Final Report on the Safety Assessment of Hypericum Perforatum Extract and Hypericum Perforatum Oil[1]

Hypericum Perforatum Extract is an extract of the capsules, flowers, leaves, and stem heads of *Hypericum perforatum*, commonly called St. John's Wort. Hypericum Perforatum Oil is the fixed oil from *H. perforatum*. Techniques for preparing Hypericum Perforatum Extract include crushing in stabilized olive oil, gentle maceration over a period of weeks, followed by dehydration and filtration. Propylene Glycol and Butylene Glycol extractions were also reported. The following components have variously been reported to be found in *H. perforatum*: hypericin, naphtodianthrones, flavonoids, terpene and sesquiterpene oils, phenylpropanes, biflavones, tannins, xanthones, phloroglucinols, and essential oils. Hypericum Perforatum Extract is used in over 50 cosmetic fomulations and Hypericum Perforatum Oil in just over 10, both across a wide range of product types. Acute toxicity studies using rats, guinea pigs, and mice indicate that the extract is relatively nontoxic. Animals fed *H. perforatum* flowers for 2 weeks showed significant signs of toxicity, including erythema, edema of the portion of the body exposed to light, alopecia, and changes in blood chemistry. In a chronic study, rats fed *H. perforatum* gained less weight than control animals. Mixtures containing the extract and the oil were not irritants or sensitizers in animals. Because of the presence of hypericin, *H. perforatum* is a primary photosensitizer. In clinical tests, a single oral administration of Hypericum extract resulted in hypericin appearing in the blood. With long-term dosing, a steady-state level in blood was reached after 14 days. The polyphenol fraction of *H. perforatum* had immunostimulating activity, whereas the lipophilic portion had immunosuppressing properties. Mixtures of the extract and the oil produced minimal or no ocular irritation in rabbit eyes. Mutagenic activity in an Ames test was attributed to flavonols in one study and to quercitin in another, but other genotoxicity assays were negative. No carcinogenicity or reproductive and developmental toxicity data were available. A mixture of the extract and the oil was not irritating in clinical studies. Adverse reactions to Hypericum extract in the clinical treatment of depression include skin reddening and itching, dizziness, constipation, fatigue, anxiety, and tiredness. Absent any basis for concluding that data on one member of a botanical ingredient group can be extrapolated to another in a group, or to the same ingredient extracted differently, these data were not considered sufficient to assess the safety of these ingredients. Additional data needs include current concentration of use data; function in cosmetics; photosensitization and phototoxicity data using visible light; gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures; dermal reproductive/developmental toxicity data; human skin irritation and sensitization data using the oil; and ocular irritation data, if available. Until these data are available, it is concluded that the available data are insufficient to support the safety of these ingredients in cosmetic formulations.

INTRODUCTION

The safety of Hypericum Perforatum Extract and Hypericum Perforatum Oil as used in cosmetic formulations is reviewed in this report. Both Hypericum Perforatum Extract and Hypericum Perforatum Oil are obtained from *Hypericum perforatum* and serve in cosmetics as biological additives (Wenninger and McEwen 1997).

CHEMISTRY

Definition

Hypericum Perforatum Extract (CAS No. 84082-80-4) is an extract of the capsules, flowers, leaves, and stem heads of the hypericum, *H. perforatum* (Wenninger and McEwen 1997). It is also known as Hypericum Extract; Extract of Hypericum; Extract of Hypericum Perforatum; and Saint John's wort Extract. The plant *H. perforatum* has the primary name Millepertuis and common names St. John's wort, Johnswort, amber, goatweed, klamath weed, tipton weed (Fleischner 1985), St. Johnswort, John's wort, herb-John, cammock, penny John, grace of god, and rosin rose (Mitich 1994).

Hypericum Perforatum Oil (CAS No. 68917-49-7) is the fixed oil from St. John's Wort, *H. perforatum* (Wenninger and McEwen 1997). It is also known as Oils, Hypericum Perforatum; St. John's Wort Oil; and Oils, St. John's Wort.

Physical and Chemical Properties

A mixture of Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%) is a red-brown oil with a specific odor (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996). It is soluble in oils, has a refractive index ($n_D 20°C$) of 1.476 to 1.470, density of 0.911 to 0.915 g/ml, and an acid value of <5. A mixture of Hypericum Perforatum Extract (10%–25%) and propylene glycol (>75%)

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is a clear, red liquid with a faint herbal odor (Grau Aromatics GmbH & Co. 1997). It is soluble in water, has a refractive index of 1.425 to 1.445 (at 20°C), density of 1.030 to 1.050 (at 20°C), and a pH value of 5.0 to 6.0. A mixture of Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) is a reddish-brown, transparent liquid (Ichimaru Pharcos Co., Ltd. 1996). It has a specific gravity (20°/20°C) of 1.01 to 1.05 and a pH of 5.5 to 6.5.

Manufacture and Production

A mixture containing Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%) is characterized as a fatty oil extract of hypericum blossoms; the vehicle used is olive oil (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996). The hypericum blossoms are "crushed and covered with stabilized olive oil. After 6 weeks maceration under influence of light the red-brown oil obtained is dehydrated and filtered."

A mixture of Hypericum Perforatum Extract (10%–25%) and propylene glycol (>75%) is prepared by extracting hypericum herbs/blossoms with 1,2-propylene glycol; the ratio of extract to botanical is 6:1 (Grau Aromatics GmbuH & Co. 1997). A preservative, 0.6% phenonip (phenoxyethanol, methylparaben, butylparaben, ethylparaben, and propylparaben), is used.

A mixture of Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) is prepared by extracting hypericum flowers with 1,3-butylene glycol solution (Ichimaru Pharcos Co., Ltd. 1996).

Composition

H. perforatum contains a red fluorescence substance called hypericin (1,3,4,6,8,13-hexahydro-10,11-dimethylphenanthro [1,10,9,8-opqra]perylene-7,14-dion e or 4,5,6,4′,5′,7′-hexahydroxy-2,2′-dimethylnaphthodianthrone), the structure of which is shown in Figure 1 (Fleischner 1985). The amount of hypericin present depends on the plant veriety, location of the plant, the portion of the plant, and the time of year (Southwell and Campbell 1991; Jensen et al. 1995), and up to 80% active hypericin is lost upon drying of the plant (Araya and Ford 1981).

H. perforatum also contains a flavonoid, quercetin 3-D-galactoside hemipentahydrate (Fleischner 1985); the terpene- and sesquiterpene-containing oils pinene, cadinene, and aromandrene; 10% to 16% catechic tannins; the flavonic compounds quercetine, quercitrine, hyperine, and rutine; the diatronic anthranolic derivatives hypercine, pseudohypericine, and aemodinanthranol (Proserpio 1976); procynaidines; biapigenin; the phloroglucine derivative hyperforin (Staffeldt et al. 1994); cholinergic acid; and epicatechine (Ollivier et al. 1985). 1-Tetracosanol, 1-hexacosanol, 1-octacosanol, and 1-triacontanol have been determined to compose 0.43% of 1 kg of dried plant material. Nahrstedt and Butterweck (1997) stated that approximately seven groups of bioactive natural products have been identified in *H. perforatum*. They reported the following components:

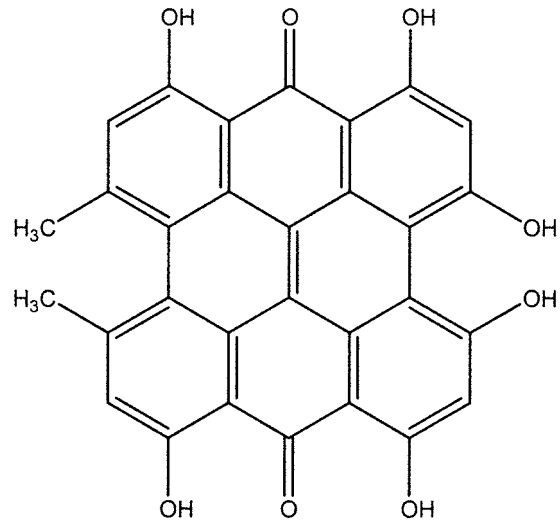

FIGURE 1
Hypericin.

phenylpropanes, mainly present as esters of hydroxycinnamic acids; flavonol glycosides, primarily quercetin (2%–4%), and also hyperin, rutin, isoquercitrin, and quercitrin; biflavones, including 13,118-biapigenin (0.1%–0.5%) and amentoflavone (0.01%–0.05%); tannins (15%) and proanthocyanidins; xanthones, including kielcorin (0.01% in the roots) and 1,3,6,7-tetrahydroxyxanthone (trace amounts in leaves and stems); phloroglucinols, including hyperforin and adhyperforin (both found only in the reproductive parts of the plant); essential oil, with α-pinene, β-pinene, myrcene, and limonene being present; amino acids, including γ-aminobutyric acid; and naphtodianthrones, including hypericin, pseudohypericin, and cyclopseudohypericin.

A supplier of a mixture containing Hypericum Perforatum Extract and propylene glycol stated that the plant is composed of essential oil, phenol acids, flavonoids, saponins, catechins, glycerides, phytosterols, cholin, dianthrones and anthranols (hypercin, pseudohypercin, and emodinanthranols), C_{21}–C_{31}-saturated hydrocarbons, C_{24}–C_{28}-saturated alcohols, carotene, vitamins, pectines, inamine, tannins, and mannitol (Grau Aromatics GmbH & Co. 1997).

Analytical Methods

Gas chromatography–mass spectrometry and cochromatography have been used to determine the alkanols present in *H. perforatum* (Brondz, Greibrokk, and Aasen 1983). Hypericin has been determined in *H. perforatum* using a Soxhlet extraction and subsequent spectrophotometry (Southwell and Campbell 1991) and by thin-layer chromatography–densitometry (Vanhaelen and Vanhaelen-Fastre 1983). High-performance liquid chromatography (HPLC) has been used to determine phenolic acids in *H. perforatum* (Ollivier et al. 1985).

A mixture of Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%) is

identified using the total of quality control data (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996).

Ultraviolet Absorption

H. perforatum in glycolic extract or lipophilic extract does not significantly absorb in the ultraviolet A (UVA) or UVB range (Proserpio 1976).

Impurities

A mixture containing Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%) contains <10 ppm heavy metals and <0.01 ppm organochlorines; organophosphoric compounds were not detectable (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996).

USE

Cosmetic

Hypericum Perforatum Extract and Hypericum Perforatum Oil function in cosmetic formulations as biological additives (Wenninger and McEwen 1997). The product formulation data submitted to the FDA in 1998 reported that Hypericum Perforatum Extract was used in 64 cosmetic formulations and Hypericum Perforatum Oil was used in 11 cosmetic formulations (FDA 1998) (Table 1).

Concentration of use values are no longer reported to the Food and Drug Administration (FDA) by the cosmetic industry (FDA 1992). One manufacturer reported that Hypericum Perforatum Extract is used at concentrations of 0.01% in hair conditioner and shampoo, 0.1% in facial mask, and 0.5% in facial cleanser, facial moisturizer, night cream, and skin freshener formulations (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1998). Another supplier reported that a mixture of Hypericum Perforatum Extract (10%–25%) and propylene glycol (>75%) is used at 1% to 10% in cosmetic products (Grau Aromatics, GmbH & Co. 1997). The product formulation data submitted to the FDA in 1984 stated that Hypericum Perforatum Extract was used in 49 cosmetic formulations, 21 at concentrations ≤5% and 28 at unknown concentrations, and that Hypericum Perforatum Oil was used in 10 cosmetic formulations, 6 at ≤5% and 4 at unknown concentrations (FDA 1984) (Table 2).

International

Hypericum Perforatum Extract, as Hypericum Extract, is listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS)* (Rempe and Santucci 1997). Hypericum Extract, which conforms to the specifications of the *Japanese Cosmetic Ingredients Codex*, has precedent for use without restriction in all *CLS* categories. Hypericum Perforatum Oil does not appear in the *CLS*. Hypericum Perforatum Extract

TABLE 1
Hypericum Perforatum product formulation data (FDA 1998)

Product category	Total no. of formulations in category	Total no. containing Hypericum Perforatum	
		Extract	Oil
Baby lotions, oils, powders, and creams	53	1	
Bath oil, tablets, and salts	124		1
Bubble baths	200	3	
Other eye makeup preparations	120	2	
Shampoos (noncoloring)	860	4	
Tonics, dressing, other hair-grooming aids	549		1
Other hair preparations	276	1	
Blushers (all types)	238	1	
Aftershave lotion	216	2	
Shaving cream	139	1	
Other shaving preparation products	60	1	
Cleansing preparations	653	5	2
Face and neck preparations (excluding shaving preparations)	263	7	1
Body and hand preparations (excluding shaving preparations)	796	7	2
Moisturizing preparations	769	4	
Night preparations	188	4	
Paste masks (mud packs)	255	3	2
Skin fresheners	184	3	
Other skin care preparations	692	14	2
Indoor tanning preparations	62	1	
1998 Total uses of Hypericum Perforatum		64	11

TABLE 2
Concentration of use data (FDA 1984)

Product category	1%–5%	0.1%–1%	0%–0.1%	Unknown	Total
Hypericum Perforatum Extract					
Bubble baths				1	1
Shampoos (noncoloring)				2	2
Shaving cream (aerosol/brushless/lather)				1	1
Skin cleansing products (cold creams/lotions/liquids/pads)		4	1	4	9
Face/body/hand preparations (excluding shaving preparations)		6	1	10	17
Night preparations		3	1	1	5
Paste masks (mud packs)				1	1
Skin fresheners			1	2	3
Other skin care preparations	1	3		6	10
1984 Total uses of Hypericum Perforatum Extract	1	16	4	28	49
Hypericum Perforatum Oil					
Bath oils/tablets/salts	1			1	2
Shaving cream (aerosol/brushless/lather)		1			1
Face/body/hand preparations (excluding shaving preparations)	1	2			3
Moisturizing products				1	1
Paste masks (mud packs)	1				1
Other skin care preparations				1	1
Suntan gels/creams/liquids				1	1
1984 Total uses of Hypericum Perforatum Oil	3	3	0	4	**10**

and Hypericum Perforatum Oil do not appear in Annex II (list of substances which must not form part of the composition of cosmetic products) or Annex III (list of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down) of the Cosmetics Directive of the European Union (1995).

Noncosmetic

The leaves, flowers, and caulis of *H. perforatum* L., as hypericin-free alcohol distillate, can be used as natural flavoring substances and natural substances used in conjunction with flavors in alcoholic beverages when used in the minimum quantity required to produce the intended effect and in accordance with good manufacturing practices (FDA 1997).

The use of Hypericum extract for treatment of depression has been examined (Harrer and Schulz 1994; Hübner, Lande, and Podzuweit 1994; Martinez et al. 1994; Sommer and Harrer 1994; Vorbach, Hübner, and Arnoldt 1994; Woelk et al. 1994; Vorbach, Arnoldt, and Hübner 1997; Wheatley 1997) *H. perforatum* is used in folk medicine as a diuretic and anthelmintic agent (Poginsky et al. 1988) and was reported to have "anti-inflammatory activity" (Fleischner 1985).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Twelve male subjects were given single oral doses of 300, 900, or 1800 mg Hypericum extract (250, 750, or 1500 μg hypericin; 526, 1578, or 3156 μg pseudohypericin) and plasma concentrations of hypericin and pseudohypericin were determined at multiple intervals up to 120 hours after dosing (Staffeldt et al. 1994). Values for hypericin after ingestion of 300, 900, and 1800 mg Hypericum extract were: the time of maximum plasma concentration (t_{max}) was 5.2, 4.1, and 5.9 hours, respectively; the maximum concentration (C_{max}) was 1.5, 7.5, and 14.2 ng/ml, respectively; the absorptive lagtime (t_{lag}) was 2.6, 2.0, and 2.6 hours, respectively; and the half-life ($t_{1/2}$) was 24.8, 26.0, and 26.5 hours, respectively. The pharmacokinetic parameters of pseudohypericin after ingestion of 300, 900, and 1800 mg Hypericum extract were: t_{max} of 2.7, 3.0, and 3.2 hours, respectively; C_{max} of 2.7, 11.7, and 30.6 ng/ml, respectively; t_{lag} of 0.6, 0.4, and 0.4 hours, respectively; and $t_{1/2}$ of 16.3, 36.0, and 22.8 hours, respectively. The areas under the curve (a measure of the amount of substance appearing in the plasma) demonstrated a nonlinear increase with increasing dose; this was statistically significant for hypericin. In long-term dosing in which subjects received 300 mg Hypericum extract three times a day for 14 days, steady-state occurred after 14 days.

Immunologic Effects

The immunomodulatory potential of polyphenol, lipophile, and water soluble fraction of *H. perforatum* was examined (Yevstifeyeva and Sibiryak 1996). The effect on macrophagal activity and humoral and cellular immune response was examined. Contact hypersensitivity was induced in BALB/c mice using 2,4-dinitrobenzene (DNFB), and a state of "high zone tolerance"

was created with a hyperimmune dose of DNFB consisting of 150 ml of a 0.5% solution and by means of sheep erythrocytes. The polyphenol fraction had immunostimulating effect on the mononuclear phagocyte system and cellular and humoral immunity. Under conditions of high dose tolerance, the polyphenol fraction "promoted regeneration of immunologic activity." The lipophilic portion of the *H. perforatum* had "immunodepressive" properties on cellular and humoral immune response. The immunosuppressive effect of the lipophilic fraction was dose dependent, with the lowest dose producing the maximum effect.

The effects of 5×10^{-4} to 5×10^{-6} mol/l Hypericum extract in propylene glycol on the expression of serotonin receptors in the rat neuroblastoma cell line PC-12 was examined (Müller and Rossol 1994). Evaluations were made 2, 4, and 6 hours after incubation. Fluorescence microscopy reported a maximum reduction in serotonin receptor expression after 6 hours, and the decrease was especially marked with the 5×10^{-5} mol/l solution.

ANIMAL TOXICOLOGY

Acute Toxicity

The oral LD_{50} for rats of a mixture containing Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%) was >20 ml/kg (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996).

The acute subcutaneous (SC) toxicity of *H. perforatum* was determined by the Reed and Vanderkleed guinea-pig method in which the minimum SC dose to kill a 250-g guinea pig within 24 hours is determined (Rogers 1914). The minimum lethal dose of *H. perforatum* was 0.1 ml.

The intraperitoneal (IP) LD_{50} values of the polyphenol, lipophile, and water soluble fractions of *H. perforatum* in mice were 780, 4300, and 2800 mg/kg, respectively (Yevstifeyeva and Sibiryak 1996).

Short-Term Toxicity

Groups of three adult Awasi sheep were fed *H. perforatum* flowers at doses of 4, 8, 12, or 16 g/kg (250, 500, 750, or 1000 g/sheep/day, respectively) for 14 days (Kako, Al-Sultan, and Saleem 1993). A control group was fed hay. Blood samples were taken on days 0, 7, and 14. Signs of toxicity appeared in the animals fed 12 and 16 g/kg *H. perforatum* 2 days prior to them appearing in the animals fed 4 or 8 g/kg; the day of onset was not specified. The signs of toxicity included restlessness, photophobia, tachycardia, polypnea, congested mucous membranes, diarrhea, hyperthermia, erythema of the exposed parts of the tail and legs, edema of the eyelids, edema, and loss of serum from the ears. These signs intensified with the appearance of salivation, alopecia of the face and around the eyes and ears, keratoconjunctivitis, severe congestion of mucous membranes, loss of eyelashes, corneal opacity, and blindness. On day 14, hemoglobin and packed cell volume values were significantly decreased in the groups fed 8, 12, or 16 g/kg. Blood urea nitrogen (BUN) values were statistically significantly increased on days 7 and 14. Most serum enzymes were elevated, but serum alkaline phosphatase was decreased. The severity of the changes generally increased with time but not with dose in all groups, except for changes in BUN, which increased significantly with both time and dose.

Groups of dd-mice were given 5, 10, or 20 ml/kg of a mixture of Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) for 14 days (Ichimaru Pharcos Co., Ltd. 1996). The LD_{50} was >20 ml/kg.

Subchronic Toxicity

Published data on the subchronic toxicity of Hypericum Perforatum Extract and Hypericum Perforatum Oil were not found.

Chronic Toxicity

Eight male Long-Evans rats were fed basal diet and *H. perforatum* for 178 days; a control group was fed basal diet only (Garrett et al. 1982). The *H. perforatum* was added at a concentration of 10% until day 12, at which time it was reduced to 5% because of lack of palatability. With the exception of four test animals that were killed on day 119, all animals were killed and necropsied on day 178. All animals survived until study termination. Average daily weight gain was statistically significantly decreased for test animals as compared to controls. No significant differences were found in the concentrations of hepatic copper, iron, or zinc between test and control animals.

Dermal Irritation

A mixture of Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%), tested at 10% in liquid paraffin, was nonirritating to rabbits in a patch test (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996).

The irritation potential of a mixture containing Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) was determined in a Draize test in which 0.5 ml of the mixture was applied to intact and abraded skin of six albino rabbits (Ichimaru Pharcos Co., Ltd. 1996). The test sites were scored 4, 24, and 48 hours after application. The mixture was not irritating. The mixture, 0.5 ml, was also applied 19 times to the skin of 10 guinea pigs over a 4-week period. Erythema and edema were not observed.

Sensitization

A mixture of Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%) was not sensitizing to guinea pigs in a Buehler test (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996).

The sensitization potential of a mixture containing Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) was determined in a maximization test using

guinea pigs (Ichimaru Pharcos Co., Ltd. 1996). Erythema and edema were not observed.

Photosensitization

H. perforatum is a primary photosensitizer in animals because of the photodynamic pigment hypericin (Mitich 1994). Hypericin causes photoactivated damage by absorbing visible light (550 to 610 nm, maximum at 585 nm), and it is poisonous to animals only by ingestion. Hypericin remains chemically intact through ingestion, digestion, absorption into the bloodstream, and passage into the liver. It is transported to the epidermal capillaries and, upon exposure to oxygen and bright sunlight, induces oxidative damage to capillary walls, particularly in areas of unpigmented skin (Jensen et al. 1995).

The phototoxicity potential of a mixture containing Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) was determined using six guinea pigs (Ichimaru Pharcos Co., Ltd. 1996). One-tenth milliliter of the test article was applied and exposed to a 15-minute minimal erythema dose. The mixture was not phototoxic.

Ocular Irritation

A mixture of Hypericum Perforatum Extract (1%–5%), olive (Olea Europaea) oil (>50%), and tocopherol (<0.1%), tested at 10% in liquid paraffin, was nonirritating to rabbit eyes in a Draize test (Chemisches Laboratorium Dr. Kurt Richter GmbH 1996).

The ocular irritation potential of a mixture containing Hypericum Perforatum Oil, butylene glycol, and water (percentages not specified) was determined in a Draize test in which 0.1 ml of the mixture was applied to the conjunctival sacs of six albino rabbits (Ichimaru Pharcos Co., Ltd. 1996). A conjunctival reaction was observed in one rabbit.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Published data on the reproductive toxicity of Hypericum Perforatum Extract and Hypericum Perforatum Oil were not found.

GENOTOXICITY

In Vitro

The mutagenic potential of a tincture of hypericum (adjusted to 20 mg of hypericum per 100 μl suspension) was determined in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 (Göggelmann and Schimmer 1986). Ethanolic solutions of 10 to 400 μl were evaluated with and without metabolic activation. The hypericum extract produced a greater than 10-fold increase in the number of revertants as compared to controls with *S. typhimurium* TA98 with and without metabolic activation and with *S. typhimurium* TA100 with metabolic activation; a 5- to 10-fold increase in revertants was seen with TA100 in the absence of metabolic activation. The researchers ascribed the mutagenic effects to the flavonols found in hypericum. It should be noted that the researchers stated that "the origin of the plant is important for the presence of essential components" and results may differ based on the district of growth and the preparation of the extraction. Also, "the mode of preparation influences the levels of mutagenic material in the suspensions and that the same amount of extracted plant material in different products can induce different revertant numbers." Göggelmann (1986) stated that "it is not possible to extrapolate from the mutagenicity of a preparation of a single plant to that of a medicine consisting of several plants." This is because "although the same amount of mutagenic activity is present in some of the drugs, different mutagenic effects have been observed. Consequently, the mode of preparation and the presence of additional plants influence the mutagenic activities."

The genotoxicity of *H. perforatum* L. was determined in an Ames test and in an unscheduled DNA synthesis (UDS) assay (Poginsky et al. 1988). In the Ames test, 20 and 40 μl undiluted *H. perforatum* L. was assayed with and without metabolic activation using *S. typhimurium* strain TA98; a negative control and two positive controls, nitrofluorene without metabolic activation and aminoanthracene with metabolic activation, were used. Mutagenic activity was observed, especially with the high dose with metabolic activation. The mutagenic potential of 10 to 40 μl of an ethanol extract, chloroform extract, and ethyl acetate extract of *H. perforatum* L. (20 g *H. perforatum* L./100 ml extract) was then evaluated in the Ames test. The ethanol and ethyl acetate extracts had mutagenic activity with and without metabolic activation. The ethyl acetate extract was fractionated by HPLC, and the fractions were assayed for mutagenic potential. The mutagenicity of the full extract was found exclusively in quercetin. Hypericin was not mutagenic.

In the UDS assay using primary rat hepatocytes, 50 to 500 μl *H. perforatum* L. in ethanol extract was evaluated. A negative control and two positive controls, 7,12-dimethylbenz(a) anthracene and UV light (4 W, 254 nm), were used. *H. perforatum* L. was genotoxic in the UDS assay. Hypericin was not genotoxic in the UDS assay.

Okpanyi et al. (1990) examined the genotoxic potential of a standardized aqueous ethanolic Hypericum extract in an UDS assay, a hypoxanthine guanidine phosphoribosyl transferase (HGPRT) test, and a cell transformation assay. ("The standardized extract [total hypericin 0.25 mg/ml] is of very high grade [total hypericin 0.314 mg/ml] for pharmaceutical preparation for systemic [oral] administration and meets the requirements of the DAB" [Okpanyi 1997]). Primary rat hepatocytes from male Wistar CF HB rats and 0.014–1.370 μl/ml Hypericum extract were used in the UDS assay. Ethanol, 1% *v/v*, was used as a negative control and 2-acetylaminofluorene was used as positive control. Hypericum extract did not increase [^{3}H]-TdR (Thymidine deoxyribose) incorporation when compared to the negative control.

In the HGPRT test, V79 Chinese hamster cell cultures were incubated with 0.65 to 4.00 μl/mg and 0.08 to 0.50 μl/mg

Hypericum extract with and without metabolic activation, respectively. Untreated V79 cells and untreated V79 cells in solvent-containing medium both with and without metabolic activation were used as the negative controls. Ethylene ethanesulfate and 9,10-dimethyl-1,2-benzanthracene were used as positive controls without and with metabolic activation, respectively. A significant difference was not observed between the test and negative control cultures in the number of 6-thioguanidine resistant cell colonies.

In the cell transformation assay, Syrian golden hamster embryo cells were used. With a 4-hour exposure with and without metabolic activation, 1.0 to 10.0 and 0.75 to 7.5 μl Hypericum extract/ml of medium were used, respectively, and with a 48-hour exposure with metabolic activation, 0.75 to 7.5 μl Hypericum extract/ml of medium was used. Positive controls were used. Cell-transforming activities were not induced by Hypericum extract with or without metabolic activation.

H. perforatum (grade and composition unknown) was evaluated in an antimutagenic assay using derivatives of *Escherichia coli* K12 (Vuković-Gačić and Simić 1993). *H. perforatum* inhibited spontaneous and UV-Induced mutagenesis. The researchers stated that the antimutagenic effect was probably due to suppression of error-prone repair.

In Vivo

Okpanyi et al. (1990) examined the genotoxic potential of a standardized aqueous ethanolic Hypericum extract in two In vivo tests, a mouse fur spot test and a chromosome aberration test. ("The standardized extract [total hypericin 0.25 mg/ml] is of very high grade [total hypericin 0.314 mg/ml] for pharmaceutical preparation for systemic [oral] administration and meets the requirements of the DAB" [Okpanyi 1997]). In the fur spot test, groups of 64, 68, and 62 female NMRI-mice were dosed orally with 1, 5, and 10 ml/kg of Hypericum extract, respectively, on day 9 of gestation. Three weeks after birth, the neonates were checked for fur spots. Ethanol, 47%, was used as a negative control and ethylnitrosourea was used as a positive control. An increase in fur spots was not observed for test animals as compared to the controls.

In the chromosome aberration test, groups of five male and five female hamsters were dosed orally with 10 ml/kg Hypericum extract at various doses. When the test was 6 hours, the extract was undiluted; when it was 24 hours, the extract was supplied as 1:10 and 1:3 dilutions and undiluted; and when it was 48 hours, the extract was undiluted. Ethanol, 46%, was used as a negative control and cyclophosphamide, 40 mg/kg, was used as a positive control. With the 6-hour exposure, a minor cytotoxic effect was observed. A genotoxic effect was not observed with Hypericum extract.

CARCINOGENICITY

Published data on the carcinogenic potential of Hypericum Perforatum Extract and Hypericum Perforatum Oil were not found.

CLINICAL ASSESSMENT OF SAFETY

Irritation

The irritation potential of a mixture containing Hypericum Perforatum Oil-butylene glycol, and water (percentages not specified) was determined using 30 subjects; the test sites were scored 48 and 72 hours after application (Ichimaru Pharcos Co., Ltd. 1996). (Details not provided.) There was a "±" reaction for one subject at 48 hours, but there were no positive reactions at 72 hours.

Sensitization

Published data on the irritation and sensitization potential in humans of Hypericum Perforatum Extract and Hypericum Perforatum Oil were not found.

Toxicity

The effects of Hypericum extract in the treatment of depression were evaluated in a number of studies, as reported in Noncosmetic Use. The information in these studies provides some human toxicity data upon dosing with 300 mg/day Hypericum extract for 4 to 6 weeks. Hübner, Lande, and Podzuweit (1994) treated 20 patients for 4 weeks; "not one patient reported any relevant adverse effects." Martinez et al. (1994) treated two groups of 10 patients with Hypericum extract and bright white light (3000 lux) or dim light (<300 lux) for 4 weeks; "none of the patients in either treatment group reported any adverse drug reactions." Sommer and Harrer (1994) treated 105 patients for 4 weeks; undesired drug effects, including skin reddening, itching, and tiredness, were seen in 2 patients. Vorbach, Hübner, and Arnoldt (1994) treated 67 patients for 6 weeks; undesired drug effects, including dry mouth, dizziness, and constipation, occurred in 8 patients. Woelk, Burkard, and Grünwald (1994) treated 3250 patients for 4 weeks; undesired drug effects, including gastrointestinal irritations, allergic reactions, fatigue, anxiety, and dizziness, occurred in 79 patients. Wheatley reported that of 83 patients given 900 mg/day for 6 weeks, 32 patients reported adverse effects, including headache (6), nausea/vomiting (6), dry mouth (4), and constipation (4) were reported. Vorbach, Arnoldt, and Hübner (1997) reported that of 107 patients given 1800 mg/day, 25 patients reported a total of 37 adverse events, including restlessness (6), gastric symptoms (5), tiredness/sedation (5), dizziness (5), and dry mouth (3).

SUMMARY

Hypericum Perforatum Extract is an extract of the capsules, flowers, leaves, and stem heads of the hypericum, *H. perforatum*. In 1998, it was reported to the FDA that Hypericum Perforatum Extract and Hypericum Perforatum Oil were used in 64 and 11 cosmetic formulations, respectively. One manufacturer reported that Hypericum Perforatum Extract is used at concentrations of ≤0.5% and it was reported by another supplier that a mixture of Hypericum Perforatum Extract and propylene glycol is used at concentrations of 1% to 10%. In 1984, Hypericum

Perforatum Extract and Hypericum Perforatum Oil were reported to be used at concentrations of ≤5% and unknown concentrations.

Using male subjects, a single oral administration of Hypericum extract resulted in a nonlinear increase, with increasing dose in the amount of hypericin or pseudohypericin appearing in the plasma, and the increase was statistically significant for hypericin. With long-term dosing of Hypericum extract, steady-state occurred after 14 days. The polyphenol fraction of *H. perforatum* had immunostimulating activity on the mononuclear phagocyte system and cellular and humoral immunity, and the lipophilic portion had immunosuppressive activity on cellular and humoral immune responses.

The oral LD_{50} values for rats and mice of mixtures containing Hypericum Perforatum Extract were >20 ml/kg. The minimum lethal SC dose of *H. perforatum* using guinea pigs was 0.1 ml. The LP LD_{50} values of the polyphenol, lipophile, and water soluble fractions of *H. perforatum* L. were 780, 4300, and 2800 mg/kg, respectively. Signs of toxicity were observed in Awasi sheep fed *H. perforatum* flowers for 14 days. In a chronic study in which Long-Evans rats were fed *H. perforatum*, average daily weight gain was statistically significantly decreased as compared to control animals. Mixtures containing Hypericum Perforatum Extract and Hypericum Perforatum Oil were not irritants or sensitizers in animals. *H. perforatum* is a primary photosensitizer in animals because of the pigment hypericin, which causes photoactivated damage by absorbing visible light. A mixture containing Hypericum Perforatum Oil, butylene glycol, and water was not phototoxic. Mixtures containing Hypericum Perforatum Extract and Hypericum Perforatum Oil were non- to slightly irritating, respectively, in rabbit eyes.

In an Ames test, a tincture of hypericum had mutagenic effects, which the researchers attributed to flavonols. However, the origin of the plant and the mode of preparation of the tincture were considered to play a role in the mutagenic potential. In another Ames test, *H. perforatum* L. had mutagenic activity; in testing fractions of three extracts, the mutagenic potential was found exclusively in quercetin, and hypericin was not mutagenic. Hypericum extract and hypericin were not genotoxic in UDS assays using primary rat hepatocytes. Hypericum extract was not mutagenic in a cell transformation assay using Syrian golden hamster embryo cells, and it was not genotoxic in a mouse fur spot test or in a chromosome aberration test.

A mixture of Hypericum Perforatum Oil, butylene glycol, and water was not irritating in clinical studies. In human testing, Hypericum extract did not appear to be toxic, although some undesirable drug effects were observed.

DISCUSSION

Section 1, paragraph (p), of the Cosmetic Ingredient Review (CIR) Procedures states that "A lack of information about an ingredient shall not be enough 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 Hypericum Perforatum Extract and Hypericum Perforatum Oil were insufficient to determine whether Hypericum Perforatum Extract and Hypericum Perforatum Oil were either safe or unsafe. The Expert Panel released a Notice of Insufficient Data Announcement on June 6, 1997, outlining the data needed to assess the safety of Hypericum Perforatum Extract and Hypericum Perforatum Oil. The types of data still required for each ingredient include[2]

1. Current concentration of use data.
2. Function in cosmetics.
3. Photosensitization and phototoxicity data using visible light (550–610 nm; 5–10 J).
4. Gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures.[3]
5. Dermal reproductive/developmental toxicity data.[3]
6. Skin irritation/sensitization data in humans on Hypericum Perforatum Oil.
7. Ocular irritation data, if available.

The Expert Panel originally also requested information on the presence of contaminants. Some data were received and summarized in the report. The Expert Panel expects that pesticide residues would be kept to a minimum.

No offer to supply the needed data was received. In accordance with Section 45 of the CIR Procedures, the Expert Panel has issued a Final Report—Insufficient Data. When the requested data are available, the Expert Panel will reconsider the Final Report in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Hypericum Perforatum Extract and Hypericum Perforatum Oil for use in cosmetic products.

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[2]All testing is to be performed on cosmetic-grade ingredients unless otherwise specified.

[3]These are data that would be expected from what is commonly referred to as a "28-day dermal toxicity study."

[4]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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