Opinion of the Scientific Committee on Food on the presence of hypericin and extracts of *Hypericum sp.* in flavourings and other food ingredients with flavouring properties

(adopted on 12 December 2001)
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Terms of Reference

The Committee is asked to advise the Commission on substances used as flavouring substances or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health.

In particular, the Committee is asked to advise the Commission on the implications for human health of the presence of hypericin in the diet.

Introduction

Hypericin occurs naturally in Hypericum (plant or crude plant extract of *Hypericum perforatum L* [St John’s wort]).

Although in the Terms of Reference the Committee is asked to advise the Commission on the implications for human health of hypericin in the diet, the Committee is aware that the major source of hypericin in the diet is *Hypericum* extract. Therefore, in this opinion data on *Hypericum* and an evaluation of these extracts are also included.

Exposure to hypericin or *Hypericum* may lead to an increased sensitivity of the skin to subsequent exposure to light. Throughout this document this phenomenon will be indicated by the term induction of enhanced photosensitivity, instead of terms like photosensitisation, photohypersensitisation, or photohypersensitivity.

Previous evaluations

The Council of Europe Committee of Experts on Flavouring Substances (CEFS) evaluated hypericin as an active principle in food flavourings (CEFS, 1996). Two major effects were distinguished, namely induction of enhanced photosensitivity and inhibition of monoamine oxidase (MAO) *in vitro*, the latter possibly resulting in psychotropic activity. CEFS concluded
that in the absence of reliable data on the MAO inhibition, a temporary TDI of 0.27 µg/kg bw/d can be established for hypericin based on a NOAEL of 54 µg/kg bw, which was found in a bovine single dose study (Araya and Ford, 1981), with a safety factor of 200 in order to take account of the uncertainty regarding MAO inhibition. CEFS (1996) and the Council of Europe (CoE, 2000) proposed a limit of 0.4 mg/l of hypericin in beverages¹ and none for foodstuffs. It suggested to request further studies on the component(s) responsible for MAO inhibition.

**Current Regulations**

Annex II of Directive 88/388/EEC on flavourings sets 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).

Hypericin free alcoholic distillate of St John’s wort (*Hypericum sp.*) is on the FDA GRAS list (121.1163) for use in alcoholic beverages only.

**Chemical characterisation of hypericin and *Hypericum perforatum L.***

![Hypericin](image)

<table>
<thead>
<tr>
<th>Name</th>
<th>Hypericin</th>
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<tr>
<td>Synonyms</td>
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¹ CEFS (1996) did not specify the type of beverages. However, the calculations on which this proposal was based assumes that hypericin is exclusively added to alcoholic beverages.
Exposure Assessment

General information on natural occurrence

Hypericin is a naturally occurring naphthodianthrone derivative in the plant species *Hypericum perforatum* (St. Johns wort). The hypericin concentration in the plant may vary, depending on place of growth, state of plant material (fresh or dried) before extraction and part of the plant studied. In the whole herb hypericin may be present in concentrations of 0.0095 to 0.466% and in flowers up to 0.086%. In addition to hypericin, in extracts of this plant several other substances can be found in various quantities, e.g. hyperoside, hyperforin, pseudohypericin, quercetin, 2-methyloctane, α-pinene, dodecanol, nonane, 3-methyl nonane, undecane, isoundecane and 6-methyl-5-hepten-2-one. In the whole herb tannin, alkaloids, ocimene and xanthone derivatives are also found (Hölzl and Ostrowski, 1987; CoE, 2000). Drying of *Hypericum* before extraction may result in a 80% reduction of the concentration of hypericin in the extract (Araya and Ford, 1981). Hypericin can be chemically synthesised and is commercially available (Bladt and Wagner, 1994; Vandenbogaerde *et al.*, 2000).

Use

Hypericin-containing *Hypericum* extracts or dried plant products are used as food flavourings or in herbal teas (CoE, 2000) and in Over-The-Counter (OTC) anti-depression medication.

Exposure estimates

*Food flavourings*

Little information about the use of hypericin or *Hypericum* extracts as food flavourings is available. Apart from the data in the CEFS evaluation (CEFS, 1996), no new data were located.

According to CEFS (1996), hypericin occurs mainly in *Hypericum perforatum* (*0.0054 % in dried plant*) and is possibly not used in foodstuffs, except in alcoholic beverages. CEFS
calculated a ‘worst case’ intake of 6.5 µg/kg bw, assuming intake via alcoholic beverages, only, and assuming that these drinks contain the maximal concentration of hypericin of 10 mg/kg (Directive 88/388/EEC).

Using consumption data from the survey of British adults (Gregory et al., 1990), a maximum daily intake for hypericin of 7.9 µg/kg bw can be calculated, assuming that high and medium strength liqueurs are the only food items that contribute to the exposure to hypericin, that these liqueurs are taken in an amount of 47.4 g/day\(^2\) and that they contain 10 mg hypericin/kg.

**Herbal teas**
In the Netherlands a herbal tea is marketed in the form of tea bags. These bags contain 2 g Hypericum (dried leaves) and provide a dose of ca. 250 µg hypericin per cup. Buyers are advised to take 1 to 2 cups for 3 times a day and may thus be exposed to up to 1500 µg hypericin per day (ca. 25 µg/kg bw/d).

**OTC anti-depression medication**
Kerb et al. (1996) mention the use of Hypericum in OTC drugs in Germany, sold in quantities of 106 million daily doses per year in 1994. A tablet (“LI 160/PK”) containing 300 mg of Hypericum extract may contain 250 µg of hypericin, resulting in a dose of 3.8 µg hypericin/kg bw/tablet. Daily doses may be 10 times higher. In the Netherlands two similar brands of tablets are marketed, containing 180 or 330 µg of hypericin in ca. 300 mg Hypericum extract per tablet\(^3\). Buyers are advised to take six or three of those tablets a day, which in both cases results in an intake of ca. 1000 µg/d (ca. 15 µg/kg bw/d).

**Summary of the exposure assessment**

1) The data on exposure to hypericin from St. John’s Wort extract, used as a flavouring substance are poor. Based on a UK estimate of intake of medium and high strength liqueurs, and assuming that these contain 10 mg hypericin/kg an intake of about 8 µg hypericin/kg bw/d can be estimated.

2) The intake via herbal teas can be estimated from a preparation sold on the Dutch market. Use of this preparation may result in an intake of 1500 µg hypericin per day (ca. 25 µg/kg bw/d).

3) Hypericum extracts are also sold as OTC preparations for the treatment of mental depression. Intake of one single tablet may result in an intake of hypericin of 3.8 µg/kg bw. Daily exposure levels to hypericin from these tablets may be 10 times higher.

\(^2\) This figure has been established in a survey in the UK in 1986-1987. It is the 97.5 percentile of the intake estimate for consumers only.

\(^3\) Several members of the Committee noted that Hypericum OTC preparations are also marketed in other EU Member States. The scientific literature also mentions the use of these preparations in the USA.
Hazard identification/characterisation

Introduction

The toxicological database is very limited. Well-defined in vivo toxicity studies and biotransformation studies (either in vivo or in vitro) with hypericin were not located. However, several studies were located with *Hypericum* extract or with whole plant material, in some of which attention was paid to hypericin:

In mice and humans kinetic studies have been performed with *Hypericum* extracts. In the human studies attention was also paid to induction of enhanced photosensitivity. Some toxicity studies in rats and ruminants, and a behavioural study in rats are available.

Various clinical trials have been carried out to evaluate the anti-depressive activity of St. John’s wort extracts. These will not be summarised here in detail. However one meta-analysis based on 23 trials will be further discussed. Recent studies have also shown that hypericin may interact with registered pharmaceuticals, possibly by modifying cytochrome P450 enzyme activities.

Two studies in humans examined the effects of St John’s wort extract on biotransformation enzymes. Three more studies focussed on the elucidation of the *Hypericum* constituent which may be responsible for MAO, catechol-O-methyl transferase (COMT) and dopamine-β-hydroxylase (D-β-H) inhibition, effects which may be related to the psychotropic activity of *Hypericum* extracts.

In “TOXLINE +” many recent publications can be found dealing with effects of hypericin on cellular systems in vitro. Many of these focus on the hypericin-mediated generation of activated oxygen species especially under influence of (UV) irradiation (e.g. Schey et al., 2000), explaining the potential of hypericin to induce enhanced photosensitivity or its ability to act as a cytostatic. Other studies refer to anti-retroviral action, especially with regards to anti HIV activity. These studies do not seem to be of direct relevance for the evaluation of the safety of hypericin in foods and beverages and are not further considered here.

Absorption, distribution, metabolism and excretion

*Study in mice*

Liebes et al. (1991) studied the kinetics of hypericin in Balb/c mice. Animals were injected i.v. with 17.5 mg hypericin/kg bw. Blood samples were collected at various time points up to 240 h post dosing and analysed for hypericin by HPLC. The blood level data were fitted to a two compartment kinetic model and showed a terminal biological half life of 38.5 h. Metabolites were not studied.
Studies in humans

12 human volunteers were dosed orally with single doses of 250, 750 or 1500 µg hypericin (as “LI 160/PK” tablets containing 300 mg Hypericum extract, with 250 µg of hypericin). The observed plasma concentration data were fitted into a two compartment kinetic model. A lag time of 2 hours was found before hypericin appeared in the blood. At the two high dose levels, the substance was eliminated with a terminal half-life of 42-48 hrs. At the lowest dose level the terminal half-life amounted to 24 hrs. The maximum concentrations (Cmax) and Areas Under the plasma-time Curve (AUC) increased more rapidly than expected from the increase in dose levels (Cmaxs: 1.3, 7.2 and 16.6 µg/l and AUCs: 41.4, 198 and 494 µgxh/L for 250, 750 and 1500 µg dose levels, respectively). Comparison of the AUC data for a single oral dose of hypericin (as tablets containing Hypericum extract) with those obtained after a single i.v. dose of 115 µg of hypericin revealed a systemic availability after oral exposure of 10 to 19%, depending on the amount that was given. Repeated dosing with 3 times 250 µg hypericin/d over 14 days did not result in changes of the terminal elimination half-life. None of the dose regimens resulted in abnormalities of laboratory parameters (not further specified) or skin reactions (Kerb et al. 1996).

13 volunteers received 0, 3, 6 or 12 “LI 160/PK” tablets (or/and placebo tablets making up a total of 12 tablets per person). Each tablet contained 363 µg of hypericin, resulting in total dose levels of hypericin of 0, 1.09, 2.18 or 4.36 mg. Average maximum plasma concentrations for hypericin were 0 and ca. 17, 35 and 90 µg/L plasma at 6 h for the various treatment groups. After a single oral dose, hypericin was eliminated from the plasma with a terminal half-life of about 28 h, respectively. Neither hypericin nor its (possible) glucuronide or sulphate conjugates were detected in the urine.

In addition, multiple dosing of 50 volunteers with 2.2 mg hypericin for 15 days resulted in trough steady state plasma levels of ca. 26 µg/L. After repeated dosing a terminal half-life of 42 h for hypericin was reported (Brockmöller et al. 1997).

Acute toxicity

Studies in rats

Two rats of 180 g bw receiving one total daily oral dose of either 30 or 60 mg hypericin showed induction of enhanced photosensitivity the next day (Pace, 1942).

In a study by Vandenbogaerde et al. (2000) male rats (8 to 12 per group) were dosed with dry Hypericum extract containing 0.11 % total hypericin or with > 98% pure hypericin by gavage. Administered doses were 0, 926, 1852 or 2778 mg extract per kg bw (0, 1, 2 or 3 mg hypericin/kg bw) or 3 mg pure hypericin/kg bw. 1 Hour post dosing the animals were tested for locomotor behaviour and anxiolytic effects. Extract-treated animals showed increased activity at the highest dose level and even at the lowest dose level an increase in number of rearings (per observation time) was observed. Pure hypericin did not influence the animals’ behaviour. The extract dose of 1852 mg/kg bw produced an anxiolytic response of the same
magnitude as 1.5 mg/kg bw diazepam (i.p.). This effect could be blocked with the benzodiazepin antagonist flumazenil (3 mg/kg i.p.). Additional electrophysiological studies in vitro, performed to gain more information on the mechanism of action, showed that hypericin reduced the GABA-activated chloride currents (GABA: gamma amino butyric acid), while hypericin inhibited the activation of NMDA receptors (NMDA: N-methyl-D-aspartate). Nevertheless, it is concluded from the in vivo studies that other constituents than hypericin in the crude Hypericum extract are contributory or responsible for the locomotor and anxiolytic effects of Hypericum.

Studies in ruminants

Single oral doses of 1, 3 and 5 g dried ground St. John’s wort/kg bw were given to calves by gavage as a watery slurry (a control group was not included in the experiment). In animals of the 3 and 5 g/kg bw group, exposure to direct sunlight produced signs of induction of enhanced photosensitivity after 3 to 4 hours. Effects were restlessness, signs of dermal irritation, skin and eye reddening of naked and white-haired skin, and in the highest dose group also scab formation and exudation. Recovery from these dermal lesions took 30 to 40 days. Blood levels of creatine phosphokinase were increased, but not of sorbitol dehydrogenase, gamma glutamyl transeptidase, glutamate dehydrogenase or arginase. No effects were observed in animals dosed with 1 g dried St. John’s wort/kg bw, equivalent to 124 μg hypericin/kg bw, or in any dosed animals which were not exposed to direct sunlight. On this study CEFS (1996) has based its proposal for a temporary TDI for hypericin. However, re-evaluation of the study revealed that the NOAEL found in this study is 124 μg hypericin/kg bw and not 54 μg/kg bw (Araya and Ford, 1981).

Groups of 11 shorn ewes were dosed once with finely ground, dried, flowering growth stage Hypericum perforatum plant material as a slurry by stomach tube at 2.85, 4.0 or 5.7 g dry plant per kg live weight. This corresponded to 2.65, 3.7 and 5.3 mg hypericin per kg live weight, respectively. After dosing they were exposed to bright sunlight for up to 5 h per day over up to 5 successive days or shorter if moderately severe clinical signs developed. Control groups were not part of the study. Clinical responses were observed and rectal temperature was measured. Ingestion of Hypericum followed by exposure to bright sunlight, frequently resulted in clinical signs attributable to skin irritation and central nervous effects, including an inappropriate increase in body temperature. A decrease in Hypericum ingestion from 5.7 to 2.85 g dry plant per kg live weight, corresponding to a decrease in hypericin ingestion from 5.3 to 2.65 mg per kg live weight, was associated with a decrease in the severity of the clinical signs, including the severity of the hyperthermia. There appears to be an absolute requirement for exposure to bright sunlight before any effects of Hypericum will develop. A single dose of Hypericum remains potentially effective for up to 4 days. A tolerance level for hypericin of < 2.65 mg per kg live weight (i.e. lowest dose tested is a LOAEL) was demonstrated (Bourke, 2000).
Sub-acute toxicity

Studies in ruminants
Sheep (3 per group) were given freshly cut Hypericum perforatum at dose levels of 0, 4, 8, 12 or 16 g plant/kg bw/d for 14 days and exposed to day light. After 7 and 14 days haematology and several clinical biochemical parameters were studied. The following clinical observations were reported: restlessness, photophobia, tachycardia, polypnea, congested mucous membranes, diarrhoea and hyperthermia. Skin redness of exposed parts of tail and legs oedema of eyelids and swelling and loss of serum from the ears. In the high dose groups the symptoms occurred two days earlier than in the low dose groups. After one week the conditions aggravated finally leading to loss of eyelashes, corneal opacity and blindness. Feeding Hypericum resulted in decreased haemoglobin, red blood cell count, packed cell volume, total protein, glucose, cholesterol, triglycerides, and serum alkaline phosphatase activities. Blood urea nitrogen, sodium, potassium, bilirubin (total and direct), and the activities of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and gamma glutamyltransferase increased. The severity of the effects increased with exposure duration but not with dose. Quantitative data were not provided. The total amount of hypericin in the feed was not determined. The authors suggested that apart from the ocular and dermal effects, dosing with Hypericum might have resulted in haemolytic anaemia and damage to the kidneys and liver (Kako et al., 1993).

Sub-chronic toxicity

Studies in rats
Male rats (8/grp) were dosed with 0 or 10% St. John’s wort meal mixed in the food. The concentration in the food was reduced to 5% after 12 days because of unpalatability. The hypericin content of the food was not specified. 4 animals per group were killed after 119 days and necropsied. No significant tissue lesions (investigated tissues not further specified) were observed. The remaining animals were sacrificed after 178 days and showed decreased average body weight gain over the entire exposure period (Garret et al., 1982).

Chronic toxicity and Carcinogenicity studies
No studies could be located in literature.

Genotoxicity

Conventional studies
In vitro tests
In an Ames test with hypericin (purity unknown) in Salmonella TA 98 and TA 100 strains, a negative result has been claimed with and without metabolic activation with S9 from Aroclor-treated rats. From the study report only limited information on the test procedure could be obtained. However, the study seems appropriate (Turek et al., 1997).
No indications for induction of gene mutations were obtained in an HGPRT-test in V79 cells in concentrations of 0 or 0.08 to 0.5 μl Hypericum extract/ml medium in absence of metabolic activation and in concentrations of 0 or 0.65 to 4 μl/ml medium in the presence of S9-mix from PCB treated rats. The test had reduced sensitivity because of a too short expression period for possible gene mutations and insufficient cytotoxicity in the highest concentrations Okpanyi et al. (1990).

No indications for induction of UDS were found in an appropriate test in primary cultures of rat hepatocytes with Hypericum extract in concentrations of 0.014 to 1.37 μl/ml medium Okpanyi et al. (1990).

A cell transformation assay with SHE cells exposed to 0, 1, 2, 5 or 10 μl/ml Hypericum extract for 4 h without S9-mix or to 0, 0.75, 1.875, 3.75 or 7.5 μl/ml Hypericum extract for 48 h with and without S9-mix did not reveal cell transformation activity (Okpanyi et al., 1990).

In vivo tests

An in vivo micronucleus test with hypericin (purity not stated) was carried out in mice. From the study report only limited technical details can be obtained (e.g. no information on number of animals used). An increase of micronuclei was observed in the treated animals, but this increase showed no dose-relationship. The relevance of this observation is difficult to assess (Turek et al., 1997).

In an appropriate mouse spot test, female NMRI mice (ca. 60 per dose level) were treated with 0, 1, 5 or 10 ml of Hypericum extract/kg bw by gavage at day 9 of gestation. No increase in the incidence of discoloured spots in the coat of the offspring was found.

Mice (5 per dose group) were exposed to 0, 1, 3 or 10 ml of Hypericum extract/kg bw by gavage. At several time points post-dosing animals were treated with colchicine and bone marrow cells were studied for the presence of chromosomal aberrations. No increase in chromosomal aberrations was found, but it can be argued that bone marrow exposure may not have been sufficient. However, as toxicity in the animals was observed, dosing at higher levels may not have been possible (Okpanyi et al., 1990).

Photogenotoxicity

Pure hypericin was studied for photogenotoxicity in a micronucleus test in V79 cells. Cells were exposed to hypericin in concentrations of 0, 10, 31.6, 100, 158, 316, 1000 or ca. 3160 ng/ml for 30 min in the dark and subsequently irradiated with 0/0, 100/3.3 or 300/10 mJ UVA/UVB per cm². No effect on cell proliferation was observed with any of the hypericin concentrations with or without UV irradiation. Phototoxicity and a doubling of the number of micronucleated cells was found at 100 and 158 ng hypericin/ml in combination with 300/10 UVA/UVB. Lower concentrations of either hypericin or UVA/UVB did not produce either phototoxicity or increased amounts of micronucleated cells, whereas phototoxicity at higher
concentrations with both low and high exposure to UVA/UVB could not be studied due to cytotoxicity (Kersten et al., 1999).

Reproductive and developmental toxicity

No adequate studies were located. However, in a pilot study by Gonzalez et al. (1998), dosing of mice with 0.75 mg dried Hypericum plant material/g food (136 mg/kg bw/d) from day 14 before mating throughout gestation resulted in reduced litter size and a reduced body size at birth. (The study was only published as an abstract).

Neurotoxicity

No data available.

Human data

A placebo-controlled randomised double blind clinical trial was performed to evaluate the induction of enhanced photosensitivity in humans after administration of high doses of Hypericum extract (“LI 160/PK”). 13 volunteers received 0, 3, 6 or 12 LI 160 tablets (or/and placebo tablets making up a total of 12 tablets per person). Each tablet contained 363 µg of hypericin resulting in total dose levels of hypericin of 0, 1.09, 2.18 or 4.36 mg. Volunteers were pre- and post-dosing exposed to simulated solar light (SSI, containing UVA + UVB light) or to UVA light only to determine minimal tanning and minimal erythema-inducing irradiation dose levels.

A slight enhancement of photosensitivity, determined as a reduction of the median minimal tanning dose of UVA, was only observed after the high dose of hypericin. No hypericin-related reduction was seen in the radiation dose level required to induce erythema after irradiation with SSI. No differences in occurrence of side effects between control and exposure groups were observed.

In addition, in 50 volunteers dosed with 2.2 mg hypericin for 15 days a slight enhancement of photosensitivity to both SSI and to UVA was observed. This enhancement was reflected in a slight reduction of the median minimal dose of SSI that was required to produce erythema and in a 20% reduction in the mean minimal tanning dose of UVA. Neither in the single dose part nor in the repeated dose part of the study a correlation between induction of enhanced photosensitivity and plasma total hypericin levels (see under the kinetics section) could be established (Brockmöller et al. 1997).

Meta-analysis of clinical trials for psychotropic effects

Linde et al. (1996) have investigated if extracts of Hypericum perforatum (St John's wort) are more effective than placebo in the treatment of depression, if they are as effective as standard anti-depression treatment and if they have fewer side effects than standard anti-depressant drugs. A systematic review and meta-analysis of 23 randomised clinical trials was carried out (20 of which double blind, one single blind and 2 open), including a total of 1757 outpatients.
with mainly mild or moderately severe depressive disorders: 15 were placebo controlled (of which 14 tested single preparations and one a combination with other plant extracts), and 8 compared Hypericum extracts with another drug treatment (e.g. amitriptylin, bromazepam, diazepam, imipramine and others, six testing single preparations and two testing combinations). The patients in the various clinical trials received 0.45 to 2.7 mg of total hypericin (approx. 6.4 to 38.6 µg/kg bw/d) for periods of 2 to 12 weeks. For the treatment of depression, Hypericum extracts were significantly superior to placebo (ratio = 2.67; 95%-confidence interval 1.78 - 4.01) and similarly effective as standard antidepressants (single preparations ratio =1.10; 95%-confidence interval 0.93 - 1.31, combinations ratio = 1.52; 95%-confidence interval 0.78 - 2.94). There were two (0.8%) drop-outs for side effects with Hypericum extract and seven (3.0%) with standard antidepressant drugs. Side effects occurred in 50 (19.8%) patients on Hypericum and 84 (52.8%) patients on standard anti-depressants.

The original studies that were used in this meta-analysis (for references see Linde et al., 1996) were examined, to see whether from these studies an indication of a NOAEL for side effects could be extracted. In these primary studies dry mouth, dizziness, gastro-intestinal symptoms, skin redness with pruritus, tiredness/fatigue, and other unspecified symptoms were noted, but it was not possible to derive a NOAEL for these effects. Other sources also mention confusion, induction of enhanced photosensitivity and interference with serotonin re-uptake inhibitors, resulting in "serotonin syndrome", but do not provide suitable data to derive a NOAEL either (Anonymous, 1997; Lantz et al., 1999; Woelk et al., 1994).

The validity of the meta-analysis by Linde et al. (1996) has been questioned by others (Anonymous, 1997) because of poor establishment of diagnosis of depression, low placebo response in comparison with other studies, low dosing of standard antidepressants in most of the studies, and wide hypericin dose range (over 6-fold). The duration of the studies taken into account varied from 2 to 12 weeks. However, some of the criticism above was at least partly overcome by the selection criteria for inclusion of a particular trial in the meta-analysis. Moreover, all studies were assessed for eligibility by at least two reviewers before inclusion in the meta-analysis.

Mechanistic studies

Psychotropic effects

It has been suggested that the psychotropic effects of St. John’s wort extract may be related to MAO inhibition. Concentrations of 68 µM and 420 µM of 80% pure hypericin produce in vitro 50% inhibition of MAO type A and B, respectively. This may be related to a xanthon contaminant, indicating that either hypericin or xanthon may give psychotropic effects in humans (CEFS, 1996).

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4 It is not clear how the percentages of patients with adverse drug reactions were calculated. However, it is clear that they were calculated for different groups of studies and therefore for different numbers of patients.
Bladt and Wagner (1994) studied the effects of various fractions of Hypericum extracts on MAO in *in vitro* and *ex vivo* systems. The test fractions consisted of 80% flavonoids with aglycones but no hypericin (EFI), 80% flavonoids without aglycones but with small amounts of hypericin (EFII) and polar substances without flavonoids or hypericin (EFIII). In addition an 80% hypericin fraction was prepared. MAO-A inhibition was studied *in vitro* in rat brain homogenate and *ex vivo* in rat brain homogenate prepared from animals treated i.p. with fractions EFI to EFIII. Results were compared with those from reference substances and from commercially obtained hypericin.

*In vitro* all fractions of Hypericum showed inhibition of MAO, whether they contained hypericin or not. Maximum inhibition was reached between 10^-4 and 10^-3 M. Lower concentrations and pure hypericin did not affect MAO activity to an appreciable extent. According to the authors, MAO-inhibition by Hypericum is insufficient to explain the psychotropic activity of this plant, because it should have occurred at concentrations in the micromolar rather than millimolar range.

Thiede and Walper (1994) studied *in vitro* inhibition of MAO and catechol-O-methyl transferase (COMT) in pork liver homogenates, by carrying out another Hypericum fractionation study. Substantial inhibition of MAO was only found at concentrations of 10^-3 M either with fractions rich in hypericin or with fractions poor in hypericin or with commercial hypericin. No MAO-inhibition at all was seen at 10^-5 M or less. Inhibition of COMT was seen in crude extract as well as in fractions containing flavonoids and xanthones at a concentration of 10^-3 M and to a lesser extent also at 10^-4 M. Lower concentrations and pure hypericin were inactive. It was concluded that neither MAO nor COMT inhibition could explain the psychotropic activity of Hypericum.

The effect of several commercially available constituents of Hypericum and of Hypericum extract on dopamine-β-hydroxylase (D-β-H) was studied in chemically defined systems. The enzyme was strongly inhibited by hypericin (IC_{50} = approx. 4 µM), whereas the IC_{50}-values of various flavonoids (quercitrin, isoquercitrin, hyperoside, rutin, quercetin, amentoflavone, kaempferol) were in the range of 50 µM or higher. The authors speculated that this inhibitory effect of hypericins on D-β-H may contribute to the psychotropic activity, but this speculation was not supported by biologically more relevant data (Denke *et al*., 2000).

Vandenbogaerde *et al*. (2000) discussed several mechanisms by which Hypericum extracts may exert psychotropic activity, among which MAO inhibition and serotonin re-uptake inhibition. In addition, inhibition of D-β-H has been mentioned as a possible explanation for this effect. Up to now no conclusive evidence is available to decide which substance is responsible for this activity and via which mechanism.

*Studies on effects on biotransformation enzymes*

Commercially available St. John's wort (*Hypericum perforatum*) extracts were examined for the potential to inhibit human cytochrome P450 (CYP) 1A2, 2C9, 2C19, 2D6, and 3A4 enzyme activities. Crude extracts demonstrated inhibition of each of these enzymes. Upon
fractionation, several fractions possessed inhibitory activity, including the fractions containing hyperforin, I3, II8-biapigenin, and hypericin. Hyperforin, I3, II8-biapigenin, hypericin, quercetin and chlorogenic acid were isolated from the extracts and inhibition constants for the five CYP activities were measured. Apart from chlorogenic acid, all substances mentioned displayed potent inhibitory activity to one or more of the cytochrome P450 enzymes mentioned. Hypericin itself inhibited CYP 2C9, 2D6 and 3A4 with Ki values of 1.4, 2.6 and 4.2 μM, respectively (Obach, 2000).

Thirteen humans participated in an unblinded, multiple-dose, single-treatment before-after trial. Each subject ingested a 300 mg tablet of reagent-grade St John's Wort extract standardised to 0.3% hypericin three times a day for 14 days. Baseline and post-treatment CYP3A4 activity was assessed with the urinary 6-β-hydroxycortisol/cortisol ratio after a 24-hour urine collection. All but one subject had an increase in the ratio after ingestion of Hypericum extract. The mean +/- SD activity increased from a baseline value of 7.1 +/- 4.5 to 13 +/- 4.9. (range: -25% to 259% increase). According to the authors, this finding suggests that St John's Wort is an inducer of CYP3A4 (Roby et al., 2000).

The Committee on Safety of Medicines of the British Medicines Control Agency (CSM-MCA, 2000) has released a warning for interactions of Hypericum preparations with the following (classes of) drugs: HIV protease inhibitors, HIV non-nucleoside reverse transcriptase inhibitors, warfarin, cyclosporin, oral contraceptives, anticonvulsants, digoxin, theophylline, triptans and SSRI (selective serotonin reuptake inhibitors). This warning is based on the observed effects on biotransformation enzymes, rather than on observations in patients. Interactions may result in a (partial) loss of effectivity or in increased incidence of adverse drug reactions. In addition, several recent publications (not further discussed) have shown that Hypericum preparations might be responsible for pharmacokinetic drug interactions in clinical studies.

Summary of the hazard identification/characterisation

1. Virtually no toxicity data are available. Studies on kinetics in mice and humans indicate a slow elimination from the body resulting in a biological half-life for hypericin of 24 - 48 h. In humans a systemic bioavailability of 10 to 19% has been established after oral intake of Hypericum, depending on the amount of extract ingested. Biotransformation has not been adequately studied. There is no indication that hypericin or hypericin- sulphate or glucuronide conjugates are excreted via the urine. Other routes of excretion were not studied.

2. It has been demonstrated that St John’s wort extract may have psychotropic effects and in particular that it may have anti-depression activity. Initially, it was thought that this activity might be related to inhibition of MAO by hypericin or a naturally occurring contaminant. However, the results from more recent fractionation studies indicate that hypericin has virtually no MAO-inhibiting potency. Moreover, the MAO-inhibiting potency of Hypericum extracts is probably insufficient to explain the psychotropic activity
of these extracts. Based on the currently available information, there is no mechanistic explanation for the presumed psychotropic activity of Hypericum (extract). It is impossible to attribute this activity of Hypericum (extract) to any of its particular constituents and certainly not to hypericin.

3. From various studies there is ample evidence that Hypericum induces enhanced photosensitivity, both in animals and in humans. This effect can be attributed to the generation of reactive oxygen species by light-excited hypericin. Single oral dose levels up to 4.4 mg hypericin, equivalent to 62 μg/kg bw for a 70 kg person do not seem to induce enhanced photosensitivity in humans in clinical studies. A single dose level of 124 μg/kg bw did not result in induction of enhanced photosensitivity in calves. Effect dose levels in animals are 372 μg/kg bw for calves and 30 to 60 mg in rats (equal to ca. 170 or 330 mg/kg bw for a rat of 180 g).

In humans a LOAEL for induction of enhanced photosensitivity was observed after 15 daily doses of 2.2 mg hypericin/d, equivalent to 31 μg/kg bw/d, indicating that prolonged exposure to hypericin or St. John’s wort extracts may well induce enhancement of photosensitivity. The dose levels mentioned above were not reported to give other side effects in humans.

4. A report of a meta-analysis of studies in which patients were treated for mild forms of mental depression with Hypericum extracts providing dose levels of 0.45 to 2.7 mg hypericin/d (approx. 6.4 to 38.6 μg/kg bw/d) for 4 to 12 weeks, mentioned that in a number of patients side effects were observed. Adverse drug reactions were also mentioned by other authors but from the primary literature sources it was not possible to derive a NOAEL for these effects.

5. Limited conventional genotoxicity studies with hypericin or Hypericum extract provided only negative results. An in vitro chromosomal aberration test is not available. For one in vivo micronucleus test with hypericin, a positive result was reported, but the relevance of this observation could not be properly assessed because of limitations in the reporting, and the absence of a dose-response relationship.

6. In vitro, hypericin may elicit micronuclei in combination with UVA/UVB irradiation, but only at levels at which cytotoxicity is seen as well.

7. Data on chronic toxicity and carcinogenicity are not available. Reproductive toxicity studies are limited up to now to a pilot study for neurobehavioural developmental toxicity in mice, in which indications were obtained that Hypericum may elicit adverse effects on foetal physical development at a dose level of 136 mg/kg bw/d, but no further study details were available.

8. In humans indications were obtained that Hypericum extract may increase the activity of at least one form of cytochrome P450 biotransformation enzyme (CYP 3A4), even after a single dose. Inhibition of at least 5 (human) forms (CYP 1A2, 2C19, 2D6, 3A4) has been demonstrated in vitro. There is evidence that these effects may result in interaction with the kinetics and/or pharmacological activities of other drugs.

There is a distinctive difference between the biological activity of hypericin, which is mainly associated with induction of enhanced photosensitivity and Hypericum (extract), which next
to a potential to induce enhanced photosensitivity, may also have psychotropic activity. The latter effect is not clearly related to hypericin. It is not clear how flavouring preparations derived from this plant species should be evaluated. Hypericin may be used as a marker substance, but the concentration of hypericin in *Hypericum* is not constant. Furthermore, the biological/toxicological activity of hypericin is at best only partly known, and is not representative for the total biological activity of *Hypericum* extract.

It is concluded that the database is too limited to allow an adequate safety assessment. This conclusion is based on the following considerations:

1) The NOAELs for induction of enhanced photosensitivity in humans and animals after single dosing are 62 and 124 μg/kg bw, respectively. Upon repeated dosing, induction of enhanced photosensitivity in humans is seen at lower dose levels (31 μg/kg bw),

2) The observation that after repeated dosing the LOAEL for the induction of enhanced photosensitivity is lower than the NOAEL for this effect is most likely related to the rather long plasma half-life of hypericin, ranging from 24 to 48 h. The slow elimination of hypericin stresses the need for appropriate (sub-)chronic studies into possible toxic, including neurotoxic effects.

3) In humans psychotropic activity of *Hypericum* extracts may have been observed at dose levels corresponding to approx. 6.4 to 38.6 μg (total) hypericin/kg bw/d for 4 to 12 weeks, without any indication that hypericin is responsible for the effect, while in some persons adverse effects were reported.

4) There is virtually no information on biotransformation, excretion or toxicity.

5) With respect to genotoxicity, only limited data are available. Some of these indicate that hypericin might have a genotoxic potential. For *Hypericum*, only negative results are available. Because of the limitations in the database the genotoxicity of hypericin or *Hypericum* cannot be adequately evaluated.

### Risk Characterisation

The variable composition of *Hypericum* extracts and the variability of the hypericin content in these prohibit to assess the safety of the use of *Hypericum* extracts on the basis of their hypericin content alone, because of the pharmacological/toxicological activity of other components in the extracts. Moreover, one effect of *Hypericum* (MAO inhibition) is not attributable to hypericin.

In the absence of adequate safety data, the Committee is of the opinion that derivation of an ADI, or any other acceptable exposure level for hypericin or *Hypericum* extracts is not feasible.
At the estimated exposure of 8 µg hypericin/kg bw per day from alcoholic beverages or 25 µg/kg bw per day from herbal teas, undesirable effects (enhanced photosensitivity) cannot be excluded.

The Committee noted especially that in comparison with the intake from alcoholic beverages, voluntarily taken OTC preparations containing Hypericum may contribute to a considerable extent (up to about 40 µg/kg bw per day) to the total exposure to hypericin and other Hypericum plant constituents.

References


**Databases and keywords used**

TOXLINE + and MEDLINE from 1991 onwards
STN Registry and STN Chemlist
Keywords: 548-04-9, hyperic*