was significantly greater in animals receiving Efamol G when compared to control animals.

Antigenotoxicity

BaP (75 mg/kg body weight) and EPO were administered to Swiss male mice (7-8 weeks old). EPO was administered an hour after BaP, either orally (0.25 mL) or via intraperitoneal injection (0.12 mL). Bone marrow smears were obtained after sacrifice to assess micronuclei formation. EPO administration reduced the number of cells with micronuclei, when compared to BaP treated animals (Das et al., 1985).

EPO significantly inhibited BaP binding to murine skin cell DNA. However, it did not affect BaP or croton oil induced increases in thymidine incorporation into skin cell DNA (Ramesh and Das, 1998 [PMID:9844986]).

9.1.7 Cytotoxicity

Over 10 articles and meeting abstracts have reviewed the cytotoxic effect of EPO in a variety of cell types. Pellegrina et al. (2005 [PMID:16004929]) showed that a phenolic fraction purified from defatted seeds of *O. biennis*, which contained gallic acid, induced selective apoptosis in human and mouse leukemic cell lines and inhibited [³H]-thymidine incorporation in CaCO₂ and WEHI164 cells. Results from several studies in Ehrlich ascites tumor cells showed that evening primrose extract induced apoptosis, decreased intracellular polyamine levels, increased the formation of reactive oxygen species, induced a dose-dependent increase in the number of cells in the G1 phase, inhibited DNA synthesis, induced an increase in the loss of mitochondrial membrane potential, and increased release of cytochrome c (Arimura et al., 2003 [PMID:12732460], 2004 [PMID:15050730], 2005 [PMID:15700107]; Matsui-Yuasa et al., 2004 abstr.). In another study, 0.05 and 0.5% (v/v) EPO was cytotoxic to MCF7 cells (Tyers et al., 1994 abstr.).

9.2 Reproductive and Teratological Effects

Male ICR mice were orally administered EPO (0.5 mL) daily for 28 days. Endpoints assessing male sexual function (e.g., testis weight, number of complete penile insertions, and mating) were determined on days 14 and 28. The body weight, testis weight, and the number of complete penile insertions during a three-hour period were increased in treated mice. Additionally, the number of sperm-positive females and testosterone level in serum were increased in treated animals (Shin and Lee, 2006).

Wistar rats were fed a diet supplemented with EPO (Efamol Ltd., Guildford, U.K.; dose not provided) from three weeks of age until mating. EPO had no effect on parturition, birth weight, postnatal growth rate, maternal weight during pregnancy, and fetal or placenta prostaglandin E₂ levels (Leaver et al., 1986).

Results from studies of the effects of EPO in zinc deficient animals have been mixed. Weanling (70 g) and pregnant (250 g) Sprague-Dawley rats were fed a zinc-deficient diet (<0.5 µg/g diet) for 21 days. Concomitant administration of EPO (250 µL) was given to some rats. EPO administration did not improve zinc deficiency-induced fetal toxicity, teratogenicity, or growth retardation (Dreosti et al., 1985). Pregnant female Hooded Lister rats (Rowett strain) were fed either control diets (20 mg zinc/kg) or zinc-deficient diets (5 mg zinc/kg or 10 mg zinc/kg for the first 15 days and 0.5 mg zinc/kg to term [10/0.5 mg zinc/kg]). Half of the rats within each group were given daily subcutaneous injections of EPO (500 µL/kg/day for the first week, 600 µL/kg/day for the second week, and 700 µL/kg/day for the third week). EPO was shown to decrease maternal fluid loss and litter size, and increase neonate survival in the control and 5 mg zinc diets. However, EPO also increased maternal mortality in those animals that received the 10/0.5 mg zinc/kg diet (Cunnane, 1982 [PMID:7082621]).

9.3 Carcinogenicity

Black female C57 mice (approximately 6 weeks old) were fed diets containing 8% EPO for 1 week before BL6 murine melanoma cells were subcutaneously injected. The mice were maintained on their diets for an additional 4 weeks. The mean tumor mass from mice fed EPO were significantly larger than those from mice fed safflower oil (1.843 ± 0.286 versus 1.343 ± 0.202 g) (Gardiner and Duncan, 1991 [PMID:1650000]). Diets supplemented with 35% EPO did not affect tumor growth in athymic mice with a murine sarcoma allograft (Ramchurren et al., 1985 [PMID:4071326]).

Dietary manipulation also affected the melanoma tumor promoting properties of EPO. Six-week-old female C57 mice were fed a diet containing 8% EPO (9.0% GLA and 70% LA) and varying levels of d- α -tocopherol (TOC) for 1 week prior to tumor injection. B16 murine melanoma cells were subcutaneously injected and mice were maintained on their diets for an additional 4 weeks. Tumor mass in mice was increased in a TOC dose-dependent manner (Gardiner and Duncan, 1990).

Male and female Sprague-Dawley rats (250 of each sex, 5-6 weeks old) and CD-1 mice (250 of each sex, 5-6 weeks old) were administered Efamol (0.3, 1.0, or 2.5 mL/kg) daily by gavage. Rats and mice were evaluated for 104 and 78 weeks, respectively. Tumor incidence was not different between control and dosed animals. In addition, no adverse effects (not specified) were noted (Everett et al., 1988b).

9.4 Initiation/Promotion Studies

No data were available.

9.5 Genotoxicity

No data were available.

9.6 Cogenotoxicity

No data were available.

9.7 Immunotoxicity

No data were available.

9.8 Other Data

Immunomodulatory Activities

Several studies and reviews have discussed the immunomodulatory properties of EPO and extract (e.g., Calder [1993; PMID:8298526] and Matsuo et al. [1996; PMID:8951990]). Below are provided a summary of two studies that demonstrate the anti-inflammatory properties of EPO. Hong et al. (1995) showed that pretreatment of mice with EPO decreased contact sensitivity elicited by 1-fluoro-2,4-dinitrobenzene in BALB/c mice by >82%. A mild inflammatory response in the dermis was noted with hematoxylin and eosin staining and keratinocyte ICAM-1 expression was decreased by EPO.

Weanling male Lewis rats (65-85 g, 3 weeks old) were fed either a low fat or high fat diet (including 20% EPO). EPO significantly inhibited natural killer (NK) cell activity in freshly

prepared lymphocytes. Incubation of spleen lymphocytes with interferon- α increased NK activity, but the stimulation was lower than observed in rats fed a low fat diet (Yaqoob et al., 1994 [PMID:8002045]).

Cardiovascular Effects

EPO exhibits a variety of cardiovascular effects. Fukushima et al. (2001 [PMID:11337981]) showed that administration (15 weeks) of EPO via diet decreased serum high density lipoprotein-cholesterol levels in male F344/DuCrj rats. Shukla and Khanuja (2004) summarized studies in rabbits and rats that showed dietary supplementation with EPO and evening primrose cake extract reduced tissue lipid peroxidation, total cholesterol and low density lipoprotein cholesterol, and increased glutathione levels. GLA reduced central venous blood pressure in normal and hypertensive animals. One proposed mechanism of action was inhibition of the angiotensin II receptor (Natural Standard, 2008).

Enzyme Effects

Administration of EPO alters the activity of $\Delta 6$ -desaturase in old rats (age, strain, sex, and dose not provided in abstract). When compared to animals given soy bean oil alone, the rate of $\Delta 6$ -desaturation of LA and α -linolenic acid was significantly greater in rats fed EPO (Biagi et al., 1991 [PMID:1674661]). In streptozotocin-induced diabetic rats, EPO increased cyclooxygenase-1 mRNA expression in the sciatic nerve (1.21 ± 0.25 versus 0.89 ± 0.32 attomol/ μ g tRNA) and retina (0.065 ± 0.017 versus 0.031 ± 0.020 attomol/ μ g tRNA) when compared to non-diabetic controls (Fang et al., 1997 [PMID:9051726]).

Endocrine Activity

In the BT-474 human breast cancer cell line, EPO (Jamieson Natural Sources) exhibited no estrogenic, androgenic, or progesterone effects (Rosenberg Zand et al., 2001 [PMID:11580929]). In MCF7 cells exposed to EPO ($3 \times 10^{-3}\%$ or $3 \times 10^{-5}\%$ v/v), both concentrations increased estrogen receptor binding capacity (B_{\max}) after a five-day exposure when compared to control cells. However, after a longer exposure (9 days) the B_{\max} was either similar to control levels ($3 \times 10^{-5}\%$) or receptor binding was not detected ($3 \times 10^{-3}\%$) (Tyers et al., 1995 abstr.).

Additional Biological Activities

Daily topical application of a cream containing 10% EPO to female Large White pigs increased cellular proliferation in the skin. EPO increased the size, but decreased the overall density, of the rete pegs. Additionally, EPO increased the labeling index of cells in the basal layer of the epidermis and the papillary dermis (Morris et al., 1997 [PMID:9501921]).

EPO also has been shown to alter the fatty acid composition of the cellular monolayer. For example, Tyers et al. (1997 abstr.) showed that supplementation of MCF7 cells with EPO significantly increased the levels of GLA and LA in the cell monolayer in a time-dependent manner. [Supplementation of MCF7 cells with GLA solely initially decreased the level of GLA, which then returned to control levels by 72 hours. Levels of LA were increased compared to control levels.]

Daily oral administration of EPO to BALB/c mice decreased skin sensitivity to radiation and antagonized radiation-induced increases in blood flow to the skin. EPO did not alter blood flow

or radiation-sensitivity to tumors present in the mice. Alterations also were observed in the plasma levels of various fatty acids including LA, GLA, DGLA, and arachidonic acid and the observed changes in fatty acid levels were irradiation-status dependent (Rahbeeni et al., 2000 [PMID:10902742]).

Extracts and preparations from evening primrose also exhibited antibacterial, antifungal, and antioxidant activities (Birch et al., 2001; Shukla and Khanuja, 2004; Wettasinghe and Shahidi, 1999 [PMID:10552455]).

10.0 Structure-Activity Relationships

No data were available that were directly applicable.

11.0 Online Databases and Secondary References Searched

11.1 Online Databases

National Library of Medicine Databases

PubMed

ChemIDplus – chemical information database that provides links to other databases such as CCRIS, DART, GENE-TOX, HSDB, IRIS, and TRI. A full list of databases and resources searched are available at <http://www.nlm.nih.gov/databases/>.

STN International Files

AGRICOLA	FSTA
BIOSIS	IPA
BIOTECHNO	MEDLINE
CABA	PASCAL
EMBASE	Registry
ESBIOBASE	TOXCENTER
FROSTI	

Information on the content, sources, file data, and producer of each of the searched STN International Files is available at <http://www.cas.org/support/stngen/dbss/index.html>.

Government Printing Office

Code of Federal Regulations (CFR)

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Appendix A: Units and Abbreviations

°C = degrees Celsius

µg/mL = microgram(s) per milliliter

attomol/µg = attomole(s) per microgram

BaP = benzo[a]pyrene

CS = cyclosporine A

DGLA = dihomo-γ-linolenic acid

DMBA = dimethylbenz(a)anthracene

EPO = evening primrose oil

FDA = U.S. Food and Drug Administration

g = gram(s)

GLA = γ-linolenic acid

HPLC = high performance liquid chromatography

HSDB = Hazardous Substances Data Bank

kg = kilogram(s)

L = liter(s)

LA = linoleic acid

LD₅₀ = lethal dose for 50% of test animals

MC = mercuric chloride

mg/kg = milligram(s) per kilogram

mL/kg = milliliter(s) per kilogram

mm = millimeter(s)

mol. wt. = molecular weight

NK = natural killer

PAD = photodiode array detector

PMID = PubMed identification

PMS = premenstrual syndrome

PUFA = polyunsaturated fatty acid

Sn = stereochemical number

TAG = triacylglycerol

TLC = thin-layer chromatography

TOC = d-α-tocopherol

U.S. = United States

Appendix B: Description of Search Strategy and Results

A preliminary search was conducted on September 23 in STN International files MEDLINE, AGRICOLA, CABA, EMBASE, ESBIODBASE, BIOTECHNO, IPA, BIOSIS, TOXCENTER, FSTA, FROSTI, and PASCAL using plant synonyms or primrose oil as keywords. The 3,065 results were saved and the available literature was categorized based on the record titles. The tallies of information by topic were summarized in a table submitted about October 8. A later attempt (October 2, 2008) to find report-subject information for all the fatty acids identified in evening primrose oil (EPO) was aborted when the total number of answers retrieved was almost 25,000. Since the Natural Standard Monograph had summarized the therapeutic uses and clinical trials, such studies were avoided when selection of articles for full record retrieval from the September results was done in December. Selection also tried to avoid duplication of results from the December 9 search described below. A total of 159 full records was printed. The major categories of retrievals were on chemical composition (44), anticarcinogenicity (33), and human studies (18).

STN International files MEDLINE, AGRICOLA, CABA, EMBASE, ESBIODBASE, BIOTECHNO, IPA, BIOSIS, TOXCENTER, FSTA, and FROSTI were searched simultaneously by another searcher on December 9, 2008. The search terms included evening primrose, *Oenothera biennis*, trade names for evening primrose oil, synonyms for gamma-linolenic acid (GLA), and CAS Registry Numbers. The resulting 14,304 records were reduced to 7,088 by deleting records with terms related to therapeutic uses of EPO. Terms related to toxicity and to studies with healthy adults were combined with the 7,088 to reduce the answer set to 652 records, 213 of which were reviews published since 2005. The history of the online session showing the actual search strategy is shown below. When the "eliminated set" was combined with the same terms in a later session, about 100 additional records were retrieved, primarily related to anticarcinogenicity. RTECS and Registry searches were done for EPO, GLA, and linoleic acid. Because GLA was searched independently of EPO, many of the articles retrieved from this search strategy included other oils that contain GLA.

```

L9          4066 S EVENING(W)PRIMROSE OR OENOTHERA(W)BIENNIS
L10         1673 S EFAMOL OR EPOGAM OR EFAMAST OR 65546-85-2
L11         4977 S L9 OR L10
L12         10482 S GAMMA(W)LINOLENIC(W)ACID
L13         92 S GAMOLENIC(W)ACID
L14         242 S GAMMALINOLENIC(W)ACID
L15         10623 S L12 OR L13 OR L14
L16         10906 S L15 OR 506-26-3 OR 1686-12-0
L17         14304 S L11 OR L16
L18         2773 S L17 AND (EFFICACY OR CLINICAL OR DOUBLE(W)BLIND)
L19         6385 S L17 AND (DEFICIEN? OR TREAT? OR THERAP? OR MANAGE? OR
PREVENT OR PROTECT?)
L20         1512 S L17 AND (DIABET? OR BRONCHITIS OR ARTHRITI? OR MASTALGIA OR
BREAST(W) PAIN)
L21         7216 S L18 OR L19 OR L20
L22         7088 S L17 NOT L21
L23         639 S L22 AND (HEALTHY(W)ADULT? OR VOLUNTEER? OR SUBCHRONIC? OR
CHRONIC? OR POISON? OR ?TOXIC?)
L24         6961 S L22 NOT ATTENUAT?
L25         6352 S L24 NOT L23
L26         144 S L25 AND (MUTAGEN? OR CARCINOGEN? OR ABORT? OR RESORB? OR
RESORP?)
L27         63 S L25 AND (CYTOTOXIC? OR ANTI(W)(TUMOR? OR TUMOUR?) OR
ANTITUMOR? OR ANTITUMOUR?)
L28         206 S L26 OR L27
L29         6146 S L25 NOT L28
SAVE L29 X590REST/A
L30         845 S L23 OR L28
SET DUPORDER FILE
L31         439 DUP REM L30 (406 DUPLICATES REMOVED)
80 ANSWERS '1-80' FROM FILE MEDLINE
11 ANSWERS '81-91' FROM FILE AGRICOLA
27 ANSWERS '92-118' FROM FILE CABA

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70 ANSWERS '119-188' FROM FILE EMBASE
10 ANSWERS '189-198' FROM FILE ESBIODBASE
1 ANSWER '199' FROM FILE BIOTECHNO
4 ANSWERS '200-203' FROM FILE IPA
80 ANSWERS '204-283' FROM FILE BIOSIS
137 ANSWERS '284-420' FROM FILE TOXCENTER
5 ANSWERS '421-425' FROM FILE FSTA
14 ANSWERS '426-439' FROM FILE FROSTI
L32 677 S L17 AND (SAFETY OR ADVERSE(W)EFFECT?)
L33 382 S L17 AND SAFETY
L34 790 S L17 AND REVIEW/DT
L35 95 S L34 AND (2007-2008)/PY
L36 145 S L34 AND (2005-2006)/PY
L37 240 S L35 OR L36
L38 237 S L37 NOT L30
L39 213 DUP REM L38 (24 DUPLICATES REMOVED)
31 ANSWERS '1-31' FROM FILE MEDLINE
182 ANSWERS '32-213' FROM FILE EMBASE
L40 439 SORT L31 1-439 TI
SAVE L40 X590TOXIC/A
L41 213 SORT L39 1-213 TI
SAVE L41 X590REVU/A

```

A very limited number of metabolism studies resulted from the two fee-based search strategies since relevant terminology had not been specified. To develop a coherent understanding, several articles were examined on general fatty acid metabolism as well as specific EPO studies identified by PubMed and Google searches in December and January.

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STN International files MEDLINE, AGRICOLA, CABA, EMBASE, ESBIODBASE, BIOTECHNO, IPA, BIOSIS, TOXCENTER, FSTA, and FROSTI were searched simultaneously on September 18, 2009, and September 24, 2009. The search conducted on September 18, 2009, focused on retrieving relevant publications published in 2009. The history of the online session showing the actual search strategy is shown below. A total of 30 titles were identified, 19 of which were duplicates. Of the remaining 11 citations, four records (2 MEDLINE, 1 TOXCENTER, and 1 EMBASE) were selected for further review.

```

L1 4273 S EVENING(W)PRIMROSE OR OENOTHERA(W)BIENNIS
L2 1744 S EFAMOL OR EPOGAM OR EFAMAST OR 65546-85-2
L3 83 S (L1 OR L2) AND 2009/PY
L4 11219 S GAMMA(W)LINOLENIC(W)ACID
L5 94 S GAMOLENIC(W)ACID
L6 251 S GAMMALINOLENIC(W)ACID
L7 362 S (L3 OR L4 OR L5) AND 2009/PY
L8 287 S (L4 OR L5 OR L6) AND 2009/PY
L9 317 S L8 OR ((506-26-3 OR 1686-12-0) AND 2009/PY)
L10 392 S L3 OR L9
L11 84 S L10 AND (EFFICACY OR CLINICAL OR DOUBLE(W)BLIND)
L12 159 S L10 AND (DEFICIEN? OR TREAT? OR THERAP? OR MANAGE? OR PREVENT OR
PROTECT?)
L13 17 S L10 AND (DIABET? OR BRONCHITIS OR ARTHRITI? OR MASTALGIA OR BREAST(W)
PAIN)
L14 179 S L11 OR L12 OR L13
L15 213 S L10 NOT L14
L16 18 S L15 AND (HEALTHY(W)ADULT? OR VOLUNTEER? OR SUBCHRONIC? OR CHRONIC? OR
POISON? OR ?TOXIC?)
L17 206 S L15 NOT ATTENUAT?
L18 192 S L17 NOT L16
L19 11 S L18 AND (MUTAGEN? OR CARCINOGEN? OR ABORT? OR RESORB? OR RESORP?)
L20 1 S L18 AND (CYTOTOXIC? OR ANTI(W)(TUMOR? OR TUMOUR?) OR ANTITUMOR? OR
ANTITUMOUR?)
L21 12 S L19 OR L20
L22 30 S L16 OR L21
SET DUP ORDER
L23 11 DUP REM L22 (19 DUPLICATES REMOVED)
L24 11 SORT L23 1-11 TI
SAVE L24 X0590UPDATE/A

```

The search conducted on September 24, 2009, focused on retrieving relevant publications added to updated in STN databases between December 1, 2008, and December 31, 2008. The history of the online session showing the actual search strategy is shown below. A total of 64 titles were identified, 10 of which were duplicates. Of the remaining 54 citations, five records (4 MEDLINE and 1 EMBASE) were selected for further review.

```
L1          22 S (EVENING(W)PRIMROSE OR OENOTHERA(W)BIENNIS) AND 20081201-2008/UP
L2          7 S (EFAMOL OR EPOGAM OR EFAMAST OR 65546-85-2) AND 20081201-2008/UP
L3          27 S L1 OR L2
L4          39 S (GAMMA(W)LINOLENIC(W)ACID) AND 20081201-20081231/UP
L5          1 S (GAMOLENIC(W)ACID) AND 20081201-20081231/UP
L6          0 S (GAMMALINOLENIC(W)ACID) AND 20081201-20081231/UP
L7          39 S L4 OR L5 OR L6
L8          41 S L7 OR ((506-26-3 OR 1686-12-0) AND 20081201-20081231/UP)
L9          64 S L3 OR L8
            SET DUP ORDER
L10         54 DUP REM L9 (10 DUPLICATES REMOVED)
L11         54 SORT L10 1-54 TI
            SAVE L11 X0590UP1208/A
```