RISK PROFILE

Hypericum perforatum, extract and oil

CAS No.84082

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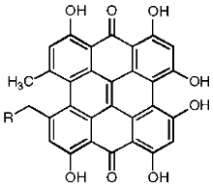
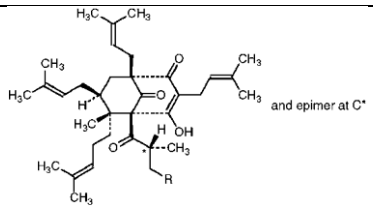
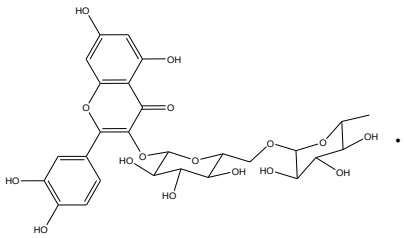
1. Identification of substance

Chemical name (IUPAC):	Not applicable.
INCI	Hypericum perforatum Extract (*HPE*) Hypericum perforatum Oil (*HPO*)
Synonyms	*HPE*: Extract of the capsules, flowers, leaves and stem heads of the St. John's wort, Hypericum perforatum L., Hypericaceae (CosIng) *HPO*: Fixed oil obtained from the flowers of St. John's Wort, Hypericum perforatum L., Hypericaceae (CosIng) The plant *Hypericum perforatum*: Millepertuis, St. John`s wort, Johnswort, amber, goatweed, Klamath weed, tipton weed, Stl. Johnswort, John`s wort, herb-John, cammock, penny John, grace of god, and rosin rose. (Johannesurt)
CAS No.	*HPE*: 84082-80-4 *HPO*: 68917-49-7
EINECS No.	*HPE*: 282-026-4 *HPO*: not available.
Molecular formula	Not applicable for extract/oil
Chemical structure	The molecular structure of some of the main constituents:

	 Hypericin: R=H CAS No: 548-04-9 Pseudohypericin: R=OH CAS No: 55954-61-5  Hyperforin R=H CAS No: 11079-53-1 Adhyperforin: R = CH_3  Rutin (aglycone: quercetin) CAS No 153-18-4
Molecular weight	Not applicable for extract/oil; *hypericin*: 504.4 *Hyperforin*: 536.8 *Rutin*: 610.5
Contents (if relevant)	The *HPE* is usually a 60-80 % hydroethanolic or hydromethanolic solution. A variety of different components have been reported in *Hypercum perforatum*. According to EMA (2009) the major characteristic constituents include: • 0.06-0.4% naphtodiantrones (pseudohypericin, *hypericin* and others), • 0.2 – 4 % phloroglucinols (*hyperforin*, adhyperforin and many more); • 2-4% flavonoids (glycosides of hyperoside, quercitrin, isoquercitrin, rutin – and also biflavones), • 7-15% procyanidines (catechin tannins and others), • 0.1-0.25 % essential oils. Rutin: 0.3 – 1.6 % (American Botanical Council). For a more detailed overview see Greeson JM *et al* (2001). Both the *HPE* and the *HPO* are commonly characterized in regard of the *hypericin* content. *Hypericin* is a red fluorescence compound. The amount of *hypericin* present depends on the location of the plant, the portion of the plant, and the time of year and up to 80 % active hypericin is lost upon drying of the plant. In the whole herb *hypericin* may be present in concentrations of 0.0095 - 0.466% and in flowers up to 0.086% (CIR, 2001).

Many of the constituents possess bio-active properties (Linde K 2009):

Component group	*Examples*	*Plant part*	*Action*
Naphthodianthrones (lipophilic)	*hypericin pseudohypericin*	*flowers, buds*	*antidepressant, antiviral, photosensitizing*
Phloroglucinols (lipophilic)	*hyperforin adhyperforin*	*flowers, buds*	*antidepressant, antibiotic*
Flavonoids (lipophilic/hydrophilic)	*quercetin hyperoside quercitrin isoquercitrin rutin*	*leaves stalk buds*	*antidepressant, antiphlogistic (3)*
Biflavonoids (lipohilic)	*biapigenin*	*flowers*	*sedating, antiphlogistic (3)*
Procyanidins (hydrophilic)	*procyanidin catechin epicatechin*	*flowers, buds*	*antiphlogistic, (3) antioxidant*
Essential oils (lipophilic)	*terpenes alcohols*	*flowers, leaves*	
Amino acids (hydrophilic)	*GABA (1)*	*flowers, leaves*	*Antidepressant (1)*
Phenylpropanes (hydrophilic)	*caffeic acid chlorogenic acid*	*flowers, leaves*	
Xanthons (2) (lipophilic)	*norathyriol*	*roots, flowers*	*Antidepressant (2)*

(1): GABA (gamma amino butyric acid) is the main inhibitory transmitter substance of central nervous system in vertebrates. Greeson JM et al (2001) inform that in the fresh plant the GABA concentration is 0.0007 % (7 ppm). Further information as to its occurrence in this plant is to be found in articles by Males Z et al (2004) and Hahn G (1992). A number of commercial sources sell formulations of GABA for use as a dietary supplement. These sources typically make claims that the supplement has a calming effect. No scientific assessment of such claims exists, but because of the extensive evidence that GABA does not cross the blood-brain barrier at significant levels, these claims are likely untrue. (2): Also xanthons occur in trace amounts only - up till 10 ppm (American Botanical Council). EMA (2009) expresses that : "Due to the low content of xanthones in the herbal substance (about 0.0004%) it is not likely that the experimentally documented inhibition of MAO A and B is of clinical relevance ". (3): Antiphlogistic means anti-inflammatory.

Impurities: the oil and extract may contain low concentrations of heavy metals and organochlorines.

Physiochemical properties

HPE and HPO

A mixture of *HPE* (1-5 %), olive oil (Olea Europaea) (>50 %), and tocopherol (<0.1 %) is a red brown oil with a specific odor. This is a fatty oil extract of hypericum blossoms, where the vehicle used is olive oil.

A mixture of *HPE* (10-25 %) and propylene glycol (>75 %), is a clear, red liquid with a faint herbal odor. This extract is added preservatives 0.6 % phenoip (phonoxyethanol, methylparaben, butylparaben, ethylparaben, and propylparaben).

A mixture of *HPO*, butylene glycol, and water (percentage not known), is a reddish-brown, transparent liquid.

For more information, see the safety assessment of Cosmetic Ingredient Review, 2001.

References:
(CIR, 2001)

Three of the main constituents

	Partition coefficient **(logPow)** [1] *Hypericin:* 0.61 (Huygens A *et al* 2005) *Hyperforin*: 6-10 (Drug bank[2]) *Rutin*: 0.15 (Drug bank) ***Water solubility*** *Hypericin* *Hypericin* is insoluble in water at pH 4-5 (Kraus GA *et al* 1995). However, the solubility of pure *hypericin* in water increased upon addition of some phenolic constituents typical for *HPE*. Most effective in solubilizing *hypericin* was hyperoside (hyperin, quercetin 3-O-beta-D-galactoside) which increased the concentration of *hypericin* in the water phase up to 400 fold in a moodl (Jurgenliemk G *et al* 2003). *Hyperforin* 0,63 mg/L *Rutin* 3540 mg/L

2. Uses and origin

Uses	➢ **Cosmetic products:** ***Functions according to:*** o CosIng database *HPE:* • "Antimicrobial"- Helps control the growth of micro-organisms on the skin • "Astringent" - Contracts the skin • "Masking" – Reduces or inhibits the basic odour or taste of the product • "Skin conditioning" – Maintains the skin in good condition • "Skin protecting" – Helps to avoid harmful effects to the skin from external factors • "Soothing" – Helps lightening discomfort of the skin or of the scalp • "Tonic" – Produces a feeling of well-being on skin and hair. *HPO:* • "Emollient" – Softens and smooth the skin (CosIng [online]).

[1] The permeability of a compound into tissue is mainly determined by its partition coefficient, while the molecular weight and the possibility of hydrogen-bond formation are less important. High pKow values indicate high penetration into tissues.

[2] http://www.drugbank.ca

Concentrations being applied

HPE:

Face cleansing products: 0.1 - 1 %
Face cream: 0 - 1 %
Body lotion: 0 - 1 %

Other product categories, such as bubble bath, shampoo, shaving cream and facial mask, may also contain HPE, but the concentrations are unknown.

HPO:

Bath oil/tablet/salt: 1 - 5 %
Shaving cream: 0.1 - 1 %
Face cream: 0.1 - 5 %
Body lotion: 0.1 – 5 %
Facial mask: 1 – 5 %

(CIR, 2001).

Some deliverers of cosmetic products ingredients currently recommend usage level of 3 % [3] and even 5 - 10% *HPE*.[4]

According to the CIR report a mixture of *HPE* (10%–25%) and propylene glycol (>75%) was used by one producer at 1% to 10% in cosmetic products. That would mean that the concentration of *HPE* in the ready to use cosmetic products varied from 0.1 to 2.5 %.

A branch inventory called the *Cosmetics & Toiletries Bench Reference* (CBR directory) list manufacturers offering cosmetic product ingredients for sale[5]. The impression is that they who deliver *HPE* mainly sell *HPE* standardized to 0.3 % *hypericin*.

Frequency of use

In search at the Codecheck.info and EWG`s Skin deep databases, *Hypericum perforatum* shows up as an ingredient in over 200 cosmetic products at both databases. *HPE* is specified as ingredient in the majority of the products, whereas *HPO* is only indicated in a few ones.

(Codecheck [online]; EWG's Skin Deep [online]).

In 1998 the FDA received information on a voluntary basis on 64 cosmetic formulations containing *HPE* and 11 containing *HPO.* Back in 1984 the numbers were smaller; 49 for *HPE* and 10 for *HPO* (CIR, 2001). In the intermediate year 1992 *HPO* was up at 22[6] formulations whereas *HPE* was up at 74 in the year 1996.[7] So, the impression is that the popularity of these two "botanical" ingredients has varied somewhat over the years.

The 193 *HPE* products mentioned in the Codecheck database are of

[3] http://www.saci-cfpa.com/site/upload/fiches/81891547047a9b252a559d.pdf
[4] http://www.specialchem4cosmetics.com/tds/actiphyte-st-johns-wort/active-organics/857/index.aspx
[5] The inventory is administrated by the American branch periodical the *Cosmetic & Toiletries Magazine.*
[6] Unwanted effects of cosmetics and drugs used in dermatology, 3rd edition 1994, editors Groot AC, Weyland JW and Nater JP
[7] Cosmetic & Toiletries magazine, Vol 113 October 1998, p. 74

the following categories:

Product type	Number of products in database	Sort of cosmetic products
Face cream	110	Leave-on
Face cleansing	27	Rinse-off
Body cream/lotion	24	Leave-on
Mask	11	Rinse-off
Hand cream	7	Leave-on
Shampoo	6	Rinse-off
Deodorant	2	Leave-on
Hair styling	2	Leave-on
Lip-product	1	Leave-on
Foot-product	1	Rinse-off
Aftershave	1	Leave-on
Shaving cream	1	Rinse-off

As concerns the face creams *HPE* is typically together with many other "botanical" ingredients (plant extracts). In one product the *HPE* figure high up in the list of ingredients; 2nd place, whereas, typically, it shows up in the middle or the lower part of the list. Only as concerns two of the creams *HPE* is featured in the sales announcement:

- Das wertvolle Johanniskrautöl beruhigt leicht irritierte oder nach einem Sonnenbad gereizte Haut (*The precious "Johanniskrauteröl" soothes irritated or easily irritated skin after sunbathing*)

- Johanniskraut wirkt entzündungshemmend und lindert den Juckreiz (z.B. Neurodermitis). (*St. John's wort reduces inflammation and relieves itching*)

We would think that the main reason *HPE* is employed in these skin creams is because the extract, according to CosIng, is an antimicrobial, astringent, skin conditioning and soothing type of ingredient. These different functions we would think is at least partly based on inherent properties that according to EMA (2009) has been documented (see page 17 in document):

Function (ref. CosIng)	Documented inherent property (activity) (ref. EMA, WHO)
Antimicrobial	Antibacterial /Antiviral
Astringent	Astringent
Skin conditioning *(Maintains the skin in good condition)*	Anti-inflammatory (1)
Soothing *(Helps lightening discomfort of the skin)*	Anti-inflammatory (Analgesic) (1)

(1) Several studies show that *HPE* taken orally has anti-inflammatory and analgesic action – and also possesses anti-inflammatory properties when applied topically. It goes about central analgesic properties (Sanchez-Mateo CC *et al* (2006), Rabanal RM *et al* (2005), Kumar *et al* (2001), Oztork Y *et al* (1996). The latter authors explain that the analgesic activity is intimately connected to the anti-depressive effect. Hence, probably, the analgesic activity is mediated by the same active constituents as those mediating the anti-depressive effect.

Further as concern the *topica*l anti-inflammatory effect EMA (2008)

informs that *HPE* has traditionally been used to treat inflammatory skin disorders (dermatitis). *In vivo* investigations by the authors Schempp CM *et al* (2000) have provided a rationale for this treatment says EMA. These studies demonstrated a substantial inhibitory effect of *HPE* and its constituent *hyperforin* on epidermal cell lymphocytes (MECLR) and on the proliferation of T lymphocytes. *Hyperforin* is abundantly present in *HPE*; > 2 % (*inter alia*).

The influencing of *HPE* and *HPO* with skin conditions possibly also connects to the behavior of the *hypericin* molecule within dermis/ epidermis. Numerous *in vitro* studies have, namely, demonstrated that *hypericin* is a potent inhibitor of protein kinase C (references No 87-92 within WHO 2004). This inhibitory effect may contribute to the anti-inflammatory effects of *HPE*, as *hypericin* also inhibited the release of arachidonic acid and leukotriene B4 (*Panossian AG et al 1996*).

Probably also important to the cosmetic effects of these face creams is the content within *HPE* of the constituent *Rutin* - which can be up to 1.6 % in the plant itself and possibly even higher in the *HPE*. This molecule also is employed in cosmetics in its own right as a separate ingredient at concentrations up to 0.2 %. *Rutin* has anti-inflammatory but also astringent properties and has a reducing effect on visible capillaries in the skin. Medicinally it was previously used topically against varicose symptoms – and was the active ingredient in topical remedies for haemorides (confer monograph in Council of Europe publication 2008 on active ingredients in cosmetics).

Employment in cosmetic products of substances that reduce inflammation also topically is questionable under a safety angel because this effect may in some cases be symptoms of underlying disease[8].

- **Medicinal products/applications**

Peroral administration

HPE is available as Over-The-Counter (OTC) anti-depression medication (Linde *et al*., 1996; Woelk *et al*., 2000; Sarris *et al*., 2009) - and as a food supplement.

In Germany, *HPE* is among the most widely prescribed antidepressant (Volz, 1997). Between October 1991 and December 1999, over 8 million patients are estimated to have been treated with Germany's leading *HPE* preparation. 130 million preparations containing *HPE* were prescribed in 1999 (American Botanical Council 2002). In 2002, 12% of U.S. adults were reported to have used *HPE* within the last 12 months (Williams JW *et al* 2005)[9].

The below table shows the development of the sale of *Hypericum* preparations (excluding combinations) in European countries 2005-2008. The numbers are in 1000 packages/year (Linde K 2009)

Country	4/2005–3/2006	4/2006–3/2007	4/2007–3/2008
Germany	5 040	4 520	3 786
Russia	2 092	2 299	2 198
Poland	1 576	1 524	1 577

[8] A marketer claiming his product helps with inflammation and/or local pain risk having the product reclassified into a medicinal product which in most cases would mean the product is illegal and has to be withdrawn. If instead he claim a skin conditioning and/ or a soothing effect he avoid this marketing obstacle

[9] Hypericum perforatum became popular in the 1990s as herbal remedy, mainly for the treatment of depression. The plant is collected from the wild, but with its increasing popularity, it has begun to be cultivated.

France	423	473	528
Ukraine	485	515	434
Switzerland	285	263	257
Other European countries	904	842	804
Total	10 805	10 437	9 583

In most countries the products are marketed as dietary supplements. Within the EU they are available both as dietary supplements and as drugs - and among the drugs, both in the categories 'well-established use' and 'traditional use' (Linde K 2009).

The drugs vary greatly in chemical content and quality, and may be standardized to *hyperforin* (commonly 3% to 5%) or *hypericin* (commonly 0.3%) content (Wurglics *et al* 2006, Linde K *et al* 2005). The American Botanical Council expressing itself in 2002 considered *hypericin* 0.3% and *hyperforin* 2.8% to be the standards for a typical standardized *HPE.* EMA (2009) mention that the content *of hyperforin* is maximum 6 %.

Ensuring protection of public health EU in 2004 adopted the directive 2004/24/EC on "Traditional Herbal Medicinal Products" – that amended directive 2001/83/EC for the purposes of regulating traditional herbal medicinal products. Products covered by the scope of the new directive include traditional OTC herbal medicinal products that are suitable for use without the intervention of a medical practitioner. An option was open to companies to ask for the effect of a "Sunset Clause" deferred if they considered transferring their product to traditional herbal registration status. The transitional period for the Directive ended 30 April 2011.

This regulative change introduced a stricter regime in the area of herbal medicines. Motivating the increased strictness also were different reports about health risks pertaining to use of traditional herbal medicinal products. Among these reports there also is one regarding the use of *HPE* – and that concerns health risks arising because of herb-drug interactions (*inter alia*). [10]

The sales figures shown above seem to indicate that the directive 2004/24/EC hasn't had a pronounced market impact. We assume that also in the years to come millions of European citizens will be exposed for *HPE* on a daily basis due to more or less continuous "self medication usage" of *HPE*-based *anti-depressives* either in the form of drugs bought without prescription in pharmacies - or in the form of dietary supplements bought in sales outlets typical for these kinds of products.

Hypericum perforatum is also used in folk medicine as a diuretic and anthelmintic agent (CIR, 2001).

External medicinal use

In France the authority AFSSAPS recognises use of high-strength hydroalcoholic *HPE* and a tincture for the following indications

[10] Because of this change of regulation at European level, the Norwegian medicinal product agency re-classified *HPE* anti-depressives from the category *"traditional use"* to the category *"well established use"*. This meant that companies had to invest in up-grading of their since long licensed/authorized *"traditional use"* products as concerns the product' efficiency assurance. With one exception they instead of investing choose to have their products de-registered with the consequence they had to withdraw them from the market. As of 2004 there were 5 brands on the market. Today there is only one. For legislative reasons any *hypericum* based dietary supplements aren't on the market in Norway nowadays.

Similar changes took place in France (information from AFSSAPS 7 March 2012). However, in that country apparently many more products remained on the market as dietary supplements.

(Council of Europe 2006):

- Traditionally used topically as a softening, anti-pruriginous adjunct treatment for skin diseases
- Used as protective nourisher in treating cracked, grazed or chapped skin and insect bites or stings
- Treatment of sunburns, small superficial burns and nappy rash
- Oral use as an antalgic in treating affections of the oral cavity and or pharynx

EMA (2009) on *"traditional use"* mention the following indication only:

> Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as *sunburn*) and as an aid in healing of *minor wounds*.

EMA (2009) conveys that the *"traditional use"* of liquid preparations of *Hypericum* for wound healing is supported by pharmacological data. Anti-inflammatory activity, analgesic activity (via oral administration), astringent activity and antibacterial activity are documented, *in-vivo* data are poor, clinical data are lacking. In contrast the *"traditional use"* for the treatment of symptoms caused by an injury or related to rheumatism is not yet plausible. Antiviral effects are documented for several types of viruses, but not for Varicella zoster. Therefore, *"the traditional use"* in the treatment of shingles cannot be supported – says EMA.

It has been demonstrated that *HPE* has antiviral properties because of the *hypericin* content. *Hypericin* and *pseudohypericin* inhibited herpes simplex virus (Ref No 75, 77-83 in WHO 2004). Patients infected with herpes communis recovered rapidly subsequent to treatment with an ointment containing *hyepricin*. The effect connects to the photodynamic properties of *hypericin* (Ref No 33 in WHO 2004).

- **Food - except for dietary supplements**

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Up until around 2008 the legislation relating to foodstuff' *aromas* was as follows:

> Annex II of Directive 88/388/EEC on flavourings set the following maximum levels for *hypericin* in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 0.1 mg/kg in foodstuffs and beverages with the exception of 10 mg/kg in alcoholic beverages and 1 mg/kg in confectionery. *Hypericin* may not be added as such to foodstuffs (EEC, 1988).(SCF 2002)

Adoption of the directive 1334/2008/EC changed this legislation so that as of now it is not allowed to make any use of *hypericin* as a flavour whatsoever (also when *hypericin* forms part of the extract or the oil). Presumably, this enhanced strictness came about because of the risk for health damage that can occur due to drug interactions (*inter alia*).

Dried leaves of *Hypericum perforatum* continues to be used in herbal

	teas in some countries we assume (Council of Europe, 2006).
Origin Natural (exo /endo) Synthetic	Natural, plant-derived.

3. Regulation

Norway	No regulation.
EU	No regulation.
Rest of the world	No regulation.

4. Relevant toxicity studies

Absorption	
Skin	Skin penetration: no available data for neither of the individual constituents *Hypericin, hyperforin, rutin* – and also other individual constituents possessing an inherent toxicity potential - are comparatively big molecules and so are expected to cross over the *stratum corneum* rather sluggishly when in water. However, a mixture of *HPE* (10-25 %) and *propylene glycol* in abundance (>75 %) is used in some ready to use cosmetic products. Because of the high content of the well known and much used vehicle in borderline products, the *propylene glycol* molecule, we think it possible that these substances are taken up in the body in significant amounts never the less. The demonstration of a phototoxic effect *hypericin* being applied to the skin (*inter alia*) shows that this molecule penetrate at least into epidermis to some extent. Further, determination of a skin LD_{50} value show that that also *hyperforin* penetrate skin to a significant extent. Due to lack of data on the skin penetration rate we apply the SCCS default value of 100 % for the individual constituents. Most probably, the real rate of penetration is fainter than the rate by which they are absorbed into the body over the epithelia of the gastric tract – i.e. it is somewhat smaller than 15 % which is the average bioavailability of *hypericin* in humans – see below.
GI tractus	In humans a systemic bioavailability of 10 % to 19% has been established after oral intake of *hypericin*, depending on the amount of extract ingested (SCF 2002). An average of 14 % for the same study is mentioned in an *HPE* assessment performed 2005 by the Council of Europe Committee of Experts on flavoring Substances – see Annex 2. The authors Greeson JM *et al* (2001) inform about an oral bioavailability of 15-20 % this pertaining to *HPE*. The bio-availability in mice is much higher than in humans (80 % for *hypericin* – see Stock S *et al* 1991).
Distribution, metabolism and excretion	For information of pharmacokinetic parameters of *hypericin* and *pseudohypericin*, it is referred to, for example, the safety assessment performed by the Cosmetic Ingredient Review in 2001 (CIR, 2001). Confer also the Council of Europe safety assessment as shown in Annex 2. Further, both a WHO (2004) and an EMA (2009) safety assessment provide pharmacokinetic data as concerns *hyperforin* as well. Investigations undertaken by Juergenliemk *et al.* (2003) indicate the ability of one of the flavonoids (miquelianin) to cross membrane barriers to finally reach the CNS. EMA (2009) referring to the studies of Wurglics *et al.* (2006) informs that *hyperforin* is the only ingredient of *HPE* that so far has be determined in the brain of rodents after oral administration of alcoholic extracts. The plasma concentrations of the *hypericins* were only one-tenth compared with *hyperforin* and until now the *hypericins* could not be found in the brain after oral

	administration of alcoholic *HPE* extracts or pure *hypericin*.
Local toxic effects Irritation Sensitivity	Skin irritation/sensitivity *Mucous membranes irritation* A 10 % mixture of *HPE* (1-5%), olive oil (<50%) with paraffin did only produce a reaction of the conjunctivae in one of six rabbits (Council of Europe, 2006). *Skin irritation* Below a concentration of 10 % *HPE* (dry extract, 0.3 % *hypericin*) in the probe applied topically in guinea pigs (sensitisation test) no irritation occurred. At 10 % desquamation was observed. Up till 3 % *HPE* (same constitution, same auxiliary ingredients) was tolerated after inter-dermal injection (Council of Europe 2006). A mixture of HPE (1- 5 %), olive oil (> 50 %) and tocopherol (< 0.1 %), tested at 10% in liquid paraffin was non-irritating to rabbits in a patch test (CIR 2001) *Sensitization (skin)* Subjection of the probe 10 % strong in *HPE* (dry extract, 0.3 % *hypericin*) to Magnusson & Kligman sensitisation testing (guinea pigs) did not produce a positive reaction. However, another study, performed with the same probe according to a photosensitisation protocol, gave some positive results upon **UVA** irradiation; 4 animals out of 20 tested positive. The probe was placed on intact skin in the guinea pigs being used. Being placed on stripped skin – e.g. on skin that is no longer fully intact - more positive results were obtained; 7 out of 20 animals reacted (Council of Europe, 2006). The phototoxicity potential of a mixture containing HPO, butylene glycol and water (percentages not specified) was determined using 6 guinea pigs. This probe was applied on the skin of the animals in a thickness of 0.1 mm and so exposed for a minimum erythema dose (MED) for 15 minutes. This test did not produce positive results (CIR 2001). These studies show that *HPE* (0.3 % *hypericin*) is not a sensitizing substance in the dark. Administrated orally it shows phototoxic effects skin being exposed to sunlight. And likewise, apparently depending upon the quality of the extract used, it shows up as a photo-sensitizing substance even upon topical administration. The test referred to by CIR has flaws: much too few animals, missing information about the content of the photodynamic compound the *hypericin* - and also missing information as to which type of irradiation was used (UVB or UVA). EMA (2009) refer to Schempp *et al.* (2000) who investigated the effects of *HPO* (*hypericin* 110 microgram/ml – i.e. ca. 0.1 %) and a *Hypericum* ointment (*hypericin* 30 microgram/ml) on skin sensitivity to solar simulated radiation. Sixteen volunteers of the skin types II and III were tested on their volar forearms with solar simulated radiation for photosensitizing effects of *HPO* (n=8) and *Hypericum* ointment (n=8). The minimal erythema dose (MED) was determined by visual assessment, and skin erythema was evaluated photometrically. With the visual erythema score, no change of the MED could be detected after application of either *HPO* or *Hypericum* ointment (P>0.05). With the more sensitive photometric measurement, however, an increase of the erythema-index after treatment with the *HPO* could be detected (P< or =0.01). The results do provide evidence for a phototoxic potential of *HPO* and *Hypericum* ointment, detectable by the clinically relevant visual erythema score. The authors thought that the detected trend towards increased photosensitivity (detected with the more sensitive photometric measurement) could become relevant in fair-skinned individuals, in diseased skin or after extended solar irradiation. EMA comments on this study pointing out that from traditional use of *HPO* it is

	known that the exposure to sunlight of treated parts of the skin would lead to skin irritations. In traditional medicine it is recommended to protect treated skin from sunlight. It is noted that the *hypericin* concentration of the *HPO* and the ointment being used in the study of Schempp *et al* was lower than the standardized concentration of 0.3 % *hypericin* within *HPE* and *HPO* ingredients going into commercial products. The Council of Europe (2006) commenting on the study of Schempp *et al* makes aware that the irradiation doses chosen for the study can test only the photosensitising potential of a molecule which is absorbing in the UVB part of the electromagnetic spectrum and not a molecule mainly absorbing in the UVA part. The *hypericin* molecule has one absorption peak at 330 nm and two others at 550 – 580 nm. The UVA area stretches from 290 to 400 nm. So *hypeicin* absorbs in the UVA and visible part of the electromagnetic spectrum – and only little in the lower-lying UVB part. Hence, the finds of Schempp *et al* must be treated with great caution. Also Schempp *et al* points out that they are aware of this weakness of their study – and that it cannot offer any assurance in case of *intense exposure to sunlight*. In another study Schempp *et al* (1999) showed that *HPE* administered by intracutaneous injection was photosensitising and as the authors points out, in the event of a skin wound transcutaneous penetration of the extract could be much greater, and hence result in concentrations in the tissue that would suffice to trigger photosensitisation, since the phototoxic effect depends on both the dose of the drug and the dose of the light received. The minimum level at which *hypericin* shows phototoxic effect is somewhere in the range 100 – 1000 ng/ ml in the skin blister fluids (EMA referring to the works of Schempp *et al* 1999, 2003). Probably, this is the threshold level also as concerns the epidermis wherein the phototoxic reaction takes place. For illustrative purposes we roughly calculated what would be the level within the epidermis upon use of a face cream the composition of which closely resembles commercial *HPE* containing face creams. We used the premises of a 0.1 mm thick epidermis skin layer, a concentration of 1 % *HPE* standardized to 0.3 % *hypericin*, a skin penetration rate of 2 % and also the SCCS default value for the area of the face. Estimated level: ca. 200 ng/ml. In harmony with the outcome of the studies of Schempp *at al* (2000) we assume this hypothetical product to be on the wedge of causing a phototoxic reaction. SCF (2002) also concluded: Exposure to *hypericin* or *Hypericum perforatum* may lead to an increased sensitivity of the skin to subsequent exposure to light.
Systemic toxic effects Acute	**Acute** In a study by Vandenbogaerde and co-workers (2000), male rats (8 to 12 per group) were dosed with dry *HPE* containing 0.11 % *hypericin* or with >98% *pure hypericin* by gavage. Administered doses were 0, 926, 1852 or 2778 mg *HPE*/kg bw (0, 1, 2 or 3 mg *hypericin*/kg bw) or 3 mg *pure hypericin*/kg bw. The rats were tested one hour after administration of the *HPE* for locomotor behavior and anxiolytic effects. The *HPE* increased the locomotor activity in the open field and showed anxiolytic activity in the light-dark test, whereas *pure hypericin* did not show any effect (Vandenbogaerde *et al*., 2000). Oral administration in rats of a dose of 5 g/kg of a 0.3 % ethanolic *hypericin* extract showed no toxic effect (Council of Europe, 2006). The oral LD_{50} for rats of *HPE* 1-5 %, olive oil >50 % and tocopherol <0.1 %) was >20 ml/kg (CIR, 2001). The subcutaneous toxic dose of *HPE* that kill a 250 g guinea pig within 24 hours was 0.1 ml (CIR, 2001). The intraperitoneal LD_{50} values of the

	polyphenol, lipophilic, and water soluble fractions of *Hypericum perforatum* in mice were 780, 4300 and 2800 mg/kg, respectively (CIR, 2001). Intravenous application of *hypericin* was well tolerated by rhesus monkeys at a dose of 2 mg/kg bw, at 5 mg/kg bw transient severe photosensitivity rash occurred. The amount of *hypericin* administered daily in usual therapeutic dosages is not more than 3 mg for adults (= 0.04 mg/kg bw) (EMA 2009). The Council of Europe safety assessment as shown in Annex 2 provides supplementary data.
Repeated dose	**Repeated dose** Groups of three adult Awasi sheep were fed *Hypericum perforatum flowers* at doses of 4, 8, 12 or 16 g/kg for 14 days. Blood samples were taken on days 0, 7 and 14. Toxicity was seen for all doses, such as decreased hemoglobin, red blood cell count, packed cell volumes, total protein, glucose, cholesterol, triglycerides, and serum alkaline phosphotase activities. Blood urea nitrogen, sodium, potassium, bilirubin (total and direct), and the activities of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and gamma glutamyltransferase increased (Kako *et al*., 1993). Oral administration of *HPE* (0.3 % *hypericin* content) to rats for 30 days, gave a NOAEL of 1090 mg *HPE*/kg/day (Council of Europe, 2006). In a 178 day study, rats were fed food containing an alcoholic *HPE*. The extract was added at a concentration of 10 % until day 12, when it was reduced to 5 % because of lack of palatability. Average daily weight gain was statistically significantly decreased for test animals as compared to controls (Council of Europe, 2006; CIR, 2001 both citing Garrett BJ *et al* 1982).
Mutagenicity /genotoxicity	**Mutagenicity /genotoxicity** According to EMA referring to studies by Leuscner 1996 and Greeson *et al* 2001 26 weeks *HPE* (80 % methanol) feeding treatment in dogs some weight loss and certain reversible pathological changes in liver and kidney occurred . The latter would be changes that EMA consider only minor ones. The *HPE* daily doses applied were either 900 mg/kg or 2700 mg/kg. Possibly, 900 mg /Kg bw may be considered a LOAEL in the dog for these effects. There are some positive findings reported for the genotoxicity of *HPE in vitro*. However, the majority of the *in vitro* assays and all *in vivo* tests show negative results for the genotoxicity of *HPE*. For detailed description of the different studies, confer the safety assessment of *HPE* and *HPO* performed by the Cosmetic Ingredient Review and the report from Council of Europe (CIR, 2001; Council of Europe, 2006, EMA 2009, Council of Europe 2008 (Annex 2).
Carcinogenicity	**Carcinogenicity** No studies found.
Reproductive toxicity / Teratogenicity	**Reproductive toxicity /Teratogenicity** Uterotonic action has been reported in animals after consumption of *HPE* (Council of Europe, 2006). In rats and dogs doses of 900 and 2700 mg/kg bw of *HPE* did not have any effect on reproduction. In a small study conducted with pregnant mice, consumption of 136 mg/kg/day of dried aerial parts of *Hypericum perforatum* led to a decrease in litter size and birth weight (Council of Europe, 2006). Another study showed that the *HPE* (*hypericin other constituents?)* is

	transferred both through breast milk and placenta. Administration of 100 or 1000 mg *HPE* /kg bw (which is comparable to the dose administered to humans) to pregnant female Wistar rats from 2 weeks before mating to 21 days after delivery, caused severe kidney and liver damage in the offspring (Gregoretti et al., 2004). So these studies in rats suggest the preparation may have *teratogenic* and toxic effects (Gregoretti B *et al* 2004, Lee A *et al* 2003) Chan LY *et al* (2001) studied the influence of *hypericin* on rat embryos. Embryos from Sprague-Dawley rats were explanted at gestational day 9.5 and were cultured in vitro for 48 hours. To the medium 0 – 142 ng/ml *hypericin* was added. At gestational day 11.5 the embryos were examined. High concentrations of *hypericin* (71 and 142 ng/ml) exhibited significant morphological changes in the embryos. The authors compared this *hypericin* concentration to that arising in the blood after ingestion of 1800 mg *HPE* (which is not unusually high intakes in some consumers that enjoy *HPE* supplements); a mean peak plasma *hypericin* concentration of 29.5 ng/ml with a range of 0-77.9 ng/ml (Schempp H *et al* 1999). Therefore, the concentrations used in the study are clinically achievable in human subjects. Hence, also the authors Chen *et al* became to think that *hypericin* is potentially teratogenic in rats in concentrations which are achievable during clinical use. EMA (2009) points out, though, that in the setting of the study *hypericin* came into direct contact with the embryos, while *in situ* embryos are protected by the placental barrier. Antenatal placebo-controlled behavioral experiments using a mouse model that received a therapeutic dosage for humans (180 mg *HPE*/kg/day) of standardized *HPE (0.3 hypericin)* didn't show any major impact on certain cognitive tasks in mice offspring. Neither were any effect on long term growth and physical maturation of exposed mouse offspring detectable (Rayburn WF et al 2000, 2001, 2001a) EMA (2009) considering the above mentioned references concluded that the data for *HPE* on reproductive toxicity are contradictory. Tests on reproductive toxicity demonstrated no differences *HPE* (108 mg/kg) and placebo in mice. However, isolated *hypericin* seems to have teratogenic properties. EMA advice that for safety reasons the oral use of *Hypericum* during pregnancy and lactation should not be recommended. Possibly, these animal experiments indicate a NOAEL of around 0.5 mg *hypericin* /kg bw as concerns this toxicity end point. The possible teratogenic effect seems, however, not to be *the* critical effect of *hypericin* since SCF in 2002 set at NOAEL of low 0,031 mg/Kg bw for the enhanced photosensitivity effect of *hypericin* (oral administration in humans).
Other effects	**Phototoxicity (oral administration)** The phototoxicity is the effect that over the years has attracted the interests of toxicologists the most. Therefore, comprehensive explanations is to be found in all the main existing safety assessments pertaining to the different use of *HPE*; CIR (2001), SCF (2002), Council of Europe (2006 and 2008), WHO (2004), EMA (2009). We, therefor, in the present assessment restrict ourselves to the following brief explanation. The plant *Hypericum perforatum* is a primary photosensitizer in animals mainly due to *hypericin*, which caused photoactivated damage by absorbing visible light (550-610 nm, maximum at 585 nm). *HPE* has demonstrated cytotoxicity and photocytotoxicity in a dose and UVA-dose dependent manner. *Hypericin* may evoke severe phototoxic effects. The molecule 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 non-pigmented skin. Dark cytotoxicity is absent, even at high *hypericin* concentration (Jensen *et al.*, 1995, EMA 2009).

Also *rutin* has demonstrated a certain phototoxic potential (EMA 2009).

Neurotoxicity / psychotropic effects (alteration of perception, mood, consciousness and behavior)

It is well established that *HPE* has psychotropic effects and in particular that it may have mild or moderate anti-depression activity (WHO 2004, EMA 2009, Linde K 2009)[11]. Seemingly, it is the constituent *hyperforin* that causes this effect (*inter alia*). A range of different adverse side effects goes with the therapeutic usage of standard anti-depressives. It is the same with the *HPE* remedies - and it even goes about the same kind of adversities. Aside from side effects affecting the digestive organs (diarrhea, for example) – that has to do with the route of administration - *nervous system disorders* of different kinds also occur. A more detailed explanation is given under the heading of "Adverse side effects from uses other than cosmetics (*therapeutic usage*)".

Drug interactions

According to Linde K (2009) drug interactions are the clearly most relevant safety issue with *HPE.* This because *HPE* is a potent activator (inducer) of the enzyme cytochrome P450 3A4 (CYP3A4). The enzyme catabolizes a large number of important medications. Induction has as a consequence more rapid breakdown of these medications so that their effectiveness is reduced to the extent that the health of patients/consumers is endangered.

In one *in vivo* experiment *HPE* induced the enzyme twofold in healthy adults who received 900 mg *HPE* per day for 16 days (references in Council of Europe monograph shown in Annex II).

Furthermore, *HPE* also increase the activity of the P-glycoprotein, an ATP-dependent drug transporter which is responsible for an increase in excretion of drugs from the organism [References No 16, 79, 80 in Linde K 2009].

In the years 2000 – 2006 medicinal products agencies all over Europe came out with warnings to the general public not to consume *HPE* supplements when on different medications. And this concerned a series of medications. Like most other agencies the Norwegian agency mentioned the following ones:

- Immune suppressive drugs (ciclosporin, tacrolimus)
- Anticoagulants (warfarin)
- HIV-drugs (saquinavir, nevirapine)
- Digoxin
- Theophylline
- Latium
- Epilepsy drugs
- Contraceptives
- Concomitant use of *HPE* and p-pills can cause breakthrough bleeding and unintended pregnancy
- Effect of SSRIs (antidepressives) and migraine drugs (triptanes) are enhanced and can lead to serious side effects

[11] Depression can be attributed to lower than normal synaptic concentrations of 5-HT (serotonin) in the brain. This concentration can be increased by the administration of a selective serotonin reuptake inhibitor. While there are a number of SSRIs available, each has a lag time of 2-6 weeks before clinical efficacy is expressed. This is the result of a feedback mechanism involving activation of the 5-HT1A somatodendritic autoreceptor by the SSRI

The British Medicines Control Agency (MCA) in response to a request from Members of Parliament additionally informed that reduced efficiency because of concomitant usage had resulted in cases of patients rejecting newly transplanted organs (heart and kidney transplants), in pregnancies in woman on contraceptives and also in other serious incidents:

Reports of suspected interactions between St John's Wort and conventional medicines received by the UK Committee on Safety Medicines for the period October 1996 to June 2002

Compound or medicine	Reports	Comment
Warfarin	4	Increased INR (2 reports); decreased INR (2 reports)
SSRIs	4	Paroxetine (3 reports); Sertraline (1 report)
Theophylline	1	Reduced serum theophylline concentration
Indinavir, lamivudine, stavudine	1	HIV viral load increased
Tacrolimus	1	Medicine ineffective
Oral contraceptives	14	Inter-menstrual bleeding (6 reports); unintended pregnancy (8 reports)
Others	15	Including: HRT (2 reports), atorvastatin (1 report), moclobemide (1 report), verapamil (1 report), enalapril (1 report), lithium (1 report), thyroxine (1 report)

† Source: Medicines Control Agency Adverse Drug Reactions Online Information Tracking (ADROIT). INR = international normalized ratio; SSRIs = selective serotonin reuptake inhibitors

More examples are to be found in a comprehensive chapter on this issue in the EMA paper as of 2009. EMA mention, for example, that Hall *et al.* (2003) studied the interactions between an oral contraceptive and *HPE* food supplements involving an intake of 900 mg *HPE* per day. This concomitant use resulted in a halving of the half-life of ethinylestradiol (23.4 ± 19.5 hours to 12.2 ± 7.1). Breakthrough bleeding occurred in 2 of 12 women in the control phase compared to 7 of 12 women in the *HPE* phase. EMA concluded that women taking oral contraceptives should be cautioned that the use of *Hypericum* might reduce the effectiveness of their birth control method.

Hyperforin seems to be mainly responsible for the interactions with other drugs. Products that do not contain substantial amounts of *hyperforin* (<1%) have not been shown to produce clinically relevant enzyme induction (Madabushi R *et al.* 2006). *Hyperforin* was found to activate a particular receptor in the liver.

Daily Intake of 900 mg HPE product containing 1 % hyperforin involves a dose of 150 microgram /kg bw. The LOAEL for the (oral) phototoxic effect is 31 – 36 microgram / kg bw.

On the other hand it seems as if *hypericin* is the P-glycoprotein inducing compound (Mannel M 2004).

Other effects

Hypericin Receptor tyrosine kinase activity of epidermal growth factor is also inhibited by *hypericin* and may be linked to the antiviral and antineoplastic effects of *HPE* (De Witte PA *et al* 1993). *Hypericin* produces a potent and irreversible inhibition of the epidermal growth factor receptor tyrosine kinase activity. The inhibition is irreversible, strictly dependent upon irradiation of the enzyme-inhibitor complex with fluorescent light and likely mediated by the formation of radical intermediates (Agostinis P *et al* 1995).

A polyphenol fraction of the plant had immune stimulating activity on mononuclear phagocyte systems and cellular and humoral immunity, and a lipophilic portion had immunosuppressive activity on cellular and humoral immune responses (CIR 2001, EMA 2009).

5. Exposure estimate and critical NOAEL / NOEL

NOAEL/NOEL critical	It is not possible to calculate a representative NOAEL/NOEL value based on the existing data on the herb *Hypericum perforatum* itself However, a LOAEL value has been set for *hypericin*; and for the enhanced photosensitivity effect of *hypericin* a LOAEL has been set to 36 µg/kg bw/day in human volunteers (Brockmöller J *et al* 1997). The Council of Europe committee of experts on Flavoring Substances in the monograph as from 2006 - as shown in Annex II - made use of this LOAEL establishing a tolerable daily intake of *hypericin* estimating a tolerable daily intake of the compound. The SCF in its safety evaluation on *HPE* as of 2002 states that in humans a LOAEL for induction of enhanced photosensitivity was observed after 15 daily doses of 2.2 mg hypericin/day, equivalent to 31 microgram /kg bw/day, indicating that prolonged exposure to *hypericin* or *HPE* may well induce enhancement of photosensitivity. We are not aware of more recent estimates as to this critical effect and chose to lay it to ground for margin of safety calculation. We make use of the conventional default value of 1/3 for the NOAEL/LOAEL ratio. Hence, the NOAEL value based on oral data in humans is set to 10 microgram *hypericin*/kg bw/day. It is the amounts of *hypericin* **ingested** that have been registered. It is, however, solely the amount taken up in the body over the epithelia of the gastric tract that causes the photosensitivity effect. The amount that only passes through the digestive tract being excretes in the feces do not contribute to the effect. In calculations of the margin of safety use is made of the estimated *systemic* exposure for *hypericin* because of occurrence in cosmetic products. It would therefore be more correct to compare this estimated systemic exposure to an "*internal NOAEL"* obtained by correcting the conventional NOAEL for the bioavailability of the *hypericin*. The medium bioavailability in humans as determined by the Council of Europe Committee of experts on Flavoring Substances is 14 %. Hence we calculate an "internal NOAEL" of (10 x 0.14 =) 1.4 microgram /kg bw /day.
Exposure cosmetic products	By making use of the above mentioned concentrations and SCCS guideline default values the systemic exposure (SED) for *hypericin* is calculated as concerns the following types of cosmetics products wherein *HPE* and *HPO* is known to be used as ingredients (CIR) and information also is available as to the in-use concentrations. This concerns the following 2 "leave-on" products • Body lotion • Face cream And the following 6 different "rinse-off" products • Face cleansing • Facial mask • Shaving cream • Shampoo • Bubble bath • Bath oil/tablet/salt

Other premises used calculating the SED

- Concentration of extract /oil in ready to use products: see data mentioned in the above (data collected from the CIR safety assessment). Instead of 5 % we use 3 % because this seems to be a recommended concentration.
- The *hypericin* content in *HPE* and *HPO* is set to 0.3 % in compliance with the apparent standard pertaining to these ingredients in the marketplace (*inter alia*).
- As concerns the skin penetration rate there are no published data as far as we can see. Therefore, in compliance with the SCCS guideline we use a rate of 100 %.

  Generally, a substance is taken up in the body more easily *via* the digestive tract than over intact skin. Reflecting this is the observation that without exceptions known skin penetration rates in humans (or a relevant animal model) are smaller than the bio-availability in humans (animal). In the present case the human bio-availability has been found to be in the range 10 – 19 % with an average of 14 % (*inter alia*). Obviously, therefore, the real skin penetration rate is much lower than 100 % - and certainly also smaller than 19 %. As an alternative we, in light of this, also calculate SED' using an illustrative skin penetration rate of 2 %.

The concrete calculations are shown in Annex I. The following SED values are arrived at – being expressed as microgram (µg) *hypericin* per Kg body weight per day:

Products	Skin penetration rate (%)			
	100		*2*	
	HPE	*HPO*	***HPE***	*HPO*
All the 8 products taken together	4.8	14	0.10	0.28
Face cream (leave-on)	0.7	2	0.01	0,04
Body lotion (leave-on)	3.6	11	0.07	0.22
All rinse-off products	0.57	0.33	0.011	0.007

The concentration premises are

Products	Concentrations (%)			
	HPE		*HPO*	
	used	*CIR*	*used*	*CIR*
Face cream (leave-on)	1	0 -1	3	0.1 - 5
Body lotion (leave-on)	1	0 -1	3	0.1 -5
Face cleansing product	1	0 -1		
Facial mask	3		3	1 - 5
Shaving cream	1		1	0.1 -1
Shampoo	1			
Bubble bath	3		3	1 - 5

Margin of Safety (MoS)

We calculate MoS values by dividing the NOAEL (10 µg *hypericin* /kg bw day) by the SED based on a skin penetration rate of 100 % (normal SCCS procedure). For the sake completion we also calculate MoS values by dividing the "internal NOAEL" (1.4 µg *hypericin* /kg bw day) by a SED obtained by use of an illustrative skin penetration rate of 2 %

Normal SCCS procedure MoS values

Products	100 % Skin penetration rate	
	HPE	*HPO*
All the 8 products taken together	**2.1**	0.7
Face cream (leave-on)	**14**	2
Body lotion (leave-on)	**2.8**	0.9
All rinse-off products	**18**	30

Alternative procedure MoS values

Products	2 % Skin penetration rate	
	HPE	*HPO*
All the 8 products taken together	**14**	5
Face cream (leave-on)	**140**	35
Body lotion (leave-on)	**20**	6
All rinse-off products	**127**	200

These MoS values should be compared to a minimum margin of safety of 10 since they are based human data.

6. Other sources of exposure than cosmetic products

Food stuffs	Directive 1334/2008/EC changed the foodstuffs legislation so that as of today all use of *HPE* and *hypericin* as flavor is prohibited. It continues to be used abundantly in food supplements, though (*inter alia*). If *hypericum* is used as herbal tea, a daily intake of 25 µg/kg bw *hypericin* has been estimated in the Netherlands (SCF 2002).
Pharmaceuticals	Antidepressant usage: The common dose is 300 mg of the standardized *HPE* (0.3% *hypericin, 2.8 % hyperforin*) taken three times daily or 200 to 1000 µg/day of *hypericin* – or 8400 to 25 200 µg/day of *hyperforin* (SCF 2002). Typically, a 4–6 week long treatment period is required to achieve a therapeutic benefit in patients (Bennett *et al* 1998). This comparatively long treatment period correlates with the low bioavailability (15 – 20 %) for relevant *HPE* constituents, a poor blood-brain barrier penetration and a slow elimination time (Bennett DA *et al.* 1998). It was once thought that the anti-depression activity could be related to the content of *hypericin* it inhibiting MAO. More recent research has shown that it at most plays “second violin” in this respect. Herbalists – and also the herbal expert committee of EMA - advocate the view that a whole range of plant constituents are involved the herb causing beneficiary CNS effects including the anti-depression one. EMA (2009) expresses it as follows: *The mechanisms of action as well as the responsible compounds of Hypericum extracts are still under discussion. Several actions contributing to clinical efficacy are reported:*

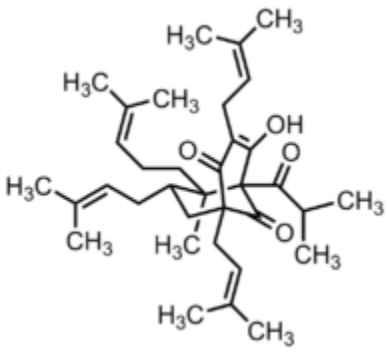
Blockade of the reuptake of serotonin (5-HT), noradrenalin and dopamine; upregulation of postsynaptic 5-HT$_1$ and 5-HT$_2$ receptors and of dopaminergic receptors; increased affinity for GABAergic receptors. Constituents which contribute to the activity are hypericin, pseudohypericin, flavonoids, and oligomeric procyanidins. The relevance of hyperforin is discussed controversially. As a consequence the entire extract has to be considered as the active substance.

Other expertise thinks otherwise. Among these are the authors Filandrinos D, Yentsch TH and Meyers KL that recently (2007) subjected all the existing different observations to a thorough analysis with the aim find out about the potency of the different constituents in relation to the CNS effects. They came out with the view that:

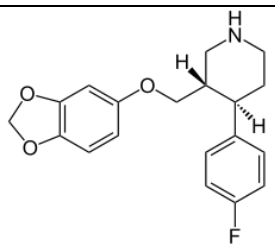
"Most evidence now implicate hyperforin as the main component responsible for the neurological activity of St John's wort."

It appears from the mentioned analytical work that *hyperforin* is the only constituent that could be determined in the brain of rodents after oral administration of alcoholic extracts. Further, it appears that the data on the relevant bio-activities pertaining to *the other* constituents in question have largely been obtained by use of *in vitro* – and not *in vivo* – studies. Especially as concerns CNS effects *in vitro* test ought to be supplemented with *in vivo* tests before a robust conclusion can be drawn. *Hyperforin* is a MAO reuptake inhibitor (WHO 2004, Chatterjee SS *et al* 1998). The effect also is dose dependent. A clinical study showed, namely, that *HPE* being 5 % strong in *hyperforin* effectively relieve depression, whereas the 0.5 % strong ones were ineffective and performed no better than placebo (Laakmann G *et al* 1998).

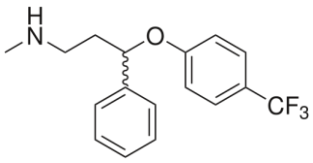
In contrast to all other antidepressants finding some therapeutic usage, the *hyperforin* molecule do not contain a nitrogen atom:



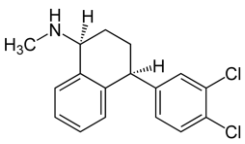
All the Selective Serotonin Reuptake Inhibitors (SSRI's) are, for example, amines. On that see below figure that display the molecular structure of the 5 SSRI's being used the most. Also contrasting *hyperforin* is the feature that their molecular structure incorporate one or two aromatic rings - and terminal halogen atoms (Evrard DA)



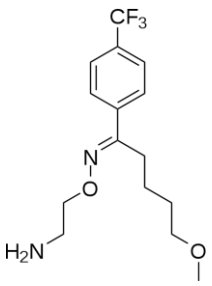
Paroxetine



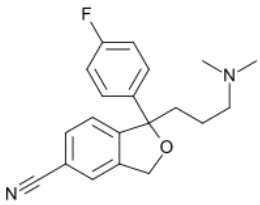
Fluoxetine



Sertaline



Fluvooxamine



Citralopram

Because of these fundamental structural differences the mechanism behind the anti-depressive effect of *hyperforin* is radically different from that of the standard antidepressants. The latter ones directly block neuronal amine uptake whereas *hyperforin* increases synaptic serotonin and norepinephrine concentrations indirectly. The indirect mechanism is not yet fully understood (Leuner K *et al* 2007)[12].

In standardized *HPE* remedies *hyperforin* is present at 2.8 % (American Botanical Council 2002). EMA (2009) mention that the content *of hyperforin* is maximum 6 %. Back in May 2000 actual measurement of the content within 8 products sold on the US market showed, however, that apart from one exceptional product, the content was very much lower (Gerlie C *et al* 2002):

Product	*Hypericin* content (%)	*Hyperforin* content (%)
A	0.29	1.89
B	0.12	0.20
C	0.22	1.16
D	0.26	0.05
E	0.17	0.29
F	0.28	0.19
G	0.03	0.01
H	0.25	0.48

Probably, the many depressed concentrations measured at the time reflect that in the past it was technically difficult to preserve a high content because of molecule' instability. The author Butterweck V (2009) informs that:

> "..major change was made since 1998, when the quite unstable component of SJW, *hyperforin*, became stabilized in many products, leading to a 10- to 20-fold amount of hyperforin in the product ."

[12] Drug bank*: "It appears to exert these effects by activating the transient receptor potential ion channel TRPC6. Activation of TRPC6 induces the entry of sodium and calcium into the cell which causes inhibition of monoamine reuptake"*.

Hence, at least as concerns the *HPE*-drugs of the category *'well-established use'* (that are approved by medicinal products agencies), the expectations are that concentration in the products offered for sale comply with the standardized level of 2.8%.

Apparently, very little is known about the toxicity of *hyperforin*. Solely, the following few data on acute toxicity are filed with the Drug bank:

Oral LD_{50} (rat):5628 mg/kg; Skin LD_{50} (rabbit): 15800 mg/kg; Subcutaneous LD_{50} (mouse):9800 mg/kg; Intraperitoneal LD_{50} (rabbit):1826 mg/kg

Other sources

The adverse side effects going with *therapeutic usage*

Oral administration

Clinical trials with dosing of 300 mg/day *HPE* have been performed (15 µg *hypericin* /Kg bw/day). A clinical trial where 3250 patients received treatment with *HPE* for 4 weeks, 79 patients (2.4 %) reported side effects such as gastrointestinal irritations (0.6%), allergic reactions (0.5%), fatigue (0.4%), restlessness (0.3 %), anxiety and dizziness (Woelk *et al*., 1994). In another clinical trial, 67 patients were treated for 6 weeks and side effects such as dry mouth, dizziness and constipation occurred in 8 patients (Vorbach *et al*., 1994). Several other clinical trials have reported unwanted side effects after treatment with *HPE* (Sommer *et al*., 1994; Vorbach *et al*., 1997; Wheatley *et al*., 1997). Two trials did not report any side effects after treatment with HPE (Hübner *et al*., 1994; Martinez *et al*., 1994).

A more recent clinical study involved dosages of 900 mg á day and made use of a *HPE* standardized to 3-6% *hyperforin* and 0.12-0.28% *hypericin*. Hence, the dosage per day was at a level of 30 µg *hypericin* /Kg bw/day – and 675 µg *hyperforin* /Kg bw/day. 251 adult outpatients with acute major depression took part in the study. The study reported as follows as concerns the safety and tolerability of *HPE* treatment for depression (Szegedi A *et al* 2005):

> "During the acute treatment phase 69/125 patients randomized to *hypericum* (55%) reported 172 adverse events. The highest incidence was found for gastrointestinal disorders (59 events in 42 patients), followed by *nervous system disorders* (35 events in 29 patients and 61 events in 43 patients, respectively). The below table shows adverse events that occurred in at least 10 patients in one group."

Side effect	HPE (n = 125)	Paroxetine (n = 126)
Upper abdominal pain	12	9
Diarrhea	12	23
Dry mouth	16	35
Nausea	9	21
Fatigue	14	16
Dizziness	9	24
Headache	13	14
Sleep disorder	5	10
Increased sweating	9	13

	Another recent investigation consisted of an open multicenter safety study with 440 out-patients suffering from mild to moderate depression. Patients were treated for up to 1 year with 500 mg HPE (0.2 % *hypericin*) per day. Evaluation criteria were safety (adverse event frequency) and influence on depression. 271 (49%) patients reported 504 adverse events, 30(6.8 %) of which were possibly or probably related to the treatment. Gastrointestinal and skin complaints were the most common events associated with treatment (Brattström A 2009). Out of the 30 cases judged to be treatment related the following ones were the most frequent: • Skin rash 4 cases • Abdominal pain 4 cases • Urticaria 3 cases • Insomnia 3 cases In addition to the 30 cases there where 25 adverse events that led to withdrawal from the study. One of these extra cases consisted of an urticarial incident that was considered serious.[13] The Norwegian medicinal products agency (MPA) informs (20 February) that there are some reports in the literature about mania relating to use of *HPE* anti-depressives. One case report in the literature is a bout a woman (76) who developed delirium and became psychotic 3 weeks after having started taking 75 mg /day. She also suffered from Alzheimer's (Laird RD *et al* 2001). Over the period 2002 – 2011 the Norwegian pharmacovigilance system received solely 5 reports about side effects judged to have been caused by use of an *HPE* anti-depressive. 2010: Anxiety reaction, palpations, sleeplessness 2006: Headache, vaginal bleeding 2005: Anxiety, difficulty sleeping 2002: Pruritus, dry skin 2002: Exanthema, pruritus MPA informs that especially as concern the "nature-medicinal" products there are serious underreporting. The low number of reports may possibly also be due to MPA in 2005 warning about using the products when on other medication. Moreover, with one exceptional product the 5 approved products on the market in 2002 were successively withdrawn up till 2010 so that at the end of the period they were no more marketed. As concerns the situation in Germany Linde K (2009) informs about a systematic review summarizing 16 observational studies including a total of 34,804 patients mostly suffering from depression. It appeared from these studies that the proportion of patients terminating treatment due to side effects varied in 14 short-term studies from 0–2.8% and was 3.4 and 5.7% in 2 long-term studies. The proportion of patients reporting side effects ranged between 0 and 5.9%. The most frequently reported side effects or adverse events were gastrointestinal symptoms. *Increased sensitivity to light and skin symptoms in general were the second most often reported side*

[13] Although urticaria – an immune system related pain full skin reaction – is associated with a good prognosis, patients with severe urticarial can suffer significant morbidity with a dramatic decline in their quality of life, productivity at work, and emotional well-being (http://allergy-book.blogspot.com/2008/09/urticaria-or-hives.html). See also the source Adverse Drug Reactions, 2nd edition (ISBN: 0 85369 601 2) © Pharmaceutical Press 2006

	effects. A variety of mental and nervous symptoms were also described in several studies. Serious adverse effects (requiring hospitalization) or interactions with other drugs were not reported in any study. Linde points out that many of these observational studies had low methodological quality and should be interpreted with caution. Linde informs about a low number of *published case reports* on clinically relevant, direct adverse effects. A systematic review published in 2004 identified a total of only 26 cases including well-documented cases reported to drug surveillance agencies. 17 cases were skin or allergic reactions (erythema, dermatitis, urticaria, hyperesthesia, and neuropathy) and 9 were psychiatric reactions (mania, psychotic episodes, or anxiety). EMA (2009) provides an overview as to the side effects referring to 24 separate studies that took place in the years 1997 – 2006. EMA also refer to a systematic review of Stevinson & Ernst (2004) as concerns the clinical evidence associating *HPE* with psychotic events. According to this work there at the time existed 17 case reports that associated the use of *HPE* with psychotic events. In 12 instances, the diagnosis was mania or hypomania. Causality was in most cases possible. These case reports raise the possibility, thinks EMA, that *HPE* may trigger episodes of mania in vulnerable patients. Beckman SE *et al.* (2000) conducted a telephone survey of 43 subjects who had taken *HPE* to assess demographics, psychiatric and medical conditions, dosage, duration of use, reason for use, side effects, concomitant drugs, professional consultation, effectiveness, relapse, and withdrawal effects. Most subjects reported taking *HPE* for depression, and 74% did not seek medical advice. Mean dosage was 475.6+/-360 mg/day (range 300-1200 mg/day) and mean duration of therapy was 7.3+/-10.1 weeks (range 1 day-5 yrs). Among 36 (84%) reporting improvement, 18 (50%) had a psychiatric diagnosis. Twenty (47%) reported side effects, resulting in discontinuation in five (12%) and one emergency room visit. Two consumers experienced symptoms of serotonin syndrome and three reported food-drug interactions. Thirteen consumers experienced withdrawal symptoms and two had a depressive relapse. **Topical administration** Apparently no reports on side effects going with topical medicinal usage seems to exist - apart from a remark by EMA (2009) that from traditional use of *HPO* (treatment of different skin disorders) it is known that the exposure to sunlight of treated parts of the skin would lead to skin irritations. **Usage of cosmetics and CNS side effects** The anti-depressive effect is mediated by a particular molecular constituent of the extract – it seems. This molecule can also be taken up in the body over the skin to some extent (*inter alia*). Hence, it would not be entirely unbelievable that even employment of *HPE* for cosmetic non-medicinal purposes causes CNS effects in the form of slight alteration of the mood of the exposed individual. In event the product in question is actually capable of doing that it could well be questioned whether that product, solely in virtue of its functioning, fall within the scoop of the medicinal product legislation – and, therefore, outside that of the cosmetic products legislation. People using a cosmetic product do expect it neither to change their

	mood nor to cause any *nervous system disorder* like becoming *dizzy* or experiencing *frequent sleep disturbances (insomnia*) . Therefore, even if such effects actually do occur the consumer affected would likely not come to think that the face cream or the body lotion involved has the faintest to do with it. Dizziness and sleep disorders can have many other causes. Further, under the normal use conditions and circumstances, we would believe these effects to be that vague/ diffuse they are hardly recognizable. Still further, the long lag time of 4-6 weeks makes it practically impossible to associate product usage with diffuse CNS disorders occurring one month later. No wonder, therefore, that such possible side effects have ever been mentioned in the literature. Millions of Europeans consume *HPE* supplements (or non – prescription *HPE*-drugs) more or less regularly because of (mild) depression or temporary mood disorders[14]. We hold it probable that thousands of these self medicating individuals also use *HPE*-cosmetics. We would believe that this concomitant usage causes more side effects – or stronger side effects - than would otherwise be the case.

7. Assessment

The Cosmetic Ingredient Review assessed the safety of use of HPE and HPO in cosmetics in 2001, and concluded that the available data are insufficient to support the safety of HPE and HPO for use in cosmetic products (CIR, 2001). As far as we know this is the view of the CIR even today. The types of data still required for included

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.
5. Dermal reproductive/developmental toxicity data.
6. Skin irritation/sensitization data in humans on HPO
7. Ocular irritation data, if available.

The data gap as concerns the toxicological profile of HPE, HPO and its essential bio-active constituents - foremost *hypericin*, *hyperforin*, *pseudohypericin* and *rutin* - is insufficient to the extent that a satisfying usual risk evaluation cannot be made. Essential data is missing 10 years after having been required. The point 3 in the above list of missing data is essential to the question about the magnitude of the NOAEL for the phototoxic critical toxicity effect. Additionally, further data on the probable teratogenic effect for hypericin is necessary so as to establish a NOAEL for this effect. The NOAEL for *hyperforin* inducing cytochrome P450 3A4 (CYP3A4) should be established so as to clear out whether this is the critical toxic effect instead of the phototoxic effect. Data on the skin penetration rate of *hypericin*, *hyperforin* and *rutin* together with the most used vehicles is missing and should be determined. EMA expresses the view that the few data on pharmacokinetics do not allow a final conclusion about absorption, distribution, metabolism and excretion of the constituents of *Hypericum* extracts.

[14] Hopelessness, dejection, loss of self esteem, difficulty in concentrating and sleep disturbance are some of the features associated with depression.

The Hypericum perforatum plant is known to contain the photodynamic molecule *hypericin* in concentrations ranging 0.0095 - 0.466 %. For the most part, due to standardisation, the concentration of *hypericin* is believed to be ca. 0.3 % in HPE and HPO ingredients going into cosmetic products currently marketed. The weigh of evidence is that use of a typical commercial HPE/HPO-containing cosmetic product causes phototoxic reactions.

The Council of Europe assessed (2006) the safety of *hypericin*, in cosmetic products and concluded that due to the potential risk of photosensitisation by cutaneous application, *hypericin* should be banned for use in cosmetic products.

One *in vitro* study on HPE showed a mutagenic potential. However, all other studies, both *in vitro* and *in vivo*, produced negative results. No carcinogenicity data were available. *Hypericin* seems to have teratogenic properties. EMA advice that for safety reasons the oral use of *Hypericum* anti-depressives during pregnancy and lactation should not be recommended.

Because of the *hyperforin* constituent a typical HPE/HPO containing product administered orally induce cytochrome P450 3A4 (CYP3A4) to the extent that concomitant use of such products and a series of important medicines may cause serious health situations. For example, people on the blood thinning warfarin remedy may risk clotting that could have fatal outcome. Inattentive women using a HPE anti-depressive together with p-pills risk unintentional pregnancy. EU banned in 2008 all use of HPE/HPO/*hypericin* for flavoring purpose in foodstuff only because of the P450 3A4 (CYP3A4) enzyme induction of the HPE/HPO.

The weigh of evidence is that the use of HPE/HPO in cosmetic products confers a significant anti-inflammation property to these products. The traditional topical use of HPE/HPO was/is for treatment of different dermatitis ailments in the skin. It's an open question whether the anti-inflammation effect make all topical creams, gels etc. that contain bio-active amounts of HPE/HPO, fall within the scope of the medicinal products legislation.

HPO/HPE may have immune modulating effects.

From the current existing systemic toxicity data, it is not possible to set a NOAEL/NOEL value. However, a LOAEL value has been set for *hypericin*; the LOAEL for the enhanced photosensitivity effect of *hypericin* have been set to 31- 36 µg/kg bw (SCF, 2002; Council of Europe 2008). A corresponding NOAEL of 10 µg/kg bw /day seems plausible. A realistic scenario of a *hypericin* content of 0.3 % of the extract and oil has been used to calculate the Margin of Safety (MoS) according to usual SCCS procedure. The NOAEL for *hypericin* is based on studies in humans (voluntaries), therefore, a MoS of at least 10 is necessary to ensure the safety. As seen above, the overall systemic exposure dose for both *HPE* and *HPO* yields a MoS much too low to ensure the safety for use in cosmetic products; 2.1 and 0.7 compared to 10 respectively.

8. Conclusion

In view of all the mentioned risks for health damage we conclude that all use of HPE and HPO is cosmetic products should be prohibited.

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10. Annex 1

Calculations of SED

HPE:

- ***Body lotion***

Calculated relative daily exposure: 123.2 mg/kg bw/day (SCCS def.)
Dermal absorption, default value, SCCS: 100%
Concentration in product: 1 %

SED: 123.20 mg/kg bw/day x 1 x 0.01 = **1.2 mg/kg bw/day**

- ***Face cream***

Calculated relative daily exposure: 24.14mg/kg bw/day (SCCS def.)
Dermal absorption, default value, SCCS: 100%
Concentration in product: 1 %

SED: 24.14 mg/kg bw/day x 1 x 0.01 = **0.24 mg/kg bw/day**

- ***Face cleansing product***

Amount applied (default): 1 mg/cm^2
Face surface area (default): 565 cm^2
Body weight (default): 60 kg
Retention factor (default): 0.01
Frequency of application: 1/day

Total amount: 1 mg/cm^2 x 565 cm^2 = 565 mg
Daily exposure to the product:
(565 mg/60 kg) x 0.01 x 1= 0.094 mg/kg bw/day

Dermal absorption, default value, SCCS: 100%
Concentration in product: 1 %

SED: 0.094 mg/kg bw/day x 1 x 0.01 = **0.00094 mg/kg bw/day**

- ***Facial mask***

Amount applied (default): 1 mg/cm^2
Surface area (default): 565 cm^2
Body weight (default): 60 kg
Retention factor: 0.1
Frequency of application: 1/day

Total amount: 1 mg/cm^2 x 565 cm^2 = 565 mg
Daily exposure to the product:
(565 mg/60 kg) x 0.1 x 1/day = 0.94 mg/kg bw/day

Dermal absorption, default value, SCCS: 100%
For illustrative purposes; concentration in product: 3 %

SED: 0.94 mg/kg bw/day x 1 x 0.03 = **0.047 mg/kg bw/day**

- ***Shaving cream***

Amount applied (default): 1 mg/cm^2
Surface area (default): 305 cm^2
Body weight (default): 60 kg
Retention factor: 0.01

Total amount: 1 mg/cm^2 x 305 cm^2 = 305 mg
Daily exposure to the product:
(305 mg/60 kg) x 0.01 = 0.051 mg/kg bw/day

Frequency of application: 1/day
Dermal absorption, default value, SCCS: 100%
For illustrative purposes; concentration in product: 1%

SED: 0.051 mg/kg bw/day x 1 x 1 x 0.01 = **0.001 mg/kg bw/day**

- ***Shampoo***

Calculated relative daily exposure: 1.51 mg/kg bw/day
Dermal absorption, default value, SCCS: 100%
For illustrative purposes; concentration in product: 1 %
Retention factor: 0.01

SED: 1.51 mg/kg bw/day x 1 x 0.01 x 0.01 = **0.000151 mg/kg bw/day**

- ***Bubble bath***

Amount applied (default): 1 mg/cm^2
Surface area (default): 16,340 cm^2
Body weight (default): 60 kg
Retention factor: 0.01
Frequency of application: 1/day

Total amount: 1 mg/cm^2 x 16,340 cm^2 = 16,340 mg
Daily exposure to the product:
(16,340 mg/60 kg) x 0.01 x 1 = 2.7 mg/kg bw/day

Dermal absorption, default value, SCCS: 100%
For illustrative purposes; concentration in product: 3 %

SED: 2.7 mg/kg bw/day x 1 x 0.03 = **0.14 mg/kg bw/day**

Overall SED for *HPE*: 1.6 mg /kg bw/day

HPO:

- ***Bath oil/tablet/salt***

Amount applied (default): 1 mg/cm^2
Surface area: 16,340 cm^2
Body weight: 60 kg
Retention factor: 0.01
Frequency of application: 1/day

Total amount: 1 mg/cm^2 x 16,340 cm^2 = 16,340 mg
Daily exposure to the product:
(16,340 mg/60 kg) x 0.01 x 1 = 2.7 mg/kg bw/day

Dermal absorption, default value, SCCS: 100% = 1
Concentration in product: 3 % = 0.03

Calculation of SED:
2.7 mg/kg bw/day x 1 x 0.03 = **0.081 mg/kg bw/day**

- ***Shaving cream***

Amount applied (default): 1 mg/cm^2
Surface area: 305 cm^2
Body weight: 60 kg
Retention factor: 0.01
Frequency of application: 1/day

Total amount: 1 mg/cm^2 x 305 cm^2 = 305 mg
Daily exposure to the product:
(305 mg/60 kg) x 0.01 x 1 = 0.051 mg/kg bw/day

Dermal absorption, default value, SCCS: 100%
Concentration in product: 1%

SED: 0.051 mg/kg bw/day x 1 x 0.01 = **0.0005 mg/kg bw/day**

- ***Face cream***

Calculated relative daily exposure (mg/kg bw/day) : 24.14
Dermal absorption, default value, SCCS: 100%
Concentration in product: 3 %

SED: 24.14 mg/kg bw/day x 1 x 0.03 = **0,72 mg/kg bw/day**

- ***Body lotion***

Calculated relative daily exposure: 123.20 mg/kg bw/day
Dermal absorption, default value, SCCS: 100%
Concentration in product: 3 %

SED: 123.20 mg/kg bw/day x 1 x 0.03 = **3,7 mg/kg bw/day**

- ***Facial mask***

Amount applied (default): 1 mg/cm^2
Surface area: 565 cm^2
Body weight: 60 kg
Retention factor: 0.1
Frequency of application: 1/day

Total amount: 1 mg/cm^2 x 565 cm^2 = 565 mg
Daily exposure to the product:
(565 mg/60 kg) x 0.1 x 1/day = 0.94 mg/kg bw/day

Dermal absorption, default value, SCCS: 100%
Concentration in product: 3 %

SED: 0.94 mg/kg bw/day x 1 x 0.03 = **0.028 mg/kg bw/day**

Overall SED for HPO: 4.6 mg/kg bw/day

Overall SED for rinse-off products that contain HPE or HPO: 0.19 mg/kg bw/day and 0.11 mg /kg bw/day respectively

Calculation of hypericin content
Hypericin content in HPE and HPO: 0.3 % (standardized concentration).

Hypericin content for HPE: 1.6 mg/kg bw/day x 0.003 = 0.0048 mg/kg bw/day – **4.8 µg/kg bw/day**

Hypericin content for HPO: 4.6 mg/kg bw/day x 0.003 = 0.014 mg/kg bw/day – **14 µg/kg bw/day**

Hypericin content in leave-on face cream for HPE: 0.24 mg/kg bw/day x 0.003 = 0,00072 mg /kg bw/day – **0.7 µg/kg bw/day**

Hypericin content in leave-on face cream for HPO: 0.72 mg/kg bw/day x 0.003 = 0,002 mg /kg bw/day – **2 µg/kg bw/day**

Hypericin content in body lotion for HPE : 1.2 mg/kg bw/day x 0.003 = 0,0036 mg /kg bw/day – **4 µg/kg bw/day**

Hypericin content in body lotion for HPO: 3.7 mg/kg bw/day x 0.003 = 0,0111 mg /kg bw/day – **11 µg/kg bw/day**

Hypericin content in rinse-off products containing Hypericum perforatum extract or oil: 0.19 mg/kg bw/day x 0.003 = 0.00057 mg/kg bw/day – **0.57 µg/kg bw/day for the extract** 0.11 mg/kg bw/day x 0.003 = 0.00033 mg/kg bw/day – **0.33 µg/kg bw/day for the oil**

Annex 2

Council of Europe / Committee of Experts on Flavouring Substances – what worked under the aegis of the previous Council of Europe Partial agreement for public health that was dissolved in 2008.

This scientific committee produced October 2005 a safety evaluation of occurrence of *hypericin* in *hypericin perforatum extracts* being used as flavour in foodstuffs. This evaluation forms part of a contribution called *"Active principles (constituents of toxicological concern) contained in natural sources of flavouring"*. This contribution can be retrieved from the internet at:

http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/flavouring_substances/Active%20principles.pdf

This Council of Europe safety evaluation is shown below:

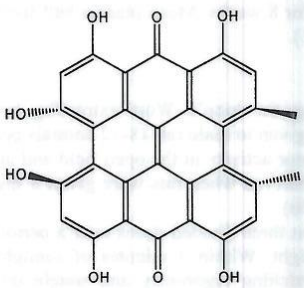
Hypericin

ACTIVE PRINCIPLE: II

SYNONYMS: Hypericum red; cyclo-werrol; cyclosan; 4,5,7,4',5',7'-hexahydro-2,2'-dimethyl-naphthodianthrone; phenanthro[1,10,9,8-opqra]perylene-7,14-dione; 1,3,4,6,8,13-hexahydroxy-10,11-dimethyl-(6CI, 7CI, 8CI, 9CI)

CAS No: 548-04-9

STRUCTURE:



REGULATORY / INTERNATIONAL STATUS: In the USA, the hypericin-free alcoholic distillate from St. John's Wort is listed as GRAS by the FDA for use as a flavouring in alcoholic beverages only (CFR 172.510). Annex II of EC Directive 88/388/EEC on flavourings specifies limits for hypericin in foods to which flavourings or food ingredients with flavouring properties have been added as follows: 10 mg/kg in alcoholic beverages, 1 mg/kg in confectionery and 0.1 mg/kg in foodstuffs and non-alcoholic beverages. Isolated hypericin may not be added to foodstuffs (EEC, 1988). The SCF recently considered the safety of hypericin in flavourings and food ingredients and concluded that the database was too limited to allow an adequate safety assessment, and no ADI was established (SCF, 2002). Regulation of the use of St. John's Wort (*Hypericum perforatum* L.) in foods and beverages in the EU is currently being under revision.

MAIN TOXICOLOGICAL STUDIES:

Metabolism:

In vitro studies: No data found.

Animal studies: A pharmacokinetics study in mice given a single i.v. dose of 17.5 mg/kg bw hypericin determined the terminal biological half-life to be 38.5 hours (Liebes et al., 1991).

Human studies: Twelve healthy male volunteers were given single oral doses of 4.2, 12.5 or 25 micrograms hypericin/kg bw (as LI 160/PK tablets containing a standardized extract of *Hypericum perforatum* L., subsequently named *Hypericum*). The median lag time for absorption was about 2 hours and median elimination half-lives were 24.5 (range 14.7-57.8), 43.1 (28.2-

57.8) and 48.2 (22.9-57.8) hours when volunteers were dosed with 4.2, 12.5 or 25 micrograms hypericin/kg bw, respectively. The pharmacokinetics of hypericin following i.v. injection was investigated in two of the subjects. Comparison of areas under the plasma-time curve (AUC) following a single oral dose (12.5 micrograms/kg bw) and a single i.v. dose (2 micrograms/kg bw) of hypericin indicated that the systemic availability following oral exposure was approximately 14%. When 13 volunteers were given 12.5 micrograms hypericin/kg bw/day (as LI 160/PK tablets containing *Hypericum* extract) orally for 14 days, plasma hypericin reached a steady state concentration of 7.9 micrograms/l after 7 days (Kerb et al., 1996). In another study in which 13 human volunteers were given single oral doses of 0, 18, 36 or 73 micrograms hypericin/kg bw/day, the half-life of hypericin was estimated to be 28 hours. Intact hypericin was not detected in the urine, nor were any possible glucuronidation or sulphation metabolites. The authors also conducted a multiple dosing study, in which 50 volunteers were dosed with 36 micrograms hypericin/kg bw/day for 15 days. The estimated half-life was 42 hours (Brockmöller et al., 1997).
Hepatitis C patients received 50 micrograms hypericin/kg bw/day (12 volunteers) or 100 micrograms hypericin/kg bw/day (7 volunteers) orally for 8 weeks. Mean plasma half-lives were 36.1 and 33.8 hours, respectively (Jacobson et al., 2001).

Toxicology:

Acute toxicity: Single doses of 926, 1852 or 2778 mg St. John's Wort extract/kg bw (equivalent to approximately 1, 2 or 3 mg hypericin/kg bw) given to male rats (8-12 animals per group) by gavage were associated with an increased locomotor activity in the open field and an anxiolytic activity in the light-dark test. No effects were observed when rats were given 3 mg pure hypericin/kg bw by gavage (Vandenbogaerde et al., 2000).
Two rats were fed total amounts of 30 or 60 mg hypericin in three divided doses over a period of 6 hours. The following day they were exposed to sunlight. Within 5 minutes of sunlight exposure they developed erythema of the ears, began scratching vigorously and sought out shade (Pace, 1942).
Calves were given single oral doses of 1000, 3000 or 5000 mg/kg bw of dried St. John's Wort (equivalent to approximately 0.124, 0.372 and 0.620 mg hypericin/kg bw, respectively) and exposed to sunlight. At the two higher doses adverse effects were noted including increased temperature and respiration rate, restlessness and skin reddening around eyes and nostrils and in white areas of the body. The lowest dose produced no adverse effects (Araya and Ford, 1981).
Groups of 11 ewes were dosed by gavage with ground, dried St. John's Wort equivalent to approximately 2.65, 3.7 or 5.3 mg hypericin/kg bw, then exposed to bright sunlight for up to 5 hours/day on 5 successive days or shorter if moderately severe clinical signs developed. All sheep showed increased body temperature and signs of skin irritation as well as restlessness, pawing of the ground, head shaking, head rubbing and oedema around the forehead and eyes. Effects persisted for up to 4 days (Bourke, 2000).
Subacute / subchronic toxicity: Groups of 8 male rats were fed a diet containing 0 or 10% dried and finely ground St. John's Wort (hypericin content not indicated) for 12 days, and then the amount was reduced to 5% due to unpalatability. After 17 weeks, 4 animals per group were sacrificed and autopsied. The remainder were sacrificed after 25 weeks. Animals treated with St. John's Wort showed a significantly decreased body weight gain compared to controls. Survival time of rats fed St. John's Wort was not decreased. No significant tissue lesions were found. Liver copper levels were not directly affected, and no major effects on liver zinc or iron levels were observed (Garrett et al., 1982).
Groups of 3 sheep were given fresh St. John's Wort at doses of 0, 4, 8, 12 or 16 g plant/kg bw/day for up to 14 days and exposed to day light. The amount of hypericin in the feed was not determined. Effects observed in all animals after 7 and 14 days of treatment included

restlessness, photophobia, tachycardia, polypnoea, congested mucous membranes, diarrhoea, hyperthermia, skin redness of exposed parts of tail and legs, oedema of the eyelids and swelling and loss of serum from the ears. Symptoms progressed after one week, with effects including crusts and ulcers of the skin, salivation, alopecia of the face and around ears and eyes, severe congestion of mucous membranes, keratoconjunctivitis, loss of eyelashes, corneal opacity and blindness. A decrease of haemoglobin, red blood cell count and packed cell volume was observed in all treated animals. Total protein, glucose, cholesterol, triglycerides and serum alkaline phosphatase activity were all decreased. Blood urea nitrogen, sodium, potassium, bilirubin, and activities of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and gamma glutamyltransferase were all increased (Kako et al., 1993).
Chronic toxicity / carcinogenicity: No data found.
Reproductive toxicity / teratogenicity: No adequate studies found. In a preliminary study which was only published as an abstract, reduced litter size and reduced body size at birth were observed when 25 CD-1 mice were dosed with approximately 136 mg dried St. John's Wort/kg bw/day via their diet (equivalent to approximately 0.4 mg hypericin/kg bw/day) from 2 weeks before mating throughout gestation (Gonzalez et al., 1998).
Forty female CD-1 mice were randomised to receive either 0 or approximately 180 mg St. John's Wort/kg bw/day (equivalent to approx. 0.54 mg hypericin/kg bw/day) in their diet from 2 weeks before mating through gestation. The impact of *Hypericum* on certain cognitive tasks was tested in the offspring. No significant differences in final performances in various neurodevelopment trials were observed, although exposed female offspring took longer to learn the Morris maze task than non-exposed offspring (Rayburn et al., 2001).
Mutagenicity / genotoxicity: *In vitro*: Hypericin was negative in an Ames test with *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation (Turek et al., 1997). St. John's Wort extract gave a negative result in an HPGRT test in Chinese hamster V79 cells with and without metabolic activation, in an unscheduled DNA assay in rat hepatocytes and in a Syrian hamster embryo cell assay with an without metabolic activation (Okpanyi et al., 1990). However, phototoxicity and a slight increase (doubling) of the number of micronucleated cells was observed in Chinese hamster V79 cells exposed to 100 and 158 ng hypericin/ml and irradiated with 300/10 mJ UVA/UVB per cm^2. Lower concentrations of 10-30 ng/ml exerted no effect whereas higher concentrations of 320-3200 ng/ml were cytotoxic (Kersten et al., 1999).
In vivo: St. John's Wort extract was negative in a mouse fur spot test and a bone marrow chromosome assay in mice (Okpanyi et al., 1990). An *in vivo* mouse micronucleus test with St. John's Wort extract was positive but showed no dose-relationship. Since no further details were provided the relevance of this observation cannot be assessed (Turek et al., 1997).

Human data: In a review article the incidence of adverse effects amongst people taking preparations of St. John's Wort was examined and adverse reactions of the skin exposed to light were described as the most common adverse effect (1 per 300'000 cases treated with St. John's Wort preparations). Less common, potentiation of coumarin-type anticoagulants, breakthrough bleeding in women taking contraceptive pills, gastrointestinal effects and reduced cyclosporine levels in organ transplant patients occurred. From the reviewed results of investigations in volunteers it was concluded that the threshold dose for an increased risk of photosensitisation is about 2-4 g/day of a usual commercial *Hypericum* extract (equivalent to approximately 5-10 mg hypericin/day and 80-170 micrograms hypericin/kg bw/day) (Schulz, 2001).
In a study in which volunteers with hepatitis C were orally administered 50 or 100 micrograms hypericin/kg bw/day for 8 weeks (hypericin as de novo synthesized substance), signs of phototoxicity were reported at both dose levels (5/12 subjects receiving the lower dose, and 6/7 subjects receiving the higher dose). Effects included dermatitis, burning and/or tingling sensations in the skin. Three volunteers in the higher dose group showed darkened coloration of

exposed skin and one patient had pruritic nodules. All symptoms resolved following discontinuation of hypericin treatment (Jacobson et al., 2001).
No adverse skin reactions were reported in a single-dose pharmacokinetics study in which 12 volunteers were given oral doses of St. John's Wort extract (standardized dried extract LI 160), equivalent to hypericin intakes of 0.25, 0.75 or 1.5 mg (corresponding to 4.2, 13 or 25 micrograms hypericin/kg bw if a bodyweight of 60 kg is assumed) (Kerb et al., 1996).
Three HIV-infected adults were given oral doses of 50 micrograms hypericin/kg bw/day (hypericin as de novo synthesized substance) in a phase I clinical trial and all withdrew from the trial within the first 8 weeks due to phototoxicity. The reaction resolved in all patients following cessation of treatment (Gulick et al., 1999).
In a placebo-controlled randomised double-blind trial to test the potential of St. John's Wort extract to produce photosensitivity, each of 13 volunteers received a single dose of 0, 900, 1800 or 3600 mg St. John's Wort extract LI 160 (equivalent to hypericin intakes of approximately 0, 18, 36 and 73 micrograms/kg bw if a bodyweight of 60 kg is assumed). Before and 4 hours after dosing, small areas of the backs of volunteers were exposed to solar simulated irradiation (containing UVA and UVB light), and, in another area, to UVA light only. A slight reduction in the median minimal tanning dose of UVA was observed at the highest dose of hypericin. No effect of hypericin on the minimal dose of solar simulated light or UVA only to produce erythema was observed (Brockmöller et al., 1997).
In a repeated-dose study, 50 volunteers were given oral doses of 600 mg/day of St. Johns Wort extract (equivalent to approximately 36 micrograms hypericin/kg bw/day based on a body weight of 60 kg) for 15 days. At the end of the trial, a slight reduction in the median minimal dose of solar simulated irradiation required to produce erythema and a 21% reduction in the mean minimal tanning dose of UVA were observed, compared to before dosing (Brockmöller et al., 1997).
St. John's Wort extract (standardized dried extract LI 160) was either given orally to 24 volunteers at an initial dose corresponding to 90 micrograms hypericin/kg bw followed by 45 micrograms hypericin/kg bw/day for 7 days or once orally to 48 volunteers at doses of 90 or 180 micrograms hypericin/kg bw. Prior to dosing and 6 hours following dosing the volunteers were tested on their forearms for skin sensitivity to UVB, UVA, visible light or solar simulated irradiation. Erythema index and melanin-index was assessed using a mexameter. In the repeated-dose study, a marginal effect on UVB-induced pigmentation ($p=0.0471$) and a possible marginal effect on visible-light induced erythema ($p=0.0568$) was observed. These effects were not dose-related. In the single dose study, there were no apparent effects on erythema or pigmentation (Schempp et al., 2003).
Other adverse effects have been in limited reports of clinical trials of St. John's Wort for treating depression, including dry mouth, dizziness, gastrointestinal symptoms, skin redness with pruritis, tiredness with fatigue and other unspecified symptoms. Estimated hypericin intakes ranged from approximately 6.7 to 45 micrograms/kg bw/day for treatment periods of 2 to 12 weeks (Linde et al., 1996; SCF, 2002; Schrader et al., 1998).
Furthermore, 5 cases of adverse effects were reported in elderly persons in the USA who combined the use of St. John's Wort extract with prescription antidepressants. Four of the cases were using a serotonin re-uptake inhibitor when they started taking 600-900 mg St. John's Wort extract/day. Within 2 to 4 days, they developed symptoms including nausea, vomiting, confusion and restlessness. The symptoms were diagnosed as being the result of a central serotonin excess or 'serotonin syndrome', characterised by Lantz et al. (1999).

Other studies: *Mechanism of toxicity:* Hypericin produced singlet oxygen *in vitro* when irradiated with light. This is thought to be at least partly responsible for the photosensitive and

phototoxic effects of hypericin (Ehrenberg et al., 1998; Fernandez et al., 1997; Wills et al., 2001).

Induction of enzyme activity: Hypericin inhibited CYP2C9, CYP2D6, CYP3A4 and dopamine-beta-hydroxylase *in vitro* (Obach, 2000; Denke et al., 2000). It also inhibited CYP1A1-catalysed diolepoxide-2-formation from benzo[a]pyrene-7,8-dihydrodiol (Schwarz et al., 2003). However, *in vivo*, St. John's Wort induced CYP3A4 activity (twofold increase) but had no significant effect on CYP2D6 in healthy human volunteers (6 men, 6 women) receiving 900 mg St. John's Wort extract per day (containing 0.3% hypericin, equivalent to 45 micrograms hypericin/kg bw/day) for 16 days (Roby et al., 2000; Markowitz et al., 2003).

Psychotropic effects and MAO-inhibition: Monoaminooxidase (MAO) inhibition has been proposed as a possible mechanism by which St. John's Wort exhibits an antidepressant activity. MAO was inhibited *in vitro* by *Hypericum* fractions either of high or low hypericin content, and it is suggested that other components than hypericin with known MAO-inhibiting properties (e.g. xanthone derivatives) could be responsible for this effect (Bladt and Wagner, 1994; Thiede and Walper, 1994; Suzuki et al., 1981; Demish et al., 1989). Hypericin on its own did not significantly affect MAO activity in either *in vitro* or *ex vivo* studies (Bladt and Wagner, 1994). However, in another study with commercially available hypericin (80% purity only) a 50% irreversible inhibition of rat brain mitochondrial type A and type B monoamine oxidase (MAO) was shown *in vitro* at concentrations of 68 and 420 micromoles/l (e.g. 34 and 212 micrograms/ml, respectively) (Suzuki et al., 1984). Up to now there is no conclusive evidence on whether hypericin has MAO-inhibiting potency and whether it is responsible for the psychotropic activity of *Hypericum*.

In vitro studies of reproductive toxicity: The potential for hypericin to cause teratogenicity was investigated *in vitro* using a whole rat embryo culture model. Rat embryos were explanted at gestation day 9.5, cultivated *in vitro* for 48 hours in medium containing 0, 14.2, 28.4, 71 or 142 ng hypericin/ml and then examined. Embryos exposed to 71 or 142 ng hypericin/ml had a significantly lower total morphological score and number of somites than controls ($p<0.05$). Trend analysis showed a negative linear trend for total morphological score ($p<0.001$), number of somites ($p<0.001$) and crown-rump length ($p=0.01$), but not for yolk sac diameter (Chan et al., 2001).

Potential to cause cataracts: Hypericin at a concentration of 50 micromoles/l (e.g. 25 micrograms/ml) caused polymerization of calf lens alpha-crystalline only when exposed to light. Mass spectrometry indicated oxidation of methionine, tryptophan and histidine residues, which increased with irradiation time (Schey et al., 2000).

TOXICOLOGICAL EVALUATION: Data on biotransformation, elimination and toxicity of hypericin is limited. From various studies there is evidence that *Hypericum* induces enhanced photosensitivity, both in animals and in humans. Several clinical studies with human volunteers have shown that the lowest doses leading to adverse skin reactions are in the range of 25 to 50 micrograms hypericin/kg bw/day. The available data indicate that single dose administration is tolerated at higher hypericin levels than repeated-dose administration. Overall, a LOAEL of 25 micrograms hypericin/kg bw/day was set for the observed adverse skin reactions in humans. Other side effects were not reported at the above mentioned dose levels.

Limited genotoxicity studies with hypericin or *Hypericum* extract provided only negative results. Data on chronic toxicity and carcinogenicity are not available. Reproductive toxicity studies are limited to a preliminary study on neurobehavioral developmental toxicity in mice indicating that *Hypericum* may elicit adverse effects on foetal physical development at very high doses of 136 mg/kg bw/day.

It has been demonstrated that St. John's Wort extract may have psychotropic and in particular an anti-depressing activity. The current knowledge does not yet explain the mechanism of the

observed psychotropic activity of *Hypericum* and *Hypericum* extract and it has been impossible to attribute this activity to any of its particular constituents and certainly not to hypericin. *Hypericum* extract (900 mg *Hypericum* extract/day for 16 days, equivalent to 45 micrograms hypericin/kg bw/day) induced CYP3A4 activity (twofold increase) in healthy volunteers, but had no significant effect on CYP2D6.
The available data indicate that photosensitivity and enzyme induction are the critical endpoints in animals and most possibly also in humans. Based on a LOAEL of 36 micrograms hypericin/kg bw/day for enhanced photosensitivity and phototoxicity in humans derived from the most reliable repeated-dose study in healthy volunteers (Brockmöller et al., 1997) a TMDI of 0.002 mg hypericin/kg bw/day was established using a safety factor of 20. The safety factor of 20 comprises a factor of 10 for interindividual differences and a factor of 2 for the use of a LOAEL instead of a NOAEL, taking into account that the observed effects were minor skin reactions.

TMDI: 0.002 mg/kg bw/day.

MAIN OCCURRENCE: Hypericin is a napthodianthrone (anthraquinone derivative) which occurs in *Hypericum perforatum* L. (St. John's Wort) at concentrations of 0.0095 to 0.466% in the whole plant (Duke, 1989) and 0.02-0.18% in dried flowers (Hölzl and Ostrowski, 1987). Drying St. John's Wort can reduce the hypericin content by up to 80% (Araya and Ford, 1981). Other major constituents in *Hypericum* are tannins (up to 16%), hyperoside, hyperforin, adhyperforin and flavonoids (up to 4%), pseudohypericin and pinene (up to 0.6%). Only traces of xanthones have been reported in *Hypericum* (USDA, 2004).
Most clinical trials have been conducted with LI 160/PK tablets (commercial product Jarsin®), containing 300 mg of a *Hypericum* extract standardized to 0.3% hypericin.

INTAKE ESTIMATION: Very limited data on the use of St. John's Wort as a flavouring substance are available. The plant and its preparations are believed to be no major food sources, but used as flavourings in some liqueurs. If it is assumed that liqueurs are the only source of hypericin, that all liqueurs contain hypericin at the current maximum limit of 10 mg/kg permitted according to EU Directive 88/388/EEC (EEC, 1988), and that 42.4 g of liqueurs are consumed per day (high intake level from a UK survey), the intake of hypericin would be 0.424 mg/day, equivalent to 7.1 micrograms/kg bw/day. If the new limit of 2 mg/kg set by the Council of Europe for hypericin in alcoholic beverages is applied, the intake of high level liqueur consumers (42.4 g/day) would be 0.085 mg/day, equivalent to 1.4 micrograms/kg bw/day, and the intake of mean consumers (10 g liqueurs/day) would be 0.020 mg/day, equivalent to 0.33 micrograms/kg bw/day. Hypericin-containing *Hypericum* extracts or dried plant products are used in herbal teas and in Over-The-Counter (OTC) anti-depression medication. A herbal tea marketed in the Netherlands has been reported to contain 2 g St. John's Wort (dried leaves) per tea bag, providing an intake of about 250 microgram hypericin per cup (SCF, 2002). Consumers are advised to take 1 to 2 cups, 3 times a day, and may thus be exposed to a maximum level of 1500 micrograms hypericin/day, equivalent to 25 micrograms hypericin/kg bw/day. Consumption of one cup per day results in a intake of 4.2 micrograms hypericin/kg bw/day for a person of 60 kg body weight.

CONCLUSIONS: Consumption of liqueurs containing the maximum level of 10 mg hypericin/kg permitted according to EU Directive 88/388/EEC, may result in hypericin intakes in high level consumers which are by a factor of 4 above the TMDI. At the maximum level of 2 mg hypericin/kg in alcoholic beverages recommended by Council of Europe, the TMDI will not be exceeded neither in high level liqueur consumers nor in mean consumers. However, the

TMDI may be largely exceeded in individuals consuming one or more cups per day of a herbal tea prepared from dried leaves of *Hypericum perforatum* L..

DATA NEEDED: Further studies on metabolism and subchronic and possibly chronic toxicity, a validated study assessing the photogenotoxicity, as well as further studies on the photosensitivity in humans, preferably using the pure substance are needed. Data on any other food uses of St. John's Wort are also required.

LIMITS: (mg/kg)

General limits in foods and beverages:	ND[a]
Exceptions:	
Alcoholic beverages	2

REFERENCES:

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[a] ND= Non-detectable based on modern analytical test methods. The limit of determination should be taken into consideration as general limit.

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DATABASES USED: Medline (1966-2003), Toxline (1969-2003). Keywords: hypericin, St. John's Wort, Hypericum, toxicity.

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