National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Risk assessment of herbal preparations containing St John's wort

RIVM report 2019-0115
L. de Wit | S. Jeurissen | W. Chen

National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Risk assessment of herbal preparations containing St John's wort

RIVM report 2019-0115

Colophon

DOI 10.21945/RIVM-2019-0115

L. de Wit (author), RIVM
S. Jeurissen (author), RIVM
W. Chen (author), RIVM

Contact:
Lianne de Wit
Voeding, Preventie en Zorg\Voedselveiligheid
Lianne.de.wit@rivm.nl

This investigation was performed by order, and for the account, of Ministry of Health, Welfare and Sport, within the framework of project 5.1.15.

Published by:
National Institute for Public Health and the Environment, RIVM
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

Risk assessment of herbal preparations containing St John's wort

People use herbal preparations (food supplements and herbal tea) with St John’s wort, amongst others to feel and sleep better. However, these herbal preparations can reduce the effect of medicines, or enhance their effect. These interactions can have serious health effects. Herbal preparations with St John’s wort, for example, reduce the effect of certain medicines prescribed for fungal or viral infections and for cancer (chemotherapy). The effect of certain consciousness-lowering agents, e.g. sedative medicines, and consciousness-stimulating agents, e.g. antidepressants, is actually enhanced.

The use of herbal preparations with St John’s wort may also pose health risks when used alone and not in combination with medicines. For example, the skin can be damaged faster (sunburn) if people sit in the sun after using St John’s wort. Other effects such as dizziness, diarrhea and anxiety have also been reported after the use of herbal preparations containing St. John's wort. It is not known what effects occur after people use these herbal preparations for a long time. There is also insufficient information available to determine whether the use of St John’s wort during pregnancy is safe for the unborn child. Moreover, the composition of herbal preparations containing St John’s wort can vary greatly, and it is often not known what exactly is in it. This makes it difficult to estimate the effects of a product. RIVM draws these conclusions based on a risk assessment on behalf of the Ministry of Health, Welfare and Sport (VWS).

RIVM advises consumers to be cautious with the use of herbal preparations containing St John’s wort, and to not use these products in combination with medicines. RIVM advises VWS to draft legislation on the use of St John’s wort in herbal preparations.

Keywords: Hypericum perforatum, dietary supplement, botanical, hypericin, hyperforin, herbal preparation

Publiekssamenvatting

Risicobeoordeling van kruidenpreparaten met sint-janskruid

Mensen gebruiken kruidenpreparaten (voedingssupplementen en kruidenthee) met sint-janskruid onder andere om zich beter te voelen en beter te kunnen slapen. Maar deze kruidenpreparaten kunnen de werking van geneesmiddelen verminderen, of juist versterken. Deze wisselwerkingen kunnen ernstige gezondheidseffecten hebben. Kruidenpreparaten met sint-janskruid verminderen bijvoorbeeld de werking van middelen die worden voorgeschreven bij schimmel- of virusinfecties en bij chemotherapie. De werking van bepaalde bewustzijns-verlagende geneesmiddelen, zoals kalmeringsmiddelen, en bewustzijns-stimulerende middelen, bijvoorbeeld antidepressiva, wordt versterkt.

Ook zonder de combinatie met geneesmiddelen kan het gebruik van kruidenpreparaten met sint-janskruid schadelijk zijn voor de gezondheid. Zo kan de huid sneller beschadigd raken (zonnebrand) als mensen na het gebruik van sint-janskruid in de zon gaan zitten. Ook worden andere effecten zoals duizeligheid, diarree en angst gemeld na het gebruik van kruidenpreparaten met sint-janskruid. Het is niet bekend welke effecten optreden als mensen deze kruidenpreparaten lang gebruiken. Ook is er onvoldoende informatie om te bepalen of het gebruik van sint-janskruid tijdens de zwangerschap veilig is voor het ongeboren kind. Bovendien verschilt de samenstelling van kruidenpreparaten met sint-janskruid sterk en is vaak niet bekend wat er precies in zit. Dit maakt het moeilijk om de effecten van een product in te schatten. Deze conclusies trekt het RIVM op basis van een risicobeoordeling, in opdracht van VWS.

Het RIVM adviseert consumenten om voorzichtig te zijn met het gebruik van kruidenpreparaten met sint-janskruid omdat deze schadelijk kunnen zijn voor de gezondheid. Daarom raadt het RIVM VWS aan om regelgeving over het gebruik van sint-janskruid in kruidenpreparaten op te stellen.

Kernwoorden: Hypericum perforatum, voedingssupplement, kruidenpreparaat, hypericine, hyperforine

Contents

Summary

Introduction

St John's wort (*Hypericum perforatum* L.) is used in food supplements and herbal teas (herbal preparations) that are marketed as mood enhancers and sleep aids, among other things. Hypericin, pseudohypericin and hyperforin are generally thought to be the most relevant constituents for the pharmacological effects of St John's wort (Linde, 2009).

St John's wort can cause interactions with several prescribed medicines, as described in a previous RIVM report (Tiesjema et al., 2013). These interactions can have serious health effects. Herbal preparations with St John's wort, for example, reduce the effect of some medicines prescribed for fungal or viral infections and for cancer (chemotherapy). They also reduce the effect of several medicines that suppress the immune system, and are used with tissue transplants. The effect of certain antidepressants (selective serotonin reuptake inhibitors, SSRIs) may be enhanced, which could result in serotonin syndrome. Other aspects of the safety of herbal preparations containing St John's wort were not addressed in that report.

Currently, there are no specific restrictions for the use of St John's wort in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act. However, a warning on all products containing St John's wort about possible interactions with medicines will be made a legal requirement.[1]

The Ministry of Health, Welfare and Sport (VWS) asked RIVM to perform a risk assessment on the safety of herbal preparations containing St John's wort to investigate whether other restrictions on the use of St John's wort in herbal preparations are needed besides warning phrases about interactions with medicines.

Use of St John's wort as a herbal medicine

St John's wort is also used in registered herbal medicines to treat mild to moderate depression (Dutch Medicines Evaluation Board (MEB), 2020). Two medicinal products containing St John's wort are registered in the Netherlands (reference date July 2020). However, only one is still available on the market and can be bought over the counter in pharmacies. This medicinal product has a recommended daily dose of 132 mg dried ethanol (68% v/v) extract, equivalent to 850 – 2600 mg fresh plant. Assuming that the extract contains 0.1 – 0.3% hypericins, this is equivalent to a daily dose of approximately 0.13 – 0.40 mg hypericins per day. In addition, 30 homeopathic products containing St. John's wort are registered in the Netherlands. Of the homeopathic medicines, 7 contain *H. perforatum* L. as the only active ingredient and the others contain one or more other active ingredients in addition to St. John's wort (MEB, 2020). St John's wort products currently have a dual

[1] https://www.rijksoverheid.nl/documenten/kamerstukken/2018/02/12/beantwoording-kamervragen-over-de-uitzending-van-radar-over-sint-janskruid

legal status, since they can be on the market as food supplements and as registered medicines.

Previous evaluations
St John's wort has previously been assessed by the Scientific Committee on Food (SCF) and the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA). In 2002, SCF evaluated St John's wort for use as a flavouring agent in alcoholic beverages and concluded that in the absence of adequate safety data, the derivation of an acceptable daily intake (ADI) or any other acceptable exposure level for hypericin or St John's wort extracts is not feasible (SCF, 2002).
In 2009, EMA published assessments of St John's wort (*H. perforatum* L., Herba; EMA, 2009a-c). The recommended daily dose of St John's wort extract for 'well-established use' is between 500 mg and 1,800 mg (EMA, 2009c). EMA concluded that the few data available on acute and subchronic toxicity do not reveal signs of a risk to the patient. Herbal preparations containing *H. perforatum* L. were considered as safe when administered in the proposed dosage. Oral use during pregnancy and lactation is not recommended though (EMA, 2009a).

St John's wort products on the Dutch market
A wide range of food supplements containing St John's wort can be found on the Dutch market. Details on the composition of these food supplements are often lacking and the composition can vary greatly. The recommended doses (for adults) for these products range from 174 – 975 mg St John's wort extract/day. The extracts with a specified hypericin content contained 0.3% hypericin according to their product information. In addition, several herbal teas containing St John's wort are being sold on the Dutch market. No information about the recommended doses and levels of hypericin and other active constituents could be found in the descriptions on Dutch websites.

Exposure assessment
Based on the recommended doses levels for food supplements, exposure to hypericin for adults ranges from 0.1 to 2.9 mg per day (i.e. 1.4–41 µg/kg bw per day for a 70 kg person). Additional exposure may result from using medicinal products with St John's wort (about 0.13 – 0.40 mg hypericin per day, approximately 1.9 – 5.7 µg/kg bw for a 70 kg person) and alcoholic beverages with hypericin as a flavouring substance (estimated exposure 0.048 mg hypericin per day) (MEB, 2020; SCF, 2002). No exposure estimate can be made for the herbal teas containing St John's wort available on the Dutch market because information on their hypericin content is lacking. SCF (2002) estimated a hypericin exposure of 1.5 mg per day (i.e. 21 µg/kg bw per day for a 70 kg person) based on data from one herbal tea manufacturer.

No exposure information is available for hyperforin or flavonoids.

Biological data
- The estimated bioavailability of St John's wort constituents is generally low: ~10% for hypericin, a comparable or higher bioavailability for hyperforin, and ~20–30% for pseudohypericin (Kerb et al., 1996; Biber et al., 1998). Plasma half-life is long for hypericin (approximately 25 hours), highly variable for

pseudohypericin (6-42 hours), and shorter for hyperforin (approximately 9 hours) (Staffeldt et al., 1994; Kerb et al., 1996; Biber et al., 1998). Steady-state plasma levels were achieved after 4-7 days for hypericin and 4 days for pseudohypericin (Staffeldt et al., 1994; Kerb et al., 1996). Limited information is available about the biotransformation, distribution and excretion.

- The acute oral toxicity of St John's wort extract in rodents is low with median lethal doses ≥ 5,000 mg/kg bw (EMA, 2009a).
- Limited short-term and sub-chronic toxicity data in rodents are available. Effects on body weight, liver and kidney were reported after exposure to hyperforin, St John's wort plant material and/or St John's wort extract (Negreş et al., 2016; Garret et al., 1982, as cited in SCF, 2002; Leuschner, 1996, as cited in EMA, 2009a).
- Acute exposure to dried plant material in farm animals (sheep, calves and steers) resulted in phototoxicity (Bourke & White, 2004; Bourke, 2000, 2003; Araya and Ford, 1981). Short-term exposure to dried plant material in sheep resulted in phototoxicity (both dermal and ocular effects), haemolytic anaemia and liver and kidney damage (Kako et al., 1993).
- No chronic toxicity and carcinogenicity data are available for St John's wort or its constituents.
- Based on the genotoxicity data available, it can be concluded that hypericin is not genotoxic, whereas hypericin irradiated with UV may cause genotoxicity. Due to limitations in the available data, it is not possible to adequately evaluate the genotoxicity of St John's wort extract.
- The available data indicate that there is a possibility of reproductive, foetal and offspring toxicity when St John's wort extract is used during pregnancy and lactation. However, no studies of reproductive toxicity or developmental toxicity performed according to international guidelines are available so no firm conclusion can be derived.
- St John's wort (extracts) can cause phototoxicity (adverse skin reactions) and a lowest-observed-adverse-effect-level (LOAEL) of 31 µg/kg bw per day was identified in humans (SCF, 2002). In addition, in vitro studies and a study in sheep showed ocular phototoxicity. It is not yet known how relevant these findings are for the human situation.
- Indicative of the occurrence of adverse effects are also the case reports of adverse effects described in literature and received by Lareb (2018) in which amongst others dizziness, diarrhoea, skin reactions and psychiatric symptoms were mentioned after use of herbal preparations containing St John's wort (Lareb, 2018).

No safe use level

Safety of a herbal preparation can be presumed when "available data would allow concluding that exposure to known levels of the botanical ingredient has occurred in large population groups for many years without reported adverse effects" (EFSA, 2009). Since it is already established that herbal preparations containing St John's wort can cause serious interactions with medicinal products at recommended dose levels, the presumption of safety does not apply in this case (Tiesjema et al., 2013).

It is not possible to establish a health-based guidance value (HBGV) for St John's wort preparations or for its main constituents, (pseudo)hypericin and hyperforin. The genotoxicity of St John's wort extract cannot be adequately addressed. No studies of reproductive and developmental toxicity performed according to international guidelines are available. The short-term studies on St John's wort do not allow a NOAEL to be derived. No chronic toxicity or carcinogenicity data are available for St John's wort. The clinical studies cannot be used as a basis for an HBGV, because not all aspects of toxicity were investigated in these studies. Since no HBGV could be established, no safe use level for food supplements containing St John's wort can be determined.

In humans, a LOAEL of 31 µg hypericin/kg bw per day was identified by SCF (2002) for enhanced photosensitivity after repeated dosing. This LOAEL can be used for assessing photosensitivity, but it cannot be used as a basis to derive a safe use level due to the limited data available and unresolved concerns on several endpoints.

Risk assessment

Based on the reported hypericin content of some food supplements containing St John's wort that are available in the Netherlands, the estimated exposure to hypericin by users ranges from 1.4 to 41 µg/kg bw per day for a 70 kg person. The estimated exposure exceeds the dose of 31 µg hypericin/kg bw at which enhanced phototoxicity was observed in humans. This indicates that phototoxicity can occur when using food supplements with St John's wort.

In addition, there are indications for genotoxicity and reproductive and developmental toxicity and chronic toxicity/carcinogenicity data are lacking for St John's wort and its constituents. Owing to omissions in the toxicological data, no firm conclusions can be drawn on these aspects.

Indicative of the occurrence of adverse effects are also the reports of adverse effects received by Lareb in which dizziness, diarrhoea, skin reactions and psychiatric symptoms were mentioned (Lareb, 2018).

Furthermore, the estimated exposure to hypericin is around or higher than the therapeutic dose of 0.13 – 0.40 mg hypericins per day (approximately 1.9 – 5.7 µg/kg bw for a 70 kg person) for the single registered medicinal product currently available in the Netherlands, indicating that a pharmacological effect can be expected for these food supplements.

The same concerns may apply to herbal teas containing St John's wort. In 2002, SCF estimated the daily exposure to hypericin by consuming these teas to be in the same range as for food supplements and medicinal products. However, more information on the hypericin content of teas containing St John's wort currently on the market would be needed for a more reliable exposure estimate.

Another concern is possible contamination of St John's wort preparations with pyrrolizidine alkaloids (genotoxic carcinogens) during the harvesting of the flower tops of St John's wort as was recently the case in the Netherlands (NVWA, 2019). According to the Dutch Herbal

Preparations Decree, herbal preparations may maximally contain 1 µg/kg toxic pyrrolizidine alkaloids. In time, this will be overruled by the amendment of Regulation (EC) 1881/2006 on contaminants (EC, 2006), which will specify maximum levels of pyrrolizidine alkaloids in several products, including herbal preparations.

Conclusions and recommendations

Food supplements

The use of food supplements containing St John's wort can cause adverse effects because:

- serious drug interactions with a wide variety of human medicinal products can occur at recommended dose levels;
- the estimated exposure to hypericin could result in enhanced photosensitivity in humans;
- a pharmacological effect can be expected for these food supplements with doses around or higher than the therapeutic dose of St John's wort;
- case reports of adverse events associated with oral use of St John's wort products at recommended use levels are described in the literature and reported by Lareb.

In addition, there are indications for genotoxicity and reproductive and developmental toxicity. Chronic toxicity/carcinogenicity data are lacking. Owing to omissions in the toxicological data of St John's wort and its constituents, no firm conclusions can be drawn on these aspects.

Details on the composition of these food supplements are often lacking and the composition can vary greatly. Therefore, the precise effects are difficult to determine.

Herbal tea

For herbal teas made from St John's wort the same concerns apply. More information on the hypericin content of teas made from St John's wort currently on the market would be needed for a more reliable exposure estimate and to draw more firm conclusions.

Given these concerns, RIVM advises consumers to be cautious with the use of herbal preparations containing St John's wort, and to not use these supplements and herbal teas in combination with medicines. RIVM considers that these concerns cannot be covered with obliging warning phrases. Therefore, RIVM advises VWS to consider to restrict the use of St John's wort in herbal preparations by law. Also, it is advised to consider which St John's wort product should be regarded as medicines and consequently would require a premarket assessment on safety, efficacy and quality.

1 Introduction

1.1 Background

St John's wort (*Hypericum perforatum* L.)[2] is used in food supplements and herbal teas (herbal preparations) that are marketed as mood enhancers and sleep aids, among other things. St John's wort is also used in registered herbal medicines to treat mild to moderate depression (Dutch Medicines Evaluation Board (MEB), 2020).

Herbal preparations containing St John's wort can cause interactions with several prescribed medicines, as described in a previous RIVM report (Tiesjema et al., 2013). They reduce the efficacy of a number of medicines prescribed for the treatment of fungal and viral infections and cancer (chemotherapy) and medicines used to suppress the immune system (in tissue transplants). In contrast, it can also strengthen (unintendedly) the effectiveness of a number of prescribed sedatives. The severity of these side effects depends on the dose of the drug as well as that of the dietary herbal supplement. The RIVM report concluded that there should be precautionary advice not to use herbal preparations containing St John's wort in combination with these prescribed medicines. In addition, it was considered important to inform consumers, physicians and pharmacists of the potentially harmful effects of the drug interactions (Tiesjema et al., 2013). Other aspects of the safety of herbal preparations containing St John's wort were not addressed at that time.

Currently, there are no specific restrictions for the use of St John's wort in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act. However, a warning on all products containing St John's wort about possible interactions with medicines will be made a legal requirement.[3]

The Ministry of Health, Welfare and Sport (VWS) asked RIVM to perform a risk assessment on herbal preparations containing St John's wort and to investigate whether other restrictions on the use of St John's wort in herbal preparations are needed besides warning phrases about interaction with medicines.

1.2 Information on existing assessments

1.2.1 *Scientific Committee on Food*

In 2002, the Scientific Committee on Food (SCF) published an opinion on the presence of hypericin and extracts of *Hypericum sp.* in flavourings and other food ingredients with flavouring properties (SCF, 2002). SCF concluded that in the absence of adequate safety data, the derivation of an acceptable daily intake (ADI) or any other acceptable exposure level for hypericin or *Hypericum* extracts is not feasible. Their conclusion was based on the following considerations (SCF, 2002):

[2] The names St John's wort, *H. perforatum* and *Hypericum* are interchangeably used throughout the report.
[3] https://www.rijksoverheid.nl/documenten/kamerstukken/2018/02/12/beantwoording-kamervragen-over-de-uitzending-van-radar-over-sint-janskruid

1. *The no-observed-adverse-effect-levels (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 per day).*
2. *The observation that after repeated dosing the lowest-observed-adverse-exposure-level (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 hours. 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 approximately 6.4 to 38.6 µg (total) hypericin/kg bw per day 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.*

1.2.2 *European Medicines Agency*

In 2009, the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) published assessments of *H. perforatum* L., Herba (EMA, 2009a-c). The substance used in herbal preparations consists of the whole of or the cut, dried flowering tops of *H. perforatum* L., harvested during flowering. It contains not less than 0.08% of total hypericins (mainly hypericin, pseudohypericin, protohypericin, protopseudohypericin and cyclopseudohypericin), expressed as hypericin calculated with reference to the dried drug. The indications for 'well-established use'[4] are 'the treatment of mild to moderate depressive episodes' and 'the short-term treatment of symptoms in mild depressive disorders'. The recommended daily dose of *H. perforatum* L. extract for 'well-established use' is between 500 mg and 1,800 mg (EMA, 2009c).

The following overall conclusion on toxicology was drawn:
The few data available on acute and subchronic toxicity do not reveal signs of a risk to the patient. The weak positive outcome of tests on mutagenicity of ethanolic extracts can be explained with the presence of quercetin in the extracts. Numerous publications deal with the potential phototoxicity of hypericin and Hypericum extracts. Extracts exert less phototoxicity than pure hypericin. Considering the outcome of clinical tests on phototoxicity herbal preparations of H. perforatum can be considered as safe when administered in the proposed dosage. The data on reproductive toxicity are contradictory. Tests on reproductive toxicity

[4] Well established use: When an active ingredient of a medicine has been used for more than 10 years and its efficacy and safety have been well established. In such cases, application for marketing authorisation may be based on results from the scientific literature (https://www.ema.europa.eu/en/glossary/well-established-use; accessed June 2020).

demonstrated no differences between Hypericum extract (108 mg/kg) and placebo in mice. However, isolated hypericin seems to have teratogenic properties. For safety reasons the oral use of Hypericum during pregnancy and lactation should not be recommended (EMA, 2009a).

H. perforatum L. is also used in veterinary medicine. The Committee for Veterinary Medicinal Products (CVMP) evaluated the topical use of Hyperici oleum, the oily extract of the flowers of *H. perforatum* L., in all food-producing species. The CVMP concluded that there was no need to establish a maximum residue level (MRL) (CVMP, 1998). In 1999, the use of *H. perforatum* L. in veterinary homeopathy was evaluated. Again, the CVMP concluded that there was no need to establish an MRL for *H. perforatum* L. for this application (CVMP, 1999).

1.2.3 *RIVM*

RIVM has published a report on interactions between herbal preparations containing St John's wort and medicinal products (Tiesjema et al., 2013; see Section 5.3.8 [Interactions] for details). No risk assessment of herbal preparations containing St John's wort has previously been undertaken by RIVM.

1.3 Information on existing legislation

The Herbal Preparations Decree of the Dutch Commodities Act prohibits to place on the market any herbal preparation that contains herbal substances in amounts that are detrimental to health[5]. It also contains a list of botanicals and botanical ingredients that are not allowed, or have maximum levels, in plant food supplements and other herbal preparations. *Hypericum perforatum* L. is not yet included in the Decree. However, a warning on all products containing *H. perforatum* L. about possible interactions with medicines will be made a legal requirement.[6]

Two medicinal products containing *H. perforatum* L. are registered in the Netherlands (reference date July 2020). These are A. Vogel Hyperiforce tablets (traditional herbal medicine, RVG 104186) and Laif 900 tablets (medicine, RVG 103963). However, only A. Vogel Hyperiforce is still available on the market and can be bought over-the-counter from a pharmacy. In addition, 30 homeopathic products containing *H. perforatum* L. are registered in the Netherlands. Of the homeopathic medicines, 7 contain *H. perforatum* L. as the only active ingredient and the others contain one or more other active ingredients in addition to *H. perforatum* (CBG, 2020). Products with *H. perforatum* L. currently have a dual legal status, since they can be on the market as food supplements and as registered medicines.

In Belgium *Hypericum perforatum* L. is included in the list of plants that should be notified, if in predosed form, before being placed on the market (list 3 of the Royal Decree on the manufacture and trade of foods composed of or containing plants or plant preparations). Notification applies to the use of the aboveground parts of *H.*

[5] https://wetten.overheid.nl/BWBR0012174/2014-12-13
[6] https://www.rijksoverheid.nl/documenten/kamerstukken/2018/02/12/beantwoording-kamervragen-over-de-uitzending-van-radar-over-sint-janskruid

perforatum, and the recommended daily dose may not lead to an intake of hypericin of more than 700 µg. Every batch must be analysed and the results made available. Every package must contain a notice stating that the user's doctor or pharmacist must be informed in cases of concomitant use of medicinal products (Koninklijk Besluit, 1997).

In Denmark, *H. perforatum* L. is included in the Drogelisten. As an indicative daily dose, 100 mg herb, corresponding to 0.1 mg total hypericin (unspecified), is mentioned. This does not mean that higher dosages are unsafe per se but these have not been evaluated. A warning about photosensibilization is also mentioned (Gry et al., 2011).

In Germany, *H. perforatum* L. is included in a list of 'Substances for which restricted use in foods is recommended'. This is because *H. perforatum* is known as both a food and a (traditional) medicinal product with a pharmacological effect demonstrated on the basis of clinical data. The pharmacological effective dose is 2–4 g drug (dried flowering tops or aerial parts of *H. perforatum* L.; WHO, 2004) per day, above which it is considered a medicinal product by function. If no significant pharmacological effect can be established, the herb may be used in a food supplement (Bundesamt, 2014).

In Annex IV of Regulation (EC) 1334/2008 on the use of flavourings in food, it is stated that flavourings and food ingredients with flavouring properties produced from *H. perforatum* L. may be used only for the production of alcoholic beverages (EC, 2008).

1.4 Reading guide

Chapter 2 describes the method used for the literature search and the process of selection of relevant articles. Chapter 3 gives a description of (the main constituents of) St John's wort extracts and an overview of the products available on the Dutch market. On the basis of this information, Chapter 4 describes the exposure resulting from the use of herbal preparations containing St John's wort as well as from other sources. Chapter 5 contains the available toxicokinetic and toxicological data about St John's wort and its main constituents (hypericins and hyperforin). The risk assessment for food supplements containing St John's wort can be found in Chapter 6, including a description of sensitive/vulnerable groups and uncertainties. Finally, Chapter 7 gives the conclusion and recommendations.

2 Methodology

The risk assessment for herbal preparations containing St John's wort was conducted using the recently developed template for the safety assessment of plant food supplements as a basis (De Wit et al., 2019).

A search strategy was developed to capture relevant literature for the risk assessment of herbal preparations containing St John's wort. To this end, search terms were formulated to describe the herb of interest, including its main constituents, to identify references describing toxicity or adverse outcomes and to include animal data as well as human data (see Annex 1). Four databases, Embase, Pubmed, Scopus and Toxcenter, were searched up to November 2018. In total, 2,778 unique references were obtained. In addition, the grey literature was searched using the internet for assessments of St John's wort by other organizations, for example EMA and SCF. In addition, information about the composition and use of St John's wort was obtained via a search of the grey literature, e.g. using the European Pharmacopeia.
The relevance of the references obtained was judged from the title/abstract. The following were excluded:

- Studies solely about beneficial effects;
- Genotoxicity studies using yeasts, algae and/or plants;
- *In vitro* studies in animal/human cell lines other than studies investigating kinetics, genotoxicity or phototoxicity;
- Studies solely about the interactions of herbal preparations containing St John's wort, as interactions are described in a previous report by the RIVM (Tiesjema et al., 2013) and the aim of the current report was to investigate whether other restrictions on the use of St John's wort in herbal preparations should be required besides a warning about interactions with medicines.

Lists of relevant articles as well as of previous evaluations were used to check that no other relevant references had been missed in the search. As St John's wort had been evaluated before by SCF (2002) and EMA (2009a), these reports were used as the starting point for the current assessment. The summaries and conclusions of these reports were used and, where necessary, supplemented by additional information from the original publications.

3 Identification and characterization

3.1 Identity and nature of the source material

H. perforatum L. is a perennial plant and belongs to the family of Hypericaceae (synonym Guttiferae) (Mullaicharam & Halligudi, 2018). In the European Pharmacopeia the basic use material of St John's wort is defined as Hyperici herba, with the definition 'whole or fragmented, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time' with a minimum content of 0.08% of total hypericins (hypericin+pseudohypericin), expressed as hypericin (dried drug) (European Pharmacopoeia, 2017). According to the WHO monograph, Hyperici herba 'consists of the dried flowering tops or aerial parts of *Hypericum perforatum* L. (Clusiaceae)' (WHO, 2004). Table 3.1 lists the classification of *H. performatum* L..

Table 3.1 Information related to the classification of St John's wort.

Scientific (Latin) names	Family: *Hypericaceae/Clusiaceae/ Guttiferae* Genus: *Hypericum* L. Species: *Hypericum perforatum L.*
Synonyms	*Hypericum officinarum* Crantz, *Hypericum vulgare* Lam, *Hypericum officinale* Gater ex. Steud
Common name	St John's wort
Parts used	Above-ground parts
Geographical origin	Indigenous to Europe, Africa, South America, Asia, Australia, New Zealand. Naturalized in the United States
Growth and harvesting conditions	Harvested when flowering

Source: European Pharmacopoeia (2017); WHO (2004).

3.2 Manufacturing process

The flowering fresh plants or the dried aerial parts are typically used as raw material for the production of medicinal products and food supplements (known as Hyperici herba) (European Pharmacopoeia, 2017; WHO, 2004). Over the long application history of St John's wort various manufacturing methods have been used (Linde, 2009). The manufacturing methods are illustrated in Figure 3.1.

The quantified dry extract of St John's wort as defined by the European Pharmacopoeia is produced from the raw material by a procedure using ethanol (50–80% V/V) or methanol (50–80% V/V) (European Pharmacopoeia, 2017).

Detailed information on the manufacturing methods used for herbal preparations on the Dutch market is not available.

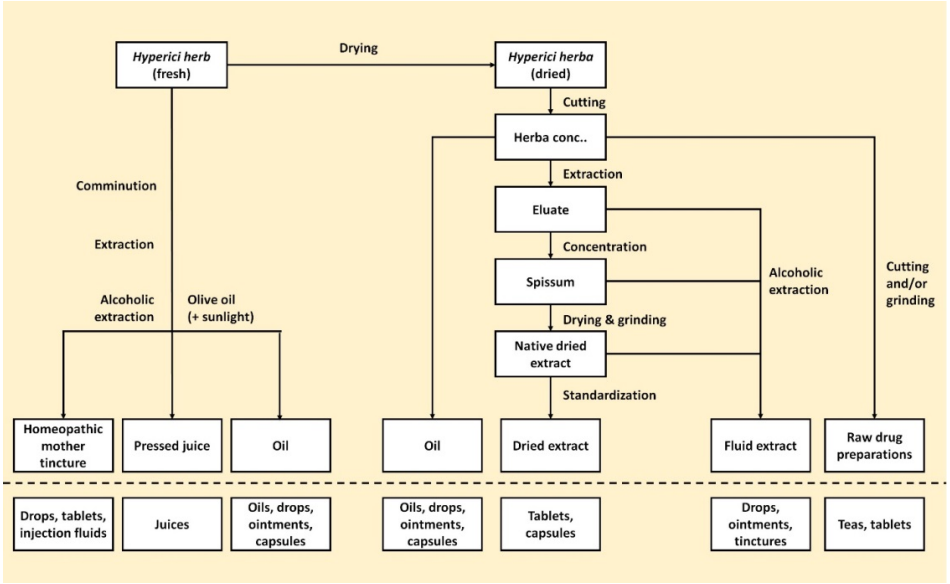


Figure 3.1 Manufacturing flow chart of St John's wort (adapted from Linde, 2009).

3.3 Chemical composition

More than 150 ingredients or groups of ingredients have been identified in *Hypericum* extracts (Linde, 2009). Table 3.2 lists some bioactive ingredients. Hypericin, pseudohypericin and hyperforin are generally thought to be the most relevant constituents for the pharmacological effects of St John's wort; however, this remains under debate (Linde, 2009). The current risk assessment focussed mainly on hypericins and hyperforin.

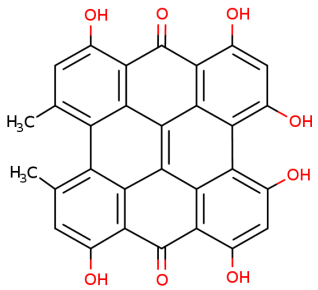
Table 3.2. Biologically active compounds found in St John's wort.

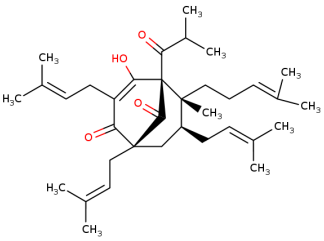
Component group	Examples	Plant parts
Naphthodianthrones	Hypericin Pseudohypericin	Flowers, buds
Phloroglucinols	Hyperforin Adhyperforin	Flowers, buds
Flavonoids	Rutoside Quercetin Hyperoside Quercitrin Isoquercitrin Rutin	Leaves, stalk, buds
Biflavonoids	Biapigenin Amentoflavone	Flowers
Procyanidins	Procyanidin Catechin Picatechin	Aerial parts, flowers, buds
Essential oil	Terpenes Alcohols	Flowers, leaves
Amino acids	GABA	Flowers, leaves
Phenylpropanes	Caffeic acid Chlorogenic acid	Flowers, leaves
Xanthones	Norathyiol	Roots, flowers

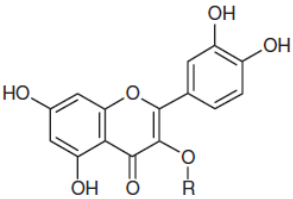
Source: Linde (2009)

According to the European Pharmacopoeia, the quantified dry extract should contain 0.10–0.30% total hypericins (hypericin + pseudohypericin) expressed as hypericin, minimum 6.0% flavonoids expressed as rutoside, and maximally 6.0% hyperforin and not more than the content stated on the label (European Pharmacopoeia, 2017).
Table 3.3 shows the structural formulas for these (groups of) substances.

Table 3.3 Chemical structures of hypericin and hyperforin.

Hypericin		
Synonyms	Hypericum red; 4,5,7,4',5',7'-hexahydro-2,2'-dimethyl-mesonaphthodianthron	
Systematic name	1,3,4,6,8,13-hexahydroxy-10,11-dimethylphenanthro[1,10,9,8-opqra]perylene-7,14-dione	
Molecular weight	504.448 g/mol	
CAS	548-04-9	

Hyperforin		
Synonyms	Hiperforina Hyperforine	
Systematic name	4-Hydroxy-6-methyl-1,3,7-tris(3-methyl-2-butenyl)-5-(2-methyl-1-oxopropyl)-6-(4-methyl-3-pentenyl) bicyclo(3.3.1)non-3-ene-2,9-dione	
Molecular weight	536.7918 g/mol	
CAS	11079-53-1	

Flavonoids and glycosides		
Examples	Quercetin (R = H) Hyperoside (R = β-D-galactoside) Quercitrin (R= α-L-rhamnosyl) Isoquercitrin (R = β-D-glycosyl) Rutin (R = β-D-rutinosyl)	

Source: ChemIDplus; Butterweck & Schmidt (2007)

It is unknown to what extent the extracts used in food supplements on the Dutch market are in compliance with this definition. Table 3.4 lists the content of total hypericins, hyperforin and in some cases flavonoids in extracts used in clinical studies evaluated by EMA (2009a). The values reported are in general in compliance with the European Pharmacopoeia.

Table 3.4 Overview of St John's wort extracts tested in clinical studies reported by EMA (2009a).

Extract	**Extraction solvent**	**DER***	**Total hypericins (unspecified)**	**Hyperforin**	**Flavonoids**	**Daily dose**
Calmigen	-	-	0.3%	-	-	-
Esbericum	60% ethanol	2–5.5:1	0.1%	-	-	-
HYP611	60% ethanol	3.5–6:1	0.18%	2.22%	-	650 mg
Hyperforat drops	50% ethanol	0.5:1	2 mg/ml	-	-	-
Hyperiforce	-	4–5:1 (shoot tips)	0.5%	-	-	-
LI 160 (Jarsin®)	80% methanol	3–6:1, initially 4–7:1	0.12–0.28%	Approx. 4.5%	Approx. 8.3%	900 mg
LoHyp-57	60% ethanol	5–7:1	0.2–0.3%	2–3%	-	800 mg
STEI 300	60% ethanol	5–7:1	0.2–0.3%	2–3%	-	1,050 mg
STW-3	50% ethanol	5–8:1	Mean 0.2%	Mean 2%	Mean 9%	612 mg
STW3-VI (Laif®)	80% ethanol	3–6:1	Mean 0.26%	Mean 3.0%	Mean 7.17%	900 mg
WS 5570	80% methanol	3–7:1	0.12–0.28%	3–6%	≥6.0%	600–1,800 mg
WS 5572	60% ethanol	2.5–5:1	-	1.5–5%	-	600–1,200 mg
WS 5573	60% ethanol	-	-	0.5%	-	-
Ze 117	50% ethanol or ethanol 49%:2-propanol (97.3:2.7)	4–7:1	0.2%	Nearly free of hyperforin	-	500 mg

* Drug extract ratio (DER): the ratio between the quantity of herbal substance used in the manufacture of a herbal preparation and the quantity of herbal preparation obtained (EMA, 2010). For instance, a DER of 2–5.5:1 means that 2–5.5 g of fresh herb is used to prepare 1 g of herbal preparation.

St John's wort does not naturally contain pyrrolizidine alkaloids (PAs), but PAs have been detected in several herbal preparations containing St John's wort. Most likely, this is due to contamination with other PA-containing plants (Mulder et al., 2015; Letsyo et al., 2017). In spring 2019, a public warning was given by the Netherlands Food and Consumer Product Safety Authority (NVWA) for six food supplements containing St John's wort that were available on the Dutch market because of the presence of high levels of PAs (NVWA, 2019a, b).

3.4 Stability

When St John's wort extract (methanolic extract (5%) in aqueous solution) was stored under light for 24 hours, levels of its active marker components decreased drastically and hyperforin, adhyperforin and protopseudohypericin completely disappeared. Storage in the dark also led to a gradual decrease of the levels of the active marker components. The instability was more severe when the pH of the solution was low (2.5–2.8) than at higher pH (4.5–6.1). The major degradation products of hyperforin in acidic aqueous solution included furohyperforin, furohyperforin hydroperoxide and furohyperforin isomer a (Ang et al., 2004).

The long-term stability of the active constituents of St John's wort at 25 °C in a primary pack (hard gelatine capsule impenetrable to light) was investigated by Bilia et al. (2001). The time point where 90% of active constituent (hypericin) is left (t_{90}) was only a couple of weeks after the start of the storage and that of hyperforin 3 months. No information was given about the degradation products. Flavonol content was still 98% of the initial value at 3 months. Adding antioxidants to the product did not significantly increase the stability of hypericins, hyperforin or flavonols. Photodegradation testing showed that both hypericins and hyperforins are very unstable when exposed to light. Testing different coloured capsules revealed that white and light blue capsules containing 2% titanium dioxide are more protective of the hyperforin content, whereas dark blue capsules, containing 0.5% indigotin, are more protective of the hypericin content, and orange capsules, containing 0.65% of yellow iron oxides and 0.65% of red iron oxides, more protective of the flavonol content (Bilia et al., 2001).

3.5 Uses and use levels

A wide range of supplements containing St John's wort can be found on the Dutch market (based on an internet search on Dutch websites, June 2019)[7]. They have different dose levels and use levels. Some examples (anonymous) are provided in Table 3.5. For some supplements, the hypericin content is declared in the product information. Recent research from the NVWA however showed that from 22 products that were analysed for their hypericin content nine contained less than 50% of the declared hypericin content and six did not contain hypericin at all although the product information stated it did (NVWA, 2019b).
In addition, several herbal teas containing St John's wort are being sold on the Dutch market. No information about dose levels and recommended

[7] The initial search was performed in June 2019, and the information on the products listed in Table 3.5 was updated in June 2020.

daily use, e.g. maximum number of cups of tea, could be found in the descriptions on Dutch websites. Nor could any information on hypericin levels.
As mentioned above, there are two registered medicinal products in the Netherlands, of which only one is still available in pharmacies. Details of these two products are given in Table 3.6.

Table 3.5 Examples of food supplements containing St John's wort available on the Dutch market with recommended daily use and recommended dose.

Supplement	Indication	Ingredients	Recommended daily use	Total recommended daily dose
Supplement 1[a]	For a good state of mind. Relaxing. Promotes healthy, natural sleep. Beneficial for bile function. Supports healthy digestion.	St John's wort extract (0.3% hypericin)	1–3 x 1 capsule	300–900 mg extract 0.9–2.7 mg hypericin
Supplement 2[b]	Not reported	St John's wort	1 tablet	333 mg extract (4:1) 1 mg hypericin
Supplement 3[c]	Beneficial for good mental balance and is supportive in case of pressure and efforts. For healthy airways. Supports kidney function and digestion. Anti-aging.	St John's wort extract (0.3% hypericin)	2 x 1 capsule	600 mg extract 1.8 mg hypericin
Supplement 4[d]	Uplifting. For depression.	St John's wort extract (0.3% hypericin)	3 x 1 capsule	975 mg extract 2.9 mg hypericin
Supplement 5[e]	Beneficial for mood. For gloomy moods and irritability. Supports a good emotional balance	St John's wort extract	3 x 10 drops (adults) 2 x 5 drops (children)	174 mg extract 0.117 mg hypericin (adults) 58 mg extract 0.039 mg hypericin (children)
Supplement 6[f]	For people feeling gloomy, listless or depressed. Calming action is beneficial for symptoms of stress such as heart palpitations, hyperventilation, headache and insomnia.	St John's wort extract	1–2 capsules	300–600 mg extract
Supplement 7[g]	For inner unrest, irritability and gloomy moods. Supports mood and has a calming effect when you experience stress.	St John's wort extract	3 x 1 capsule*	900 mg extract

[a] https://www.vitaminesperpost.nl/sint-janskruid-extra-sterk (Accessed June 2020)
[b] https://www.lambertshealthcare.co.uk/herbs/other-herbs/st-johns-wort-oneaday/ (Accessed June 2020)
[c] https://www.vitortho.nl/product/sint-janskruid-extract-300-mg/(Accessed June 2020)
[d] https://www.new-care.nl/st-janskruid (Accessed June 2020)
[e] https://www.vitaminbottle.nl/sint-janskruid-druppels.html (Accessed June 2020)
[f] https://www.livinggreensshop.nl/?s=janskruid (Accessed June 2020)
[g] https://www.hollandandbarrett.nl/shop/product/nature-s-garden-sint-janskruid-300mg-60012492?skuid=012492&gclid=EAIaIQobChMIteL0gO_y4gIVibTtCh3Ztgz2EAQYAiABEgLgOPD_BwE (Accessed June 2020)
* Can also be used as tea by opening the capsule and dissolving the contents in boiling water.

Table 3.6 Medicinal products containing St John's wort registered in the Netherlands with daily use and dose.

	Indication	**Ingredients**	**Recommended daily use**	**Total recommended daily dose**
A. Vogel Hyperiforce	To relieve temporary nervous tension and complaints of depression	Dry extract of St John's wort (68% ethanol v/v)	2 x 1 tablet	66 mg dry extract
Laif900*	Mild to moderate depression	Dry extract of St John's wort (DER 3–6:1, 80% ethanol v/v)	1 x 1 tablet	900 mg dry extract

* No longer available on the Dutch market.

4 Exposure: extent and duration

4.1 Exposure from food supplements

Based on the recommended use levels of the food supplements described in Table 3.5, exposure to hypericin for adults ranges from 0.1 to 2.9 mg per day (i.e. 1.4–41 µg/kg bw per day for a 70 kg person).

In 2014, RIVM performed a specific plant food supplement (PFS) consumption survey among 739 PFS users in 8 age and gender subgroups of the Dutch population (Jeurissen et al., 2018). Consumption of PFS containing St John's wort was reported 24 times, ranging from 1 consumer in the subgroup of children aged 1–8 years to 7 consumers in the subgroup of women aged 19–50 years.
In total, 22 different PFS containing St John's wort were reported to be used. For 6 PFS, information on the concentration of hypericin was available. These 6 PFS were used by adults only. The daily exposure resulting from the use of these supplements ranged from 0.45 to 2 mg hypericin per day (i.e. 6–29 µg/kg bw per day for a 70 kg person). Detailed information can be found in Annex 2.
Although only few data are available from the consumption survey, the estimated exposure to hypericin by respondents was within the range of the total recommended daily dose for the food supplements listed in Table 3.5.

No exposure information is available for hyperforin or the flavonoids.

It should be noted that the recommended and estimated exposure for some food supplements is equal to or higher than the recommended dose of 1 mg hypericin per day (approximately 17 µg/kg bw) for the single medicinal product containing St John's wort that is available in the Netherlands.

4.2 Possibility of additional human exposure

As well as being used in food supplements, St John's wort is used in medicinal products (see Table 3.6). Only one registered medicinal product containing St John's wort is available on the Dutch market. The recommended daily dose for this product is 132 mg dry extract. The hypericin content is however not specified. In Table 3.4 can be seen that comparable extracts contain about 0.1 – 0.3% hypericins. Assuming therefore that the dry extract contains about 0.1 – 0.3% hypericins, the recommended daily dose of the registered medicinal product is equivalent to 0.13 – 0.40 mg hypericins per day (approximately 1.9 – 5.7 µg/kg bw for a 70 kg person). If consumers use both food supplements and medicinal product containing St John's wort, their exposure will increase.

As mentioned in Chapter 1, flavourings and food ingredients with flavouring properties produced from St John's wort may also be used in the production of alcoholic beverages. SCF has estimated an exposure of 0.048 mg hypericin per day (for a person weighing 60 kg; 0.8 µg/kg bw per day) from its use as a flavouring substance in alcoholic beverages

(SCF, 2002). The estimated exposure to hypericin from the consumption of alcoholic beverages is about 10% of the lower end of the exposure resulting from the food supplements described in section 4.1 (6–29 µg/kg bw per day for a 70 kg person).

In addition, herbal teas containing St John's wort are sold on the Dutch market. However, as described in Chapter 3, no information about hypericin content or recommended use is available. Hence, no exposure assessment could be done for herbal teas. Previously, SCF (2002) estimated hypericin exposure from a herbal tea that was available on the Dutch market. Based on the assumption that a tea bag contains 2 g dried leaves of St John's wort, which provides a dose of approximately 250 µg hypericin per cup, and a recommended daily dose of 1–2 cups three times a day, they estimated a daily exposure of up to 1.5 mg hypericin (0.025 mg/kg bw per day for a person weighing 60 kg). The consumption of herbal tea may result in exposure to hypericin at levels that are comparable to exposure resulting from food supplement use. It may be assumed that people combine the use of these two products. If that is the case, their exposure to hypericin will increase.

No information on exposure to hyperforin or flavonoids resulting from the use of medicinal products, alcoholic beverages or herbal tea is available.

5 Biological data

5.1 Introduction

Section 5.2 describes the data on kinetics and section 5.3 on toxicity of St. John's wort (extract) and its constituents hypericins and hyperforin, and for some flavonoids. A summary of these data can be found in section 5.2.6 and 5.3.9, respectively.

5.2 Toxicokinetics

5.2.1 *Absorption*

In vitro

Sattler et al. (1997) studied the transport across, the binding to and the uptake in Caco-2 cell monolayers of hypericin (>93%). When hypericin was present as a cyclodextrin complex (thereby increasing its solubility), transport of hypericin across Caco-2 cell monolayers was measurable. Hypericin was found to bind to cell surface membranes and was found in the cell nucleus membrane, suggesting transport across the intestinal epithelium via passive transcellular diffusion.

Kamuhabwa et al. (1999) studied the *in vitro* transport and uptake of protohypericin and hypericin in Caco-2 cells at concentrations of 80 and 200 µM. Specific light conditions were used to prevent the photoconversion of protohypericin into hypericin and the photosensitization of the cells. Transport of both compounds from the apical to the basolateral side was very low and was reduced with increasing concentration. A lag time of about 2–3 hours was observed. Uptake of both compounds (4–8% of the original amount incubated) was observed to be saturable after 3 hours. Protohypericin and hypericin showed similar absorption characteristics.

Animal data

Different formulations of St John's wort extracts and consequences for pharmacokinetic parameters were investigated by Hatanaka et al. (2011) in male ICR mice. Mice received St John's wort extract or St John's wort as a nano-emulsion (St John's wort-NE) in a single oral dose of 5.2 mg/kg hyperforin, and blood samples were taken up to 6 hours post-dose. In addition, brain samples were collected for analysis of hyperforin concentrations. For determination of bioavailability, other mice received the St John's wort extracts intravenously. Oral administration of St John's wort-NE resulted in a statistically significantly higher Area under the Curve over the first 6 hours ($AUC_{0\text{-}6h}$) and maximum plasma concentration (C_{max}) values of hyperforin in plasma and brain than when 'normal' St John's wort extract was given. The bioavailability of hyperforin increased from 10% for St John's wort extract to 26% for St John's wort-NE.

Tablets containing 300 mg alcohol/water extract from St John's wort with a hyperforin content of 5% (WS 5572) or placebo were orally administered to male Sprague-Dawley rats (Biber et al., 1998). The rats (n=5) received a single dose of 300 mg/kg dissolved in 10 ml of the St John's wort extract or vehicle only, and blood samples were collected at

several time points up to 24 hours, where for each time point a separate group of animals was used. The mean maximum plasma concentration (C_{max}) was approximately 370 ng/ml. Other pharmacokinetic parameters could not be reliably determined due to limitations in the analytical method. However, based on the plasma concentration curve of orally given St John's wort extract, a multi-compartmental behaviour was assumed.

Fox et al. (2001) investigated the pharmacokinetics of hypericin in non-human primates after an intravenous dose of 2 mg/kg (n=3) or 5 mg/kg (n=1). Plasma and cerebrospinal fluid samples (CSF, see also Section 5.2.2) were obtained prior to and at several time points after administration. After administration of 2 mg/kg, the mean peak plasma concentration was 142±45 µM, the mean AUC value 646±146 µM*h and the mean clearance 6±2 ml/kg/h. The elimination in plasma was bi-exponential, with a terminal half-life of 26±14 h. Transient, severe photosensitivity rash occurred in the animal dosed with 5 mg/kg. Therefore, only limited plasma and CSF samples were obtained from this animal and no pharmacokinetic modelling could be performed.

Human data
In a randomized double-blind study by Staffeldt et al. (1994), 12 healthy male volunteers were given three different single doses (with a 2-week interval) of 300, 900 or 1,800 mg St John's wort extract (LI 160) via 1, 3 or 6 tablets containing 900 µg total hypericin per tablet (250 µg hypericin, 526 µg pseudohypericin) and placebo tablets (up to a total of 6 tablets) under fasting conditions. Blood samples were taken up to 120 hours after intake. In addition, in the second part of the study, the subjects were given 300 mg St John's wort extract (1 tablet) three times a day for 14 days, and blood samples were collected. The kinetic parameters obtained for hypericin are presented in Tables 5.1 and 5.3 for single and multiple dosing, respectively, and in Tables 5.2 and 5.3 for pseudohypericin.

Table 5.1 Kinetic parameters (median + range) of hypericin after different single oral doses of St. John's wort extract.

Parameter	**Dose extract (mg)/ hypericin (µg)**		
	300 / 250 (n=4)	900 / 750 (n=3)	1,800 / 1,500 (n=4)
C_{max} (ng/ml)	1.5 (1.0–2.0)	7.5 (6.1–12.5)	14.2 (8.6–19.3)
T_{max} (h)	5.2 (4.0–8.0)	4.1 (4.0–6.0)	5.9 (5.0–6.0)
$AUC_{0\text{-}72h}$ (ng/ml*min)	1,920 (978–2,720)	8,820 (8,740–11,890)	23,090 (12,790–29,020)
t½ (h)	24.8 (10.3–37.0)	26.0 (13.0–41.0)	26.5 (21.5–40.5)

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life

Table 5.2 Kinetic parameters (median + range) of pseudohypericin after different single oral doses of St John's wort extract.

Parameter	Dose extract (mg) / pseudohypericin (µg)		
	300 / 526 (n=4)	900 / 1,578 (n=3)	1,800 / 3,156 (n=4)
C_{max} (ng/ml)	2.7 (2.0–5.4)	11.7 (9.4–15.0)	30.6 (23.0–35.8)
T_{max} (h)	2.7 (2.0–3.5)	3.0 (3.0–3.5)	3.2 (2.0–4.0)
$AUC_{0\text{-}72h}$ (ng/ml*min)	1900 (1,150–2,600)	7,130 (4,800–9,870)	19,910 (15,420–23,740)
t½ (h)	16.3 (6.0–30.5)	36.0 (18.0–42.0)	22.8 (9.0–34.5)

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life

Table 5.3 Kinetic parameters (mean) of hypericin and pseudohypericin after multiple dosing with 300 mg St John's wort extract, three times a day for 14 days.

Parameter	Hypericin (n=2)	Pseudohypericin (n=2)
C_{max} (ng/ml)	8.5	5.8
C_{basal} (ng/ml)	5.3	3.7
t½ (h)	28.0	23.5

C_{max} = maximum plasma concentration; C_{basal} = basal concentration; t½ = plasma half-life

After a single dose, there was a lag time of about 2–3 hours before plasma concentrations of hypericin started to rise, while no lag time was observed for pseudohypericin. Both hypericin and pseudohypericin displayed disproportional kinetics, as the C_{max} and AUC increased more than dose-proportionally with increasing dose. This suggests saturation of elimination processes. Plasma concentrations of hypericin were still measurable 3 days after administration at all dose levels. This is in line with the generally long half-life, which showed large interindividual variation (see Table 5.1). No changes in T_{max} or plasma half-life were observed across doses, which would be expected in view of the disproportional kinetics. After multiple dosing 3 times a day for 14 days, steady state was reached for both compounds after 4 days. No changes occurred in plasma half-life compared with single dosing (Staffeldt et al., 1994).

In the first part of a similar study, a double-blind random-order trial, 12 healthy volunteers received a single dose of 300, 900 or 1,800 mg St John's wort extract (LI 160) via 1, 3 or 6 tablets supplemented by placebo tablets up to a total of 6 tablets (Kerb et al., 1996). Every volunteer received all three doses, separated by a 10-day washout period. Blood samples were taken up to 120 hours post-dose, and urine samples were collected at four intervals up to the next morning. The kinetic parameters are presented in Tables 5.4 and 5.5 for hypericin and pseudohypericin, respectively.
In the second part of the study, 13 healthy volunteers received 300 mg St John's wort extract three times a day for 14 days, and blood and urine samples were collected at different time points (see Table 5.6). In addition, in one volunteer 750 µg pure hypericin was orally administered,

and in two other volunteers St John's wort extract (115 g hypericin, 38 µg pseudohypericin) was given intravenously (see Table 5.7).

Table 5.4 Kinetic parameters (median + range) of hypericin after different single oral doses of St John's wort extract.

Parameter	**Dose extract (mg)/ hypericin (µg)**		
	300 / 250 (n=12)	900 / 750 (n=12)	1,800 / 1,500 (n=12)
C_{max} (µg/L)	1.3 (0.9–3.3)	7.2 (4.1–17.3)	16.6 (4.1–66.3)
T_{max} (h)	5.5 (4.0–8.0)	6.0 (4.1–8.1)	5.7 (3.5–6.1)
$AUC_{0-\infty}$ (µg/L*h)	41.4 (17.5–120)	198 (127–452)	494 (139–826)
t½ (h)	24.5 (14.7–57.8)	43.1 (28.2–57.8)	48.2 (22.9–57.8)
CL/F (ml/min)	101 (34.7–238)	63.3 (27.7–98.3)	51.0 (30.3–180)
V_d/F (L)	111 (32.3–280)	69.6 (41.0–147)	73.3 (18.5–297)

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability; V_d = volume of distribution

Table 5.5 Kinetic parameters (median + range) of pseudohypericin after different single oral doses of St John's wort extract.

Parameter	**Dose extract (mg)/ pseudohypericin (µg)**		
	300 / 526 (n=12)	900 / 1,578 (n=12)	1,800 / 3,156 (n=12)
C_{max} (µg/L)	3.4 (1.1–7.1)	12.1 (6.8–28.4)	29.7 (8.9–48.0)
T_{max} (h)	0.5 (0.2–0.9)	0.4 (0.3–1.0)	0.4 (0.3–0.5)
$AUC_{0-\infty}$ (µg/L*h)	45.0 (17.2–98.2)	140 (87.1–481)	285 (89.7–498)
t½ (h)	18.2 (13.9–27.9)	24.8 (13.9–69.3)	19.5 (13.9–41.9)
CL/F (ml/min)	195 (89.2–511)	188 (54.7–302)	185 (106–586)
V_d/F (L)	117 (40.6–519)	61 (24.1–134)	50.0 (28.8–209)

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability; V_d = volume of distribution

Table 5.6 Kinetic parameters (mean) of hypericin and pseudohypericin after multiple dosing with 300 mg St John's wort extract, three times a day for 14 days.

Parameter	**Hypericin (n=13)**	**Pseudohypericin (n=13)**
$C_{ss,max}$ (µg/L)	8.8 (5.7–22.1)	8.5 (4.3–20.7)
$C_{ss,min}$ (µg/L)	7.9 (3.4–13.6)	4.8 (1.1–10.1)
$AUC_{0-\infty}$ (µg/L*h)	61.5 (39.6–152)	50.9 (30.7–108)
t½ (h)	41.3 (30.1–71.4)	18.8 (13.9–46.2)
CL/F (ml/min)	68.2 (27.4–105)	172 (81.3–286)
V_{ss}/F (L)	162 (34.0–346)	63.0 (29.2–158)

$C_{ss,max}$ = maximum plasma concentration in steady-state; $C_{ss,min}$ = minimum plasma concentration in steady-state; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability; V_{ss} = volume of distribution in steady-state

Table 5.7 Kinetic parameters of hypericin and pseudohypericin after intravenous dosing with St John's wort extract.

Parameter	**Hypericin (115 µg)**		**Pseudohypericin (38 µg)**	
	Volunteer 1	*Volunteer 2*	*Volunteer 1*	*Volunteer 2*
C_{max} (µg/L)	29.5	24.6	6.8	6.5
C_{24h} (µg/L)	1.5	1.6	ND	ND
$AUC_{0-\infty}$ (µg/L*h)	205 (243)	194 (12.3)	19.1 (0.74)	11.9 (0.45)
t½ (h)	39.9 (149)	43.9 (12.2)	22.8 (2.8)	17.4 (3.9)
CL (ml/min)	9.3 (11.1)	9.2 (1.28)	33.3 (0.76)	53.3 (2.15)
V_d (L)	18.5 (52)	20.9 (3.9)	44 (4.6)	34.5 (6.4)

Values in parentheses are asymptotic standard errors of estimated parameters
C_{max} = maximum plasma concentration; C_{24h} = plasma concentration at 24 hours; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; V_d = volume of distribution; ND = not detectable

After a single dose with St John's wort extract, there was a lag time of about 2 hours before hypericin was measurable in plasma. This was also the case after oral administration of pure hypericin (no tabulated data presented), suggesting that hypericin is most likely absorbed at the distal end of the intestines. No lag time was observed for pseudohypericin. In addition, no pseudohypericin was detectable after the intake of pure hypericin, suggesting that pseudohypericin is not formed out of hypericin. Maximum concentrations of hypericin in plasma were reached after approximately 6 hours, indicating a slow absorption. Hypericin displayed non-linear kinetics with a greater-than-dose-proportional increase in C_{max} and AUC, suggesting saturation of elimination processes. Kinetics for pseudohypericin were generally linear. For both hypericin and pseudohypericin large interindividual variability was observed.
Hypericin was still detectable in plasma after 72 hours at the lowest dose, and after 120 hours at the two higher doses. Pseudohypericin was generally undetectable after 72 hours. This is consistent with the longer observed half-life of hypericin compared with pseudohypericin. The elimination half-life of hypericin increased significantly with increasing dose but it is not clear what the cause was. No changes in half-life with dose were found for pseudohypericin.
Steady state was achieved after approximately 6–7 days for hypericin and 4 days for pseudohypericin. Four volunteers showed little difference between peak and trough plasma levels of hypericin.

Kinetics after intravenous dosing showed comparable elimination half-lives compared with oral dosing. The absolute bioavailability from the extracts, calculated by RIVM, is very low, approximately 10% for hypericin and 20–30% for pseudohypericin.
Neither pseudohypericin nor hypericin was detected in urine samples, irrespective of incubation with glucuronidase and sulfatase. Based on the chemical structure and molecular size (>500 Da), conjugation with glucuronic acid and subsequent excretion via bile is expected (Kerb et al., 1996).

Brockmöller et al. (1997) conducted a single and multiple dose study with tablets containing 300 mg St John's wort extract (LI 160, 363 µg hypericin and 574 µg pseudohypericin) per tablet in healthy male volunteers. In the double-blind randomized cross-over design single-dose study, each volunteer received a total of up to 12 tablets (placebo or with St John's wort extract), resulting in a dose of 0, 900, 1,800 or 3,600 mg St John's wort extract with a washout period of 14 days between each dose. Blood samples were drawn up to 72 hours after dosing. In the multiple-dose study, 23 healthy female and 27 healthy male subjects received two tablets three times a day, corresponding to a daily dose of 2,180 µg hypericin and 3,440 µg pseudohypericin during 2 weeks, with the last dose in the morning of day 15. Blood samples measuring trough blood concentrations were drawn on several days up to and including day 15, as well as on days 1 and 15, 4 hours after dosing. The kinetic parameters for hypericin and pseudohypericin obtained after single and multiple dosing are presented in Tables 5.8–5.10.

Table 5.8 Kinetic parameters (median + range) of hypericin after different single oral doses of St John's wort extract.

Parameter	**Dose extract (mg)/ hypericin (µg)**		
	900 / 1,089 (n=13)	1,800 / 2,178 (n=13)	3,600 / 4,356 (n=13)
C_{max} (µg/L)	18 (14–22)	36 (29–44)	91 (71–111)
T_{max} (h)	7.1 (4.0–10.1)	6.0 (5.9–6.1)	6.5 (5.8–7.1)
$AUC_{0-\infty}$ (µg/L*h)	435 (334–537)	993 (809–1,177)	2,503 (1,960–3,049)
t½ (h)	27.8 (24.6–31.0)	29.1 (26.0–32.2)	27.5 (25.0–30.0)
CL/F (L/h)	2.7 (2.3–3.1)	2.4 (1.9–2.9)	2.2 (1.2–3.1)

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability

Table 5.9 Kinetic parameters (median + range) of pseudohypericin after different single oral doses of St John's wort extract.

Parameter	**Dose extract (mg)/ pseudohypericin (µg)**		
	900 / 1,722 (n=13)	1,800 / 3,444 (n=13)	3,600 / 6,888 (n=13)
C_{max} (µg/L)	10 (7–12)	25 (20–30)	68 (52–83)
T_{max} (h)	3.3 (3.0–3.6)	3.2 (3.0–3.4)	3.5 (3.1–3.8)
$AUC_{0-\infty}$ (µg/L*h)	138 (108–168)	334 (265–402)	835 (628–1041)
t½ (h)	19.4 (15.7–23.2)	16.1 (13.4–18.8)	17.5 (13.8–21.2)
CL/F (L/h)	14.1 (10.8–17.4)	12.3 (7.7–16.9)	11.4 (4.7–18.1)

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability

Table 5.10 Kinetic parameters (mean + 95% CI) of hypericin and pseudohypericin after multiple dosing with 600 mg St John's wort extract (2,180 µg hypericin, 3,440 µg pseudohypericin per day) three times a day for 14 days.

Parameter	**Hypericin (n=50)**	**Pseudohypericin (n=50)**
$C_{ss,max}$ (µg/L)	29 (25–33)	15 (12–17)
$C_{ss,trough}$ (µg/L)	26 (22–29)	12 (9–14)
$AUC_{0-\infty}$ (µg/L*h)	270 (232–308)	165 (137–139)
t½ (h)	41.7 (40.1–42.2)	22.8 (20.0–25.7)
CL/F (L/h)	2.7 (2.4–3.1)	7.0 (5.9–9.0)

$C_{ss,max}$ = maximum plasma concentration in steady-state; $C_{ss,trough}$ = minimum plasma concentration in steady-state; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability

After a single dose, maximum plasma concentrations of hypericin were reached after 6–7, hours indicating slow absorption. Absorption of pseudohypericin was faster. No statistically significant differences were found between dose-normalized C_{max} and AUC values. However, although not statistically significant, the results seem to indicate a saturation of elimination at higher doses, as was observed in other studies. Elimination of both compounds was slow.
After multiple dosing, steady-state concentrations of pseudohypericin were reached after approximately 3 days and of hypericin after 5-6 days. When comparing multiple-dose with single-dose kinetics, the terminal half-life of hypericin was longer (Brockmöller et al., 1997).

Tablets containing 300 mg alcohol/water extract from St John's wort with a hyperforin content of 5% (WS 5572) or 0.5% (WS 5573) or a placebo were orally administered to male volunteers (Biber et al., 1998). In the first study, a randomized four-way crossover study, six healthy volunteers received a single dose of 300, 600 or 1,200 mg of WS 5572 by taking 1, 2 or 4 tablets with water 2 hours before breakfast after fasting for 10 hours. The washout period between doses was three days. In the second study, a double-blind, randomized, placebo-controlled, parallel-group study, 54 healthy volunteers received a dose of 900 mg WS 5572 or WS 5573 or a placebo once a day for eight consecutive days. The tablets were taken

2 hours before breakfast after fasting overnight on days 1 and 8, and with breakfast after fasting overnight on days 2 to 7. Blood samples were collected before and at several time points after administration during 24 hours, and pharmacokinetic parameters were determined. The pharmacokinetic parameters after single and repeated dosing are presented in Tables 5.11 and 5.12, respectively.

Table 5.11. Pharmacokinetic parameters of hyperforin (mean ± SEM) after a single dose of St John's wort extract in human volunteers.

Parameter	**Dose extract / hyperforin (mg)**		
	300 / 14.8 (n=6)	600 / 29.6 (n=6)	1,200 / 59.2 (n=6)
C_{max} (ng/ml)	153.15 ± 21.3	301.8 ± 47.2*	437.3 ± 101.3
T_{max} (h)	3.58 ± 0.6	3.5 ± 0.3	2.83 ± 0.3
$AUC_{0-\infty}$ (ng/ml*h)	1,335.9 ± 145.3	2,214.6 ± 278.6*	3377.9 ± 670.1
t½ (h)	9.46 ± 1.1	8.52 ± 0.7	9.65 ± 0.8
CL/F (ml/min)	199.3 ± 28	238.2 ± 25.2	340.3 ± 49.3*

* Statistically significant difference compared with 300 mg dose group, $p<0.05$
C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability

Table 5.12 Pharmacokinetic parameters of hyperforin (mean ± SEM) after repeated doses with 900 mg St John's wort extract WS 5572 (5% hyperforin, 45 mg) or WS 5573 (0.5% hyperforin, 4.5 mg) for eight consecutive days in human volunteers.

Parameter	**WS 5573 (n=7)**		**WS 5572 (n=9)**	
	Day 1	*Day 8*	*Day 1*	*Day 8*
C_{max} (ng/ml)	18.2 ± 2.8	20.7 ± 1.6	300 ± 23.2	246 ± 22.3
T_{max} (h)	3.9 ± 0.6	3.0 ± 0.5	2.9 ± 0.3	3.1 ± 0.4
$AUC_{0-\infty}$ (ng/ml*h)	227 ± 28.3	-	3352 ± 329	-
AUC_{0-T} (ng/ml*h)	-	254 ± 21.3	-	2336 ± 303*
t½ (h)	9.1 ± 0.5	16.0 ± 3.5[a]	7.2 ± 0.3	11.2 ± 1**
CL/F (ml/min)	327 ± 31.7	283 ± 21.2	266 ± 17	342 ± 38*

* Statistically significant difference compared with corresponding day 1 values, $p<0.05$
** Statistically significant difference compared with corresponding day 1 values, $p<0.01$
[a] n=6
C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability

After a single dose of hyperforin (WS 5572) there was a lag time of approximately 1 hour before hyperforin reached detectable levels. Plasma concentrations could best be described following a two-compartment model. Peak plasma levels were reached after 3-4 hours, irrespective of dose. Hyperforin displayed kinetics approximately proportional with dose up to a dose of 600 mg extract, while at the higher dose of 1,200 mg extract C_{max} and $AUC_{0-\infty}$ increased less than dose-proportionally, suggesting saturation of the absorption process.
Repeated once daily dosing for up to eight days showed no accumulation of hyperforin. As with the single-dose study, disproportional kinetics were observed between extract WS 5573 with 0.5% hyperforin and extract WS 5572 with 5% hyperforin. At the high hyperforin dose

(WS 5572), a statistically significant, but unexplainable, increase in both clearance and plasma half-life, together with a decrease in AUC, was observed at day 8 compared with day 1 (Biber et al. 1998).

In a double-blind, placebo-controlled, balanced-order study conducted by Franklin et al. (1999), 12 healthy male volunteers received a single oral dose of 9 tablets each containing 300 mg St John's wort extract (LI 160, 0.3% total hypericin (unspecified)), equivalent to 8.1 mg of total hypericin, or a placebo, and blood samples were collected up to 4 hours post-dose. The treatments were separated by a wash-out period of at least one week. Plasma concentrations of hypericin rose after approximately 150 minutes and were still rising at 4 hours post-dose. Plasma concentrations of hyperforin generally increased at 60 minutes post-dose, but in three subjects significant amounts were already observed at 30 minutes. Peak plasma levels were reached at 210 minutes.

Agrosi et al. (2000) studied the rate and extent of the oral absorption of hyperforin and hypericin in 12 healthy human volunteers using 300 mg dry St John's wort extract in soy oil per softgel capsule or 300 mg dry St John's wort extract per solid state hard shell capsule in a single-dose, two-way, open, balanced, randomized, cross-over study with a 1-week washout period. The hypericin and hyperforin content of the extract was 0.3% and 5%, respectively.
Significant levels of hyperforin were found in the blood of all subjects. Hypericin was detected at over 100 times lower levels, and in five of the subjects who received the softgel capsules and six of those who received the reference capsules the blood levels were below the level of detection (LOD). Pseudohypericin was not detected. Table 5.13 shows the kinetic parameters after dosing with the two types of capsules. Data for hypericin were too limited to derive kinetic parameters due to the high level of non-detects (LOD was 0.29 µg/l). However, the available plasma levels showed a similar trend as with hyperforin, with a higher absorption after taking the softgel compared with the hard shell capsule formulation.

Table 5.13 Pharmacokinetic parameters of hyperforin after a single dose of 300 mg St John's wort extract (containing 5% hyperforin and 0.3% hypericin) via softgel or reference capsules in human volunteers (n=12).

Parameter	**Type of capsule**	
	Softgel capsule	*Hard shell capsule*
C_{max} (ng/ml)	168.35 ± 57.79	84.25 ± 33.51
T_{max} (h)	2.5 ± 0.83	3.08 ± 0.79
$AUC_{0-\infty}$ (ng/ml*h)	1,482.7 ± 897.13	583.65 ± 240.29

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve

When corrected for their presence in the extract, hyperforin had approximately a one order of magnitude higher bioavailability than hypericin. In addition, the softgel capsule led to a higher absorption of hyperforin, and to a lesser extent of hypericin, compared with the hard shell capsule, as indicated by the pharmacokinetic data (Agrosi et al., 2000).

A single- and multiple-dose clinical trial was conducted in which 18 healthy male volunteers were given tablets containing 612 mg dry extract of St John's wort (STW-3, Laif®) (Schulz et al., 2005). The tablets contained approximately 600 µg hypericin, 1,200 µg pseudohypericin, 13.5 mg hyperforin and 73.2 mg flavonoids (among which quercetin and isorhamnetin). In the single-dose study, subjects were fasted for 10 hours and then given 1 tablet with water. In the multiple-dose study, subjects were given 1 tablet after breakfast each day for 13 days, and under fasting conditions at day 14. In both studies, blood samples were collected pre-dose and for up to 24 (multiple-dose study) or 48 (single-dose study) hours. The kinetic parameters after a single dose and after multiple dosing are presented in Tables 5.14 and 5.15, respectively.

Table 5.14 Kinetic parameters (mean ± SD) of hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin after a single oral dose of 612 mg dry extract of H. perforatum.

Parameter	**Compound**				
	Hypericin	**Pseudohypericin**	**Hyperforin**	**Quercetin**	**Isorhamnetin**
C_{max} (ng/ml)	3.14 ± 1.57	8.50 ± 4.35	83.5 ± 27.8	47.7 ± 22.5[a] 43.8 ± 23.1[b]	7.6 ± 2.8[a] 9.0 ± 6.1[b]
T_{max} (h)	8.1 ± 1.8	3.0 ± 1.4	4.4 ± 1.5	1.17 ± 0.52[a] 5.47 ± 1.38[b]	1.53 ± 0.67[a] 6.42 ± 1.90[b]
$AUC_{0-\infty}$ (ng/ml*h)	75.96 ± 23.52	93.03 ± 29.40	1009.0 ± 203.4	318.70 ± 130.82	97.99 ± 107.28
t½ (h)	23.76 ± 5.46	25.39 ± 10.18	19.64 ± 6.35	4.16 ± 2.97	4.45 ± 3.27

[a] first maximum
[b] second maximum
C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life

Table 5.15 Kinetic parameters (mean ± SD) of hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin after a multiple oral dosing of 612 mg dry extract of H. perforatum for 14 days.

Parameter	**Compound**				
	Hypericin	**Pseudohypericin**	**Hyperforin**	**Quercetin**	**Isorhamnetin**
C_{max} (ng/ml)	4.43 ± 1.49	8.51 ± 3.29	97.4 ± 30.0	45.1 ± 21.1[a] 59.1 ± 27.2[b]	8.7 ± 5.5[a] 9.8 ± 8.1[b]
T_{max} (h)	6.8 ± 1.8	3.3 ± 1.7	4.3 ± 1.0	1.33 ± 0.59[a] 5.33 ± 1.25[b]	1.47 ± 0.67[a] 5.82 ± 1.19[b]
AUC_{0-24h} (ng/ml*h)	76.50 ± 24.74	87.63 ± 30.33	825.5 ± 176.4	272.34 ± 157.32	84.96 ± 106.89

[a] first maximum
[b] second maximum
C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve

In general, there are no differences in the kinetic parameters for hypericin, pseudohypericin, hyperforin and the flavonoids after single or multiple dosing. No signs of accumulation in plasma were observed. The elimination of hypericin, pseudohypericin and hyperforin is slow, as indicated by the long plasma half-life. On the other hand, the absorption and elimination of the flavonoids is fast (Schulz et al., 2005).

A phase I dose escalation study to determine the safety of hypericin in patients with a chronic hepatitis C virus infection was conducted by Jacobson et al. (2001). Nineteen patients received either 0.05 mg/kg bw hypericin (n=12) or 0.1 mg/kg bw hypericin (n=7) orally once a day for eight weeks. Blood samples were collected weekly from week 0 to 4 and for detailed pharmacokinetic analyses at week 8 at 0, 6, 9, 12, 24 and 48 hours. The kinetic parameters obtained are presented in Table 5.16.

Table 5.16 Kinetic parameters (mean ± SD) of hypericin after repeated oral dosing with 0.05 or 0.1 mg/kg bw per day hypericin for 8 weeks.

Parameter	**Hypericin dose (mg/kg bw)**	
	0.05 (n=7)	0.1 (n=4)
C_{max} (ng/ml)	30.6 ± 12.6	64.9 ± 39.1
T_{max} (h)	4.4 ± 2.7	4.4 ± 2.7
$AUC_{0-\infty}$ (µg/ml*h)	1.5 ± 0.7	3.1 ± 1.3
t½ (h)	36.1 ± 22.6	33.8 ± 18.8
CL/F (ml/min/m^2)	5.8 ± 2.3	13.4 ± 6.4
V_d/F (L)	26.7 ± 12.3	84.3 ± 32.5

C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; AUC = area under the curve; t½ = plasma half-life; CL = total clearance; F = bioavailability; V_d = volume of distribution

Both C_{max} and AUC increased proportionally with dose at steady state. The time to reach maximal plasma concentration did not alter over the treatment weeks. Both CL/F and V_d/F increased with increasing dose, which may indicate a secondary tissue absorption that is more apparent at the higher concentration. No hypericin metabolites were observed, as no other peaks than for hypericin were found with high-performance liquid chromatography (HPLC).

5.2.2 *Distribution*

In vitro

The ability to cross the membrane barriers of the intestine (using Caco-2 cells), the blood–brain barrier (using porcine brain capillary endothelial cells) and the blood–cerebrospinal fluid barrier (using porcine epithelial cells of the plexus chorioidei) was investigated for the flavonols miquelianin, hyperoside and quercitrin by transport and/or permeability experiments (Juergenliemk et al., 2003). Uptake into Caco-2 cells was highest for miquelianin followed by hyperoside and then quercitrin, and uptake values increased when the MRP-2 inhibitor MK-571 was added, indicating involvement of the MRP-2 transporter in the efflux of these compounds. Miquelianin was able to cross the small intestine membrane, the blood–brain barrier and the blood–cerebrospinal barrier. No active transport was found for crossing the blood–cerebrospinal barrier.

In 2001, Delaey et al. studied the *in vitro* plasma–protein binding of different hypericins, including hypericin, tetrasulfonhypericin, fringelite D, hexamethylhypericin, pentamethylhypericin, hypericin PEG-3 and dibenzyltetramethylhypericin. Hypericin was mainly bound to low-density lipoproteins, whereas dibenzyltetre amethylhypericin had approximately an equal affinity for different plasma proteins. The other hypericin analogues were mainly bound to high-density lipoproteins and/or heavy proteins, mainly albumin (Delaey et al., 2001).

Animal data
Keller et al. (2003) investigated the distribution of hyperforin into the brain in female NMRI mice (n=8) 3 hours after administering 300 mg/kg *H. perforatum* extract (WS 5572) containing 5% hyperforin, or 15 mg/kg hyperforin via a single oral gavage. A control group was included. An average brain concentration of 28.8±10.1 ng/g hyperforin was found in the hyperforin group. In the group gavaged with the extract, the brain concentration was 15.8±10.9 ng/g hyperforin, which was significantly lower than in the hyperforin group. No hyperforin was detected in the brains of the control group.

Fox et al. (2001) investigated the distribution of hypericin in non-human primates after an intravenous dose of 2 mg/kg (n=3) or 5 mg/kg (n=1). Plasma (see also Section 5.2.1) and CSF samples were obtained prior to and at several time points after administration. Hypericin was not detected (<0.1 µM) in the CSF of any of the animals, indicating that the penetration of hypericin into CSF was less than 1% of the plasma exposure.

Cervo et al. (2002) studied two *H. perforatum* extracts with different hyperforin contents, i.e. 0.5% and 4.5%, and the resulting plasma and brain concentrations in male CD-COBS rats (n=4 per group). The extracts and hyperforin dicyclohexylammonium (DCHA) were administered intraperitoneally three times in a 24-hour period, at 24 hours, 5 hours and 1 hour (extracts) or 0.5 hour (hyperforin DCHA) before behavioural experiments at doses of 3.12 mg/kg or 6.25 mg/kg for the extract with 4.5% hyperforin, at 6.25 mg/kg or 12.50 mg/kg for the extract with 0.5%, or at 0.19 mg/kg or 0.38 mg/kg for hyperforin DCHA. Afterwards, plasma and brain tissue were examined for hyperforin content. Dose-related increases in hyperforin plasma concentrations were observed with the 4.5% extract, while the 0.5% extract resulted in non-detectable concentrations. Administration of 0.38 mg/kg hyperforin DCHA resulted in a rapid increase in hyperforin plasma levels and also rapid clearance, as concentrations reached the limit of quantification (LOQ) within 120 minutes. The mean C_{max} and AUC were comparable to those found after a dose of 6.25 mg/kg of the extract with 4.5% hyperforin. Brain concentrations of hyperforin were below the LOD. An additional dosing with 12.5 mg/kg hyperforin DCHA resulted in brain concentrations approaching the LOQ, yielding a mean brain:plasma concentration ratio of 0.04±0.02.

Paulke et al. (2008) studied the distribution into the brain of flavonoids present in *H. perforatum* extract in rats. In the single-dose study, rats were given either vehicle (agarose, n=6), 100 mg/kg isoquercitrin (n=30) or 1,600 mg/kg St John's wort extract (WS 5572 containing 1.35%

isoquercitrin, 0.38% quercitrin, 3.26% rutin and 1.83% hyperoside, n=30). Five days before and during the study the rats received a flavonoid-free diet. Blood and brain samples were collected 2, 4, 8, 24 and 48 hours after administration in the treated groups. In the repeated-dose study, rats received either vehicle (agarosegel 0.2%, n=6) or 1,600 mg/kg St John's wort extract (n=18) once daily for eight days. Blood and brain samples were taken every 4 hours after the last feeding on days 1, 5 and 8 from the treated group.
Following a single dose of St John's wort extract, the plasma concentration of quercetin increased rapidly, reaching a maximum concentration of 700 ng/ml after 4 hours, followed by a slow continuous decrease over the next 44 hours. The plasma concentrations of the flavonoid metabolites isorhamnetin and tamarixetin increased much more slowly and reached a C_{max} of 903 ng/ml after 24 hours. Higher levels of the compounds were found after dosing with pure isoquercitrin. In addition, isorhamnetin/tamarixetin maximum plasma concentrations were reached much faster (8 hours) after dosing with pure isoquercitrin than after dosing with St John's wort extract. Brain levels for quercetin reached their maximum 4 hours after dosing – 340 ng/g protein for dosing with St John's wort extract and 870 ng/g protein for dosing with isoquercitrin – and were not quantifiable after 24 hours.
For isorhamnetin/tamarixetin when administered via St John's wort extract, brain concentrations were less than a fifth of the plasma concentrations and remained stable over 44 hours.
After repeated dosing with St John's wort extract, the plasma levels of isorhamnetin/tamarixetin increased continuously from day 1 to 8, and that of quercetin only from day 1 to 5, after which it remained constant. For all compounds a five-fold increase in plasma concentration was observed. With respect to brain concentrations, only an accumulation of isorhamnetin/tamarixetin was observed over days 1 to 8, which was approximately five-fold as well.

Human data
The study by Schempp et al. (1999), reported by EMA (2009a), describes the HPLC detection of hypericin (purity >98%) and semiquantitative detection of pseudohypericin in human serum and skin blister fluid after oral single-dose (1 x 6 tablets) or steady-state (3 x 1 tablet per day, for 7 days) administration of the *Hypericum* extract LI 160 (300 mg per tablet, standardized to 900 µg of total hypericin) in healthy volunteers (n=12). Serum levels of hypericin and pseudohypericin were always found to be significantly higher than skin blister fluid levels ($p \leq 0.01$). After oral single-dose administration of *Hypericum* extract, the mean serum level of total hypericin (hypericin + pseudohypericin) was 43 ng/ml and the mean skin blister fluid level was 5.3 ng/ml. After steady-state administration, the mean serum level of total hypericin was 12.5 ng/ml and the mean skin blister fluid level was 2.8 ng/ml. These skin levels are far below hypericin skin levels that are estimated to be phototoxic (>100 ng/ml).

5.2.3 *Biotransformation*

In vitro
Cui et al. (2004) studied the *in vitro* metabolism of 0.36 mM hyperforin (>99%) in male and female rat liver microsomes. Several metabolites were formed, of which four major ones were identified as hydroxymethyl

derivatives. As the rate of formation was higher in male than in female rats and was increased when dexamethasone- or phenobarbital-induced microsomes were used, and when NADH was added to the NADPH-regenerating system, this may indicate that CYP3A is involved in the hydroxylation pathway of hyperforin.

Hokkanen et al. (2011) studied the *in vitro* metabolism of hyperforin in human liver microsomes. In total, 57 metabolites were detected. Reactions observed were monohydroxylation, dihydroxylation, trihydroxylation, and hydroxalation in combination with dehydrogenation. Monohydroxylation metabolites were the most abundant. CYPs mainly involved in the *in vitro* metabolism are 2C8, 2C9, 2C19 and 3A4. In addition, hyperforin was found to inhibit CYP2D6 and 3A4.

5.2.4 *Excretion*

Human data

Kerb et al. (1996) collected urine samples at four intervals up to the morning after individuals were given three single oral doses of 300, 900 or 1,800 *Hypericum* extract. In addition, 24-hour urine samples were obtained in four fractions on days 1 and 14 from subjects who received 300 mg *Hypericum* extract three times a day for 14 days. Hypericin or pseudohypericin were not detectable in the urine after incubation with glucuronidase and sulfatase. No metabolic formation of pseudohypericin was observed after administration of purified hypericin.

Klier et al. (2002) investigated the excretion of St John's wort constituents into the breast milk of a 33-year-old mother who had taken 300 mg of a St John's wort preparation (Jarsin®; hyperforin content of batch not specified) three times a day for eight weeks. Four milk samples were collected over an 18-hour period. Blood samples from the mother were collected 5 hours after administration, and blood samples from the infant 3 hours later. All samples were analysed for hypericin and hyperforin content. Hypericin was not quantifiable in breast milk (see Table 5.16). Hyperforin was found in both fore- and hindmilk in generally low concentrations. The milk:plasma ratio was far below 1 for both components. No quantifiable amounts of hypericin or hyperforin were found in the plasma of the infant.

Table 5.16 Hypericin and hyperforin levels in foremilk, hindmilk and plasma of mother and her breastfed infant after intake of 300 mg St John's wort extract three times a day for 8 weeks by the mother.

Time	**Matrix**	**Hypericin** (ng/ml)	**Hyperforin** (ng/ml)
20:30	Foremilk	BLQ	0.58
	Hindmilk	BLQ	1.24
06:00	Foremilk	BLQ	1.01
	Hindmilk	BLQ	18.20
10:00	Foremilk	BLQ	BLQ
	Hindmilk	BLQ	0.86
12:45	Plasma mother	10.71	151
13:15	Foremilk	BLQ	BLQ
	Hindmilk	BLQ	2.76
15:30	Plasma infant	BLQ	BLQ

BLQ = below the limit of quantification (LLOQ: 0.20 ng/ml hypericin, 0.50 ng/ml hyperforin)

In 2006, Klier et al. studied the excretion of hyperforin into breast milk further by analysing the blood and breast milk of five mothers who had been taking 300 mg St John's wort extract (Jarsin®; average hyperforin content 7.48 mg) three times a day for at least four weeks. In addition, the plasma hyperforin levels from two infants were studied. The milk samples were obtained during an 18-hour period before (foremilk) and after (hindmilk) breastfeeding. The blood samples were drawn 5 hours post-dose.
None of the mothers had used St John's wort extracts during pregnancy and no other drugs were taken during breastfeeding. The hyperforin concentrations in the mothers' milk and plasma and in the plasma of two of the infants are presented in Table 5.17. Hyperforin was measurable in the breast milk but the milk:plasma ratio was low, indicating that only a small amount of the ingested dose is excreted via milk. This is also reflected in the low plasma levels of hyperforin observed in the infants that were at LOQ.

Table 5.17 Concentrations of hyperforin in mother's milk and plasma and in the plasma of two infants breastfed by mothers who took 300 mg St John's wort extract three times a day for at least 4 weeks.

	Hyperforin concentration (ng/ml)		Maternal plasma hyperforin concentration (ng/ml)	Milk/plasma concentration ratio	Infant plasma hyperforin concentration (ng/ml)	Relative dose of hyperforin received by infant (%)[a]
Patient	**Foremilk**	**Hindmilk**				
1	3.4 (1.0–11.7)	7.3 (5.3–8.1)	60.2	0.09	0.1[b]	2.5
2	3.3 (1.3–6.0)	2.0 (0.2–5.6)	65.2	0.04	0.1[b]	1.1
3	3.3 (0.3–10.1)	1.6 (0–31.5)	32.2	0.08	NA	0.9
4	5.6 (0.6–8.6)	2.4 (0.6–4.1)	34.8	0.11	NA	1.6
5	2.1 (0.1–5.1)	4.0 (2.6–10.3)	22.8	0.13	NA	1.3

[a] infant hyperforin dose per kg bw expressed as percentage of the maternal hyperforin dose per kg bw
[b] LOQ = 0.1 ng/ml
NA = not available

5.2.5 *Effects on enzymes*

In vitro

Godtel-Armbrust et al. (2007) studied different St John's wort extracts and commercial preparations with different levels of major compounds for their effect on CYP3A4 induction by exposing human colon adenocarcinoma LS174T transfected cells to these preparations. To this end, six commercial St John's wort preparations available on the German market were bought together with 10 St John's wort dry extracts and purified hyperforin (>93.75%). For the commercial St John's wort preparations, the hyperforin content varied 62-fold, the hypericin content 37-fold and the flavonoids content 11-fold. The dry extracts contained hyperforin in the range of 0.07–4.61%. A correlation was observed between the hyperforin content of the preparation or extract and the induction of CYP3A4, whereas the correlation values between the hypericin and flavonoids content and CYP3A4 induction were low. The EC_{50} value of hyperforin was 92 nmol/L.

In the study of Hellum et al. (2007), male human hepatocytes were exposed to 8, 80 or 800 µg/ml total St John's wort constituents to assess CYP1A2, 2D6 and 3A4 induction and activity. CYP3A4 activity was induced by St John's wort and increased in a dose-dependent manner at the two lowest concentrations, whereas it dramatically decreased at the highest concentration, possibly due to cytotoxicity. No effect on CYP2D6 activity was observed, and for CYP1A2 there was a slight but statistically significant induction.

Silva et al. (2016) investigated the effects of St John's wort extract, hypericin and hyperforin on CYP1A2 and CYP2D6 expression in different human hepatic cell lines. WRL-68, HepG2 and HepaRG cells were incubated with 1 or 10 µM St John's wort extract, hypericin or hyperforin for 24 or 72 hours. The 10 µM concentration was cytotoxic in most circumstances, while the 1 µM concentration showed significant cytotoxicity in the WRL-68 cell line for all three test substances and for hyperforin in the HepG2 and HepaRG cell lines. Hence, expression of CYP1A2 and CYP2D6 was only investigated at 1 µM. The extract had an inductive effect on CYP1A2 as well as CYP2D6 in all three cell lines. Hypericin exposure led to a delayed CYP1A2 induction in HepG2 cells but not in the other cell lines. With respect to CYP2D6 expression, hypericin had an inductive effect comparable with that of the extract in Hep2G cells, whereas in WRL-68 and HepaRG cells the expression was inhibited (delayed in HepaRG cells). Hyperforin exposure resulted in an inductive effect on CYP1A2 expression in WRL-68 cells, while in the other two cell lines there was an inductive effect after a 24-hour incubation but an inhibitory effect after a 72-hour incubation. This may be due to the metabolism of hyperforin or the result of increased cytotoxicity. For CYP2D6, its expression was induced in HepG2 cells but inhibited in WRL-68 cells and no effect at 24 hours and an inhibitory effect at 72 hours was observed in HepaRG cells, probably linked to cytotoxicity.

Animal data

Male B6 mice were gavaged with water or extract of St John's wort at doses of 100–900 mg/kg bw per day for different periods (14, 21 or 28 days), and the effect on the activity of different enzyme systems was investigated using substrates. Analysis of the extract showed a

hyperforin content of 1.3 mg/g (0.13%). The minimal dosing regimen to observe a response in hepatic Cyp3a activity was treatment with 600 mg/kg per day for 21 days, and for hepatic Cyp2c for 28 days. After these periods, stimulation of hepatic and renal Cyp3a and hepatic Cyp2c function was observed. No changes were observed in hepatic NADPH-P450 reductase, cytochrome b_5, or Cyp1a, 2b, 2d or 2e1. In addition, no changes were found for glutathione S-transferase (GST), NAD(P)H-quinone oxidoreductase (NQO) or UDP-glucuronosyltransferase (UGT) (Yang et al., 2018).

Dostalek et al. (2005) studied the effect of St John's wort extract on CYP2C6, 2D2 and 3A2 activity in male rats. The rats were given 100 mg/kg bw of standardized St John's wort extract, containing 280 µg hypericin, 300 µg pseudohypericin, 6.5 mg hyperforin and 10 mg hyperosid, intraperitoneally, once daily for 10 days. The livers were isolated 24 hours after the last administration and were perfused with the marker substrates tolbutamide, dextromethorphan and midazolam to measure CYP2C6, 2D2 and 3A2 activity. St John's wort extract significantly inhibited the biotransformation of tolbutamide, indicating inhibitory activity towards CYP2C6. The biotransformation of both dextromethorphan and midazolam was significantly induced, indicating that the St John's wort extract is an inducer of CYP2D2 and 3A2 (Dostalek et al., 2005).

In 2011, Dostalek et al. studied the effect of standardized St John's wort extract on CYP1A2 activity in a similar experiment using phenacetin as a marker compound. The metabolism of phenacetin was significantly inhibited by St John's wort extract, indicating that St John's wort extract is an inhibitor of CYP1A2 as well (Dostalek et al., 2011).

Human data
Wang et al. (2004) investigated the effect of long-term use of St John's wort extracts on CYP2C19 activity (as measured by the metabolism of mephenytoin) in 12 healthy male volunteers, with the activity of CYP1A2 (as measured by the metabolic ratio of caffeine) as a control.
Six individuals had the wildtype genotype 2C19*1*1, four individuals 2C19*2/*2, and two 2C19*2/*3. The study design was a two-phase crossover with a 5-week interval. Each individual received a placebo or a tablet containing 300 mg St John's wort extract (0.3% hypericin and a minimum of 4% hyperforin) three times a day for 14 days. At day 15, the individuals received a single oral dose of 100 mg mephenytoin and 300 mg caffeine. Blood samples (6 h post-dose) and urine (0–8 h post-dose) were collected for analysis. Except for a little dizziness in two volunteers, no adverse effects were reported. No effects were found on caffeine metabolic ratio and thus on CYP1A2 activity. Urinary recovery of 4-OH-mephenytoin was significantly increased in treated individuals compared with the placebo in CYP2C19 wildtype individuals but not in individuals with CYP2C19*2/*2 or CYP2C19*2/*3, indicating induction of CYP2C19.

Hennesy et al. (2002) studied the effect of St John's wort extracts on P-glycoprotein expression and function after giving healthy male and female volunteers 600 mg St John's wort extract (0.15% hypericin, standardized extract) three times a day for 16 days (n=12) or a placebo

(n=7). Blood samples were drawn at baseline, day 16 and day 32, and peripheral blood mononuclear cells (PBMCs) were isolated for P-glycoprotein (P-gp) investigation.
A mean 4.2-fold increase in P-gp expression was observed in PBMCs from subjects treated with the St John's wort extract at day 16 compared with the baseline. There was large interindividual variability in the P-gp expression. P-gp expression had returned to baseline at day 32. The function of P-gp was also enhanced, as indicated by an increase in P-gp-mediated rhodamine efflux compared with the baseline. Furthermore, the efflux of rhodamine was significantly more inhibited by ritonavir before treatment than after treatment with St John's wort extract. No change in P-gp expression or function was found for the placebo group.

The effect of St John's wort extract on CYP1A2, CYP3A4, CYP2D6, N-acetyltransferase 2 (NAT2) and xanthine oxidase (XO) activity was studied in healthy males and females (n=16) by giving them 300 mg St John's wort extract (900 µg hypericin) via capsules three times a day for 14 days (Wenk et al., 2004). Assessment of the enzyme activities was conducted at day 1 before intake (baseline) and at day 15. Results were as follows: induction of CYP3A4 activity with indications of gender differences, though not consistently pointing in one direction; induction of CYP1A2 activity but only in females; and no influence on CYP2D6, NAT2 or XO activity. No adverse effects were reported by the volunteers.

5.2.6 *Summary on toxicokinetics*

Absorption of hypericin after a single dose showed a lag time of about 2–3 hours. Maximum plasma concentrations were reached after approximately 4–6 hours. Both C_{max} and AUC increased more than dose-proportionally over the dose range 250–1,500 µg, indicating saturation of elimination processes (Staffeldt et al., 1994; Kerb et al., 1996). Hypericin has a long plasma half-life of about 25 hours (Staffeldt et al., 1994). The estimated bioavailability for hypericin from St John's wort extract is very low, ~10% (Kerb et al., 1996).
The absorption of pseudohypericin showed no lag time. Both C_{max} and AUC increased more than dose-proportionally over the dose range 526-3,156 µg, indicating saturation of elimination processes, while over the dose range ~1,700–6,900 µg the kinetics were linear. The plasma half-life was found to be highly variable at between 6 and 42 hours (Staffeldt et al., 1994; Kerb et al., 1996). Its estimated bioavailability is a little higher than for hypericin but still low, ~20–30% (Kerb et al., 1996).
The repeated dose kinetics (3 x 300 mg St John's wort per day) showed that a steady state was achieved after 4–7 days for hypericin and after 4 days for pseudohypericin (Staffeldt et al., 1994; Kerb et al., 1996). Generally, the kinetics did not alter after repeated dosing. However after dosing with 600 mg three times a day, the plasma half-life of hypericin was longer than after a single dose (Brockmöller et al., 1997).

After a single dose, the absorption of hyperforin showed a lag time of approximately 1 hour and maximum plasma concentrations were reached after 3–4 hours. Hyperforin has a comparable or higher bioavailability than hypericin. After dosing of up to 600 mg of St John's wort extract (WS5572, hyperforin content 5%, i.e. 30 mg hyperforin) linear kinetics

were found, whereas AUC and C_{max} increased less than dose-proportionally up to a dose of 1,200 mg extract (i.e. 60 mg hyperforin). Saturation of absorption processes may play a role here (Biber et al., 1998). Plasma half-life was around 9 hours. Repeated dosing with 900 mg extract (WS5572, hyperforin content 5%; or WS5573, hyperforin content 0.5%) for eight consecutive days showed no accumulation of hyperforin. Disproportional kinetics were observed between extract WS 5573 with 0.5% hyperforin and 900 mg extract WS 5572 with 5% hyperforin, since the AUC levels for the latter extract were approximately 15-fold instead of 10-fold higher than for the extract with 0.5% hyperforin. In addition, repeated dosing with the 5% hyperforin extract resulted in an increase in total clearance (CL) accompanied by a decrease in AUC. One reason for this might be induction of metabolizing enzymes (Biber et al., 1998).

St John's wort constituents are able to cross the blood–brain barrier, as demonstrated for several flavonoids and hyperforin (Cervo et al., 2002; Fox et al., 2001; Keller et al., 2003; Paulke et al., 2008). For hyperforin, brain levels are generally low, indicating a poor passage across the blood–brain barrier (brain levels equal 2–4% of plasma levels).

There is not much information available about the biotransformation of the active ingredients in St John's wort extracts. Upon incubation of hyperforin with human liver microsomes, 57 metabolites were found, of which monohydroxylation metabolites were the most abundant. CYP enzymes involved included 2C8, 2C9, 2C19 and 3A4 (Hokkanen et al., 2011).

There is limited information available about the excretion of hypericin and hyperforin in breast milk. Hypericin could not be quantified in breast milk. Only a small amount of the ingested dose of hyperforin is excreted via milk (Klier et al., 2002; 2006). Further information about the excretion pathways of hyperforin and hypericin, for example via urine or faeces, was not found.

In humans using St John's wort extracts, induction of P-gp, CYP2C19, 3A4 and 1A2 (in females only; no induction observed in males) was found (Hennesy et al., 2002; Wang et al., 2004; Wenk et al., 2004). In addition, no influence on CYP2D6, NAT2 or XO activity was observed (Wenk et al., 2004).

5.3 Toxicological studies

5.3.1 *Acute toxicity*

SCF (2002) described two oral acute toxicity studies with hypericin and/or St John's wort extract in rats, one study with ground St John's wort in sheep and one study with dried ground St John's wort in cows. EMA (2009a) described one additional acute toxicity study in rhesus monkeys and an overview of LD_{50} values in mice and rats. These studies, as well as four newly identified studies, are described below.

Mice

Li et al. (2012) administered non-radioactive iodinated hypericin (>98.6%) intravenously to male and female CD-1 mice at a single dose of 0.1 or 10 mg/kg bw with toxicity investigations at 24 hours or

14 days, respectively, post-dosing (n=5 per dose group). Clinical signs including mortality, body weight change, change in general activity level, and increased/decreased food and water intake were recorded, as well as skin changes and increased sensitivity to light. Blood samples were collected for clinical biochemistry analysis and the liver, heart, kidney and lungs were subjected to close inspection and histopathology. No adverse effects were observed 24 hours or 14 days post-dosing (Li et al., 2012).

Negreş et al. (2016) administered hyperforin ethylene diammonium salt (HY-EDS) to male white NMRI mice (n=6) at a single oral dose of 2,000 or 5,000 mg/kg bw. The animals were observed once a day for signs of toxicity (pathological changes in skin and/or mucous membranes, changes in appearance, pathological impairment in organs and systems, changes in behaviour) during 14 days. After 14 days, biochemical parameters including liver transaminases, creatinine and serum glucose were determined. No lethality was observed. A statistically significant increase in aspartate transaminase (AST) and alanine transaminase (ALT) levels was observed at both dose levels, compared with the control group. The graphs in the publication indicate that the increases in AST were approximately 2-fold and the increases in ALT approximately 4-fold (Negreş et al., 2016).

Rats
Two rats of 180 g bw receiving one total daily oral dose of either 30 or 60 mg hypericin (equal to 333 mg/kg bw) showed induction of enhanced photosensitivity the next day (Pace, 1942, as cited in SCF, 2002).

In a study by Vandenbogaerde et al. (2000) male rats (8 to 12 per group) were given dry St John's wort extract (containing 0.11% hypericin and 0.43% pseudohypericin) at dose levels of 0, 926, 1,852 or 2,778 mg/kg bw (equal to 0, 1, 2 or 3 mg hypericin/kg bw and 4, 8 and 12 mg pseudohypericin/kg bw) by oral gavage. Two additional groups of rats were given 15 mg/kg bw of a mixture of hypericin and pseudohypericin (equal to 3 mg/kg bw hypericin and 12 mg/kg bw pseudohypericin; purity >98%) or 2 mg/kg bw of protohypericin (purity >99%).
One hour post-dosing, the animals were tested for locomotor behaviour in an open-field test and for anxiolytic effects in a light-dark test. St John's wort extract statistically significantly increased the total length of pathway and the number of crossings and rearings measured during the open-field test at the highest dose tested, and the number of rearings in all treatment groups in comparison with the control. No effects were observed with the hypericin/pseudohypericin mixture and with protohypericin.
The mid dose of the extract produced an anxiolytic response of the same magnitude as 1.5 mg/kg bw diazepam (i.p.), but this was not observed at the high dose. This effect could be blocked by the benzodiazepin antagonist flumazenil (3 mg/kg i.p.). Since hypericin/pseudohypericin and protohypericin failed to alter the different parameters evaluated during the open-field test, these results suggest that other constituents than hypericin are contributory to or responsible for the locomotor and anxiolytic effects of St John's wort extract (Vandenbogaerde et al., 2000).

LD_{50} values published in the EMA assessment report for *Hypericum* extract LI 160 (LI 160, extraction solvent methanol 80%, DER 3–6:1) (EMA, 2009a) and Li et al. (2012) are provided in Table 5.18.

Table 5.18 LD_{50} values for Hypericum extract LI 160 in mice and rats after oral, intravenous or intraperitoneal administration.

Species	**Test substance**	**Route**	**LD_{50}** (mg/kg bw)	**Reference**
Mouse	Hypericum extract LI 160	oral	≥5,000	EMA, 2009a
Rat	Hypericum extract LI 160	oral	≥5,000	EMA, 2009a
Mouse	Hypericin	i.v.	21.23 (males); 19.30 (females)	Li et al., 2012
Mouse	Hypericum extract LI 160	i.p.	1,780	EMA, 2009a
Rat	Hypericum extract LI 160	i.p.	1,000	EMA, 2009a

Sheep
Groups of 11 shorn ewes were given a single dose of finely ground, dried, flowering-growth-stage St John's wort plant material as a slurry by stomach tube at 2.85, 4.0 or 5.7 g dry plant/kg bw. This corresponded to 2.65, 3.7 and 5.3 mg/kg bw hypericin, respectively. After dosing, the animals were exposed to bright sunlight for up to 5 hours per day over up to five successive days or shorter if moderately severe clinical signs developed. Control groups were not included. Clinical signs were recorded and rectal temperature was measured. Ingestion of St John's wort followed by exposure to bright sunlight frequently resulted in clinical signs attributable to skin irritation and central nervous effects, including an adverse increase in body temperature. The severity of the clinical signs and hyperthermia was dose-related. The LOAEL in this study was 2.65 mg/kg bw per day, the lowest dose tested (Bourke, 2000, based on citation by SCF, 2002).

Bourke (2003) investigated the effect of shade, fleece length and wool type in the protection of Merino sheep (10 per group) against St John's wort poisoning. Poisoning was defined as a rectal temperature of >40°C after 5 hours outdoors. During late spring and summer a series of successive, replicate experiments were conducted, each using one group and lasting 5 days. The sheep carried 14 to 24 weeks' wool growth. In each experiment the treatments tested were: St John's wort+, sunlight+, (n=7); St John's wort, sun- (n=1); St John's wort-, sun+ (n=1); and St John's wort-, sun- (n=1). In addition, two groups of 12 sheep consisting of 9 recently (1 to 3 weeks previously) shorn and 3 wool-covered (25 to 26 weeks' growth) were tested. The treatments tested were St John's wort+, sunlight+, fleece- (n=9); St John's wort+, sun-, fleece+ (n=1); St John's wort-, sun+, fleece+ (n=1); and St John's wort-, sun-, fleece+ (n=1). Finely milled St John's wort was administered by gavage to provide a single dose of 3 mg/kg bw hypericin. The sheep were sheltered from direct sunlight or were exposed for 5 hours per day for four successive post-treatment days. Of

the wool-covered sheep that were exposed to sunlight, 26.5% developed St John's wort poisoning, while none of the fully shaded sheep showed signs of poisoning. The percentages of poisoned sheep based on wool type were: 14% for superfine wool, 28.5% for fine wool and 33.3% for medium wool. Of sheep that were treated similarly but were recently shorn, 94% displayed St John's wort poisoning (Bourke, 2003).

Calves
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 (no control group was included). In animals of the 3 and 5 g/kg bw groups, exposure to direct sunlight produced signs of enhanced photosensitivity after 3–4 hours. Effects were restlessness, signs of dermal irritation, reddening of the skin around the eye and reddening of naked and white-haired skin, and in the highest dose group also scab formation and exudation. Recovery from these dermal lesions took 30–40 days. Blood levels of creatine phosphokinase were increased, but sorbitol dehydrogenase, gamma glutamyl transpeptidase, glutamate dehydrogenase and arginase were not increased. 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 that were not exposed to direct sunlight. The NOAEL was 124 µg hypericin/kg bw (Araya and Ford, 1981, as cited in SCF, 2002).

Steers
Finely milled St John's wort (of Australian origin) was administered orally to groups of Hereford (n=18) and Angus (n=18) steers using gelatine capsules to provide a dose of 1.5 mg/kg bw hypericin (Bourke & White, 2004). All animals were then exposed to direct sunlight for 5 hours per day for five days. None of the treated animals showed a rectal temperature of >40°C or displayed other clinical signs. The authors therefore question the oral toxic dose of 124 µg hypericin/kg bw reported by Araya & Ford (1981) and suggest that the hypericin content of the extract used by Araya & Ford (1981) was probably higher than they assumed (Bourke & White, 2004).

Monkeys
Rhesus monkeys were given a dose of 2 or 5 mg/kg bw hypericin intravenously. A dose of 2 mg/kg bw was well tolerated, whereas at a dose of 5 mg/kg transient severe photosensitivity rash occurred (Fox et al., 2001, as cited in EMA, 2009a).

5.3.2 *Short-term and sub-chronic toxicity*

Two studies (in rats and in sheep) were identified by SCF (2002) and two additional studies (in rats and dogs) by EMA (2009a). Their summaries are provided below. Two additional studies on the short-term or sub-chronic toxicity of St John's wort have since been identified.

Mice
As part of a study on the anti-influenza virus effect of St John's wort extract, Pu et al. (2009) administered St John's wort extract to female BALB/c mice at dose levels of in total 0, 125, 250, 500, 1,000 or 2,000 mg/kg bw per day, given twice daily via oral gavage for 5 days. Body weights were determined before treatment and 18 hours after the

last treatment. Animals were observed for mortality for 14 days. All animals survived except one mouse in the 125 mg/kg per day dose group. No attempt was made to determine the cause of death. In the highest treatment group a weight loss of 0.4 g was observed. In the other treatment group, the weight gain varied between 0.1 and 1.2 grams, compared with 2.4 g in the control group (Pu et al., 2009).

Negreş et al. (2016) administered 0, 50, 75 or 100 mg/kg bw per day hyperforin ethylene diammonium salt to male white NMRI mice (n=20 per group) by gastric intubation once daily for 28 consecutive days. Lethality, body weight, feeding behaviour, motor behaviour, aggressiveness and appearance were recorded. At days 15 and 22, motor behaviour was tested by the Activity Cage system. At the end of the observation period, samples were collected, including blood, serum, liver, kidney and brain. There were no differences in body weight gain across dose groups. Vertical motor activity was statistically significantly reduced at day 22 in all treated groups, but not horizontal motor activity. No significant differences in leukocyte, thrombocyte or erythrocyte count, or in haemoglobin, haematocrit, total bilirubin or serum creatinine levels were observed. AST, ALT and alkaline phosphatase (ALP) levels were statistically significantly increased in all dose groups. In the 50 mg/kg bw dose group, histopathological examinations of the liver revealed granulovacuolar hepatosis in all animals, while kidney and brain were generally unaffected except the appearance of nonpurulent encephalitis in one animal. Adverse effects observed in the 75 mg/kg bw dose group included granulovacuolar hepatosis (n=1), extramedullary haematopoiesis (n=7), hepatic lipidosis (n=3), neuronal necrosis (n=2) and glial nodules (n=1, not treatment-related according to the study authors). At the highest dose of 100 mg/kg, granulovacuolar dystrophy of the liver (n=6), extramedullary haematopoiesis (n=1), lipidosis (n=1) and necrotic hepatocytes (n=1) were recorded. The kidney and brain did not reveal any histopathological changes in the high-dose group (Negreş et al., 2016). From this study, a LOAEL of 50 mg/kg bw per day for hyperforin ethylene diammonium salt could be derived. This amounts to approximately 45 mg/kg bw hyperforin.

Rats
Male rats (8 per group, initial body weight ± 90 g) were given 0% or 10% St John's wort meal mixed in the feed. The concentration in the feed was reduced to 5% (50,000 mg/kg feed, equivalent to 5,000 mg/kg bw per day) after 12 days because of decreased feed intake. The hypericin content of the food was not specified. Four animals per group were killed after 119 days and necropsied. No significant tissue lesions (investigated tissues not specified) were observed. The remaining animals were killed after 178 days and showed decreased average body weight gain over the entire exposure period (Garret et al., 1982, as cited in SCF, 2002).

In rats exposed to St John's wort extract (LI 160, extraction solvent methanol 80%, DER 3–6:1) at dose levels of 900 and 2,700 mg/kg bw per day for 26 weeks only minor non-specific symptoms (weight loss, minor pathological changes in liver and kidney) were observed. All organs reverted to normal when treatment was stopped (Leuschner, 1996, as cited in EMA, 2009a). No further details were given.

Dogs
The same study was also performed in dogs. In dogs exposed to St John's wort extract (LI 160, extraction solvent methanol 80%, DER 3-6:1) at dose levels of 900 and 2,700 mg/kg bw per day for 26 weeks only minor, reversible, non-specific symptoms (weight loss, minor pathological changes in liver and kidney) were observed (Leuschner, 1996, as cited in EMA, 2009a). No further details were given.

Sheep
Sheep (3 per group) were fed freshly cut St John's wort at dose levels of 0, 4, 8, 12 or 16 g plant/kg bw per day for 14 days and were exposed to daylight. After 7 and 14 days, several haematological and clinical biochemical parameters were studied. The following clinical observations were reported: restlessness, photophobia, tachycardia, polypnea, congested mucous membranes, diarrhoea and hyperthermia. Skin redness on exposed parts of the tail and legs was observed, as well as oedema of the eyelids and swelling of 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 St John's wort 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 (no explanation was given by the study authors). 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, as cited in SCF, 2002).

5.3.3 *Genotoxicity*

Several genotoxicity assays with St John's wort extract, hypericin or photoactivated hypericin are described by SCF (2002) and EMA (2009a) and in the literature. These studies are summarized below and the results are presented in Tables 5.19 (St John's wort extract), 5.20 (hypericin) and 5.20 (photoactivated hypericin).

In vitro tests

Bacterial reverse mutation assay
In a bacterial reverse mutation assay with hypericin (5, 10 and 50 µg/plate; purity unknown) in *Salmonella typhimurium* strains TA98 and TA100, negative results were observed with and without metabolic activation. No information was provided on cytotoxicity (Turek et al., 1997; abstract only). Hypericin (20–100 µg/plate; purity unknown) did not induce reverse mutations in *S. typhimurium* TA97 in the absence or presence of metabolic activation (Miadokova et al., 2010).

Hypericin (purity 98%) and photoactivated hypericin (20–100 µg/plate) were tested in *S. typhimurium* strains TA97, TA98 and TA100 in the absence and presence of metabolic activation using the preincubation

method. After administration of the test substances to the test tubes, half of the tubes were exposed to a light dose of 3.65 J/cm^2 for 30 min at 37 °C followed by an additional 30 min incubation at 37 °C. The other half of the test tubes were incubated for 60 min at 37 °C before plating. No increase in the number of revertants was observed after hypericin or photoactivated hypericin treatment (Feruszová et al., 2016).

St John's wort extracts prepared with ethanol, chloroform and ethylacetate were tested in *S. typhimurium* strain TA98, with and without metabolic activation. Positive results were obtained for the ethanol and ethylacetate extracts, tested at 20 and 40 µL extract/plate. After chromatographic separation of the extract, the effect could be assigned to quercetin (Poginsky et al., 1988). The same results were obtained for an ethanol extract tested in *S. typhimurium* strains TA98 and TA100 by Schimmer et al. (1994, as cited in EMA, 2009a).
In none of the tests was information on cytoxicity at the dose levels tested provided.

Gene mutation in mammalian cells
No indications for induction of gene mutations were obtained in a hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) test in which V79 cells (Chinese hamster lung fibroblasts) were exposed for 4 hours to *Hypericum* extract (ethanol extract, DER 1:5–7, 0.2–0.3% hypericin, 0.35 mg/g quercetin) in concentrations up to 0.5 µl extract/ml medium in the absence of metabolic activation and up to 4 µl/ml medium in the presence of rat liver S9 homogenate. Cells were cultured for three more days before they were stained. No information on cytotoxicity was provided (Okpanyi et al., 1990).

Chromosomal aberrations
Hypericin (purity not specified) was tested in chromosomal aberration assays in HepG2 cells (human hepatoma cells), V79 cells (Chinese hamster lung fibroblasts) and VH10 cells (human primary foreskin fibroblasts). The cells were exposed to 100, 500, 750 and 1,000 ng/ml hypericin for approximately 1.5 cell cycle (HepG2 cells 43h, V79 23h and VH10 3h). No significant differences were observed in the number of aberrant metaphases between the hypericin treatment groups and the solvent controls. The highest dose tested in the VH10 cells could not be evaluated due to cytotoxicity. No toxicity was observed in the other cell lines. The positive control benzo(a)pyrene gave a positive response only in the HepG2 cells, since the other cell lines lacked the biotransformation enzyme capacity needed for activation of benzo(a)pyrene (Miadokova et al., 2010).

Feruszová et al. (2016) tested hypericin (purity 98%) and hypericin irradiated with UV in a chromosomal aberration assay in human liver HepG2 cells. The experiments were reported to be based on the OECD Test Guideline No. 473 (In vitro mammalian chromosome aberration test, 1994). HepG2 cells were exposed to 0.005–1.0 µg/ml for 16 hours. Then the medium was replaced by fresh medium and half of the samples were irradiated with UV using a specially modified lamp for 25 min (3.65 J/cm2). The other half of the samples was not irradiated with UV. The cells were then cultivated for additional 36 hours. Colchicin was added into the media 3 hours before the cells were prepared for microscopic

examination for chromosomal aberrations. For each sample, 100 metaphases were analysed, if possible. No increase in the number of cells with chromosomal aberrations was observed after exposure of the cells to hypericin. No increase in cells with chromosomal aberrations was observed after exposure of the cells to hypericin irradiated with UV in concentrations up to 0.05 µg/ml. After treating HepG2 cells with hypericin irradiated with UV at the concentration of 0.05 µg/ml, only 32 evaluable mitoses were recorded. Higher concentrations could not be analysed because no evaluable mitoses were recorded at higher concentrations of hypericin irradiated with UV (0.1–1.0 µg/ml) due to the inhibition of cell proliferation by hypericin irradiated with UV (data not shown) (Feruszová et al., 2016).

Micronucleus test
Hypericin (purity not specified) was studied for photogenotoxicity and photocytotoxicity in a micronucleus test in Chinese hamster lung V79 cells. Cells were incubated with different concentrations of hypericin (up to ~10^{-6} M) for 30 min in the dark and were subsequently irradiated with 0/0, 100/3.3 or 300/10 mJ UVA/UVB per cm^2. Following irradiation, the treatment medium was removed and the cells were washed twice and further incubated in the dark for 24 hours prior to harvesting. No effect on cell proliferation was observed with any of the hypericin concentrations without UV irradiation, whereas in the presence of UV irradiation, cell proliferation was clearly inhibited at concentrations from approximately 10^{-7} M hypericin onwards. A doubling of the number of micronucleated cells was found at 10^{-7} M hypericin in combination with 300/10 mJ UVA/UVB. Lower concentrations of either hypericin or UVA/UVB did not produce increased amounts of micronucleated cells. Higher concentrations could not be analysed due to toxicity (Kersten et al., 1999).

Comet assay
Traynor et al. (2005) used human HaCaT keratinocytes to investigate the photoclastogenic ability of hypericin (purity not specified) upon irradiation with UVA in a comet assay. The cells were incubated with hypericin in concentrations of up to 1 µM for 1 hour and then irradiated with either 0.4 J/cm^2 or 4 J/cm^2 UVA. After irradiation, cells were incubated for 1 more hour. Hypericin uptake by the cells was confirmed by spectrofluorometry. Phototoxicity was determined by neutral red uptake. Irradiating the treated cells with either 4 or 0.4 J/cm^2 UVA resulted in pronounced phototoxicity, with IC_{50} values of 0.06 and 0.36 µM, respectively. Without irradiation, the percentage of viable cells at the highest dose of hypericin tested was approximately 80%. Irradiating hypericin with 4 J/cm^2 UVA resulted in dose-related, statistically significant increases in DNA damage in doses from approximately 0.10 µM onwards. At a dose of 0.4 J/cm2, a statistically significant increase was observed at the highest concentration tested only (Traynor et al., 2005).

The ability of hypericin and hypericin irradiated with UV (0.05-0.75 µg/ml; purity 98%) to induce single-strand DNA breaks in human lymphocytes was evaluated by an alkaline comet assay. Cells were exposed to hypericin for 60 min at 37 °C. As a positive control, 100 µmol/L hydrogen peroxide (H_2O_2) was used. In the case of samples irradiated with UV, after the incubation with hypericin, the cells were washed and placed on the diffuser glass of a specially modified lamp for 10, 15 or 25 min, which is

equivalent to doses of energy of 1.46 J/cm^2, 2.19 J/cm^2 and 3.65 J/cm^2, respectively. Hypericin gave negative results in the comet assay, whereas hypericin irradiated with UV showed an increase in DNA damage that was dose-dependent and dependent on the intensity of the light energy (Feruszová et al., 2016).

Unscheduled DNA synthesis
No indications for induction of UDS were found in primary rat hepatocytes incubated with *Hypericum* extract (DER 1:5–7, 0.2–0.3% hypericin, 0.35 mg/g quercetin) in concentrations of 0.014–1.37 µl/ml medium for 3 hours (Okpanyi *et al.,* 1990).

In vivo tests

Micronucleus test
An *in vivo* micronucleus test with hypericin (2, 4 and 8 g/kg bw; purity not stated) was carried out in mice. From the publication (in Czech) only limited technical details can be obtained. A statistically significant increase in cells with micronuclei was reported in the treated animals, but this increase showed no dose relationship. Results from the negative control were not reported. The relevance of this observation is difficult to assess (Turek et al., 1997).

Chromosomal aberration test
Okpanyi et al. (1990) tested an ethanolic extract of St John's wort (DER 1:5–7, 0.2–0.3% hypericin, 0.35 mg/g quercetin) in a chromosome aberration test in Chinese hamsters. Groups of 5 male and 5 female hamsters were given 10 ml undiluted extract. Bone marrow cells were collected after 6, 24 or 48 hours. In addition, groups of animals were given 1:3 and 1:10 diluted extract, and bone marrow cells were collected after 24 hours. No increases were observed in the percentage of aberrant cells. The authors indicate that weak cytotoxic effects were observed in samples collected after 6 hours, but not after 24 or 48 hours (data not shown).

A chromosomal aberration test in Wistar rats was performed with St John's wort extract by Peron et al. (2013). St John's wort was extracted from capsules (300 mg; equivalent to 0.9 mg of hypericin) and dissolved in water at three concentrations: 0.3, 3.0 and 30.0 mg St John's wort extract/ml. Cyclophosphamide was used as a positive control. For the acute treatment, groups of 3 male and 3 female rats were dosed intraperitoneally with 1 ml/100 g bw of 0, 0.3, 3.0 and 30.0 mg/ml St John's wort extract (equal to 0, 3, 30 or 300 mg St John's wort extract/kg bw) or via gavage with 1 ml/100 g bw of 0, 3.0 and 30.0 mg/ml St John's wort extract (equal to 0, 30 or 300 mg St John's wort extract/kg bw). For the subchronic treatment, groups of 3 male and 3 female rats were given 1 ml of 0, 0.3, 3.0 and 30.0 mg/ml St John's wort extract for 7 days by gavage. Rats were killed 24 h after the last treatment, 30 minutes after intraperitoneal administration of 0.5 ml/100 g bw of colchicine, and bone marrow cells were collected. No statistically significant differences in the mitotic index were observed between the control and the treatment groups. After acute intraperitoneal treatment, a statistically significant increase in cells with chromosomal aberrations was observed at the lowest treatment group only (percentage of cells

with chromosomal aberrations was 2.5% versus 0.3% in the controls). After acute treatment via gavage, the percentage of cells with chromosomal aberrations was statistically significantly increased in both treatment groups. However, this increase was not dose-related (0.6% and 0.5% in the low and high treatment group, respectively) and the percentage of cells with chromosomal aberrations in the control group was low (0.01%). After subchronic treatment via gavage, lower percentages of cells with chromosomal aberrations were observed in the treatment groups than in the control group. These differences were not statistically significant (Peron et al., 2013). Since the statistically significant increases in cells with chromosomal aberrations were observed at the lowest dose only (acute IP treatment) or due to a very low percentage of cells with chromosomal aberrations in the controls, and no increases in cells with chromosomal aberrations were observed after subchronic oral treatment, the results of this study are considered negative.

Mouse spot test
In a mouse spot test, female NMRI mice (about 60 per dose level) were treated with 0, 1, 5 or 10 ml of St John's wort extract/kg bw by gavage at day 9 of gestation. The extract used was an ethanolic extract (DER 1:5–7, 0.2–0.3% hypericin, 0.35 mg/g quercetin). Three weeks after birth, the F1 mice were examined and no increase in mice with discoloured spots in the coat of the offspring was found. No signs of toxicity were observed in the mice (Okpanyi et al., 1990).

Table 5.19 Studies of genotoxicity with St John's wort extract.

Test	Test system	Test substance	Results	Ref.*	Remarks
In vitro					
Reverse mutation	*S. typhimurium* TA98	Ethanol extract	+	a	No cytotoxicity reported. Only 1 strain tested. Positive result attributed to quercetin.
Reverse mutation	*S. typhimurium* TA98	Chloroform extract	—	a	No cytotoxicity reported. Only 1 strain tested.
Reverse mutation assay	*S. typhimurium* TA98	Ethylacetate extract	+	a	No cytotoxicity reported. Only 1 strain tested. Positive result attributed to quercetin.
Reverse mutation assay	*S. typhimurium* TA98 and TA100	Ethanol extract	—	b	No cytotoxicity reported. Only 2 strains tested.
Gene mutation (HGPRT)	V79 cells	Ethanol extract	—	c	No cytotoxicity reported.
Unscheduled DNA Synthesis	Primary rat hepatocytes	Ethanol extract	—	c	No cytotoxicity reported.
In vivo					
Chromosomal aberrations	Chinese hamsters	Ethanol extract	—	c	Weak cytotoxic effects observed in bone marrow samples collected after 6 h, but not after 24 or 48 h.
Chromosomal aberrations	Wistar rats (i.p., single dose)	Extracted from capsules	+/—	d	Positive at the lower dose only.
Chromosomal aberrations	Wistar rats (gavage, single dose)	Extracted from capsules	+/—	d	Positive results statistically significant but not dose related. Negative control extremely low.
Chromosomal aberrations	Wistar rats (gavage, repeated dose)	Extracted from capsules	—	d	No cytotoxicity reported.
Mouse spot test	NMRI mice (gavage)	Ethanol extract	—	c	No cytotoxicity reported.

* a) Poginsky et al. (1988); b) Schimmer et al. (1994); c) Okpanyi et al. (1990); d) Peron et al., 2013

Table 5.20 Studies of genotoxicity with hypericin (without irradiation with UV).

Test	Test system	Concentration /dose	Results	Ref.*	Remarks
In vitro					
Reverse mutation	*S. typhimurium* TA98 en TA100	5–50 µg/plate	—	a	No cytotoxicity reported. Only 2 strains tested.
Reverse mutation	*S. typhimurium* TA97	20–100 µg/plate	—	b	No cytotoxicity reported. Only 1 strain tested.
Reverse mutation	*S. typhimurium* TA97, TA98 and TA100	20–100 µg/plate	—	c	No cytotoxicity reported.
Chromosome aberration test	HepG2, V79 and VH10	100–1,000 ng/ml	—	b	No cytotoxicity reported.
Chromosome aberration test	HepG2	0.005–1.0 µg/ml	—	c	No cytotoxicity reported.
Micronucleus test	V79	Up to ~10^{-6} M	—	d	No effect on cell proliferation reported.
Comet assay	HaCaT keratino-cytes	Up to 1 µM	—	e	Cell viability 80% at highest dose tested.
Comet assay	Human lymphocytes	0.005–1.0 µg/ml	—	c	No cytotoxicity reported.
In vivo					
Micronucleus test	Mice	2, 4 and 8 mg/kg bw	+	a	Results not dose-dependent. Limited information (article in Czech).

a) Turek et al. (1997); b) Miadokova et al. (2010); c) Feruszová et al. (2016); d) Kersten et al. (1999); e) Traynor et al. (2005)

Table 5.21 Studies of genotoxicity with hypericin irradiated with UV.

Test	Test system	Concentration	Results	Ref.*	Remarks
In vitro					
Reverse mutation	*S. typhimurium* TA97, TA98 and TA100	20–100 µg/plate	–	a	No cytotoxicity reported.
Chromosome aberration test	HepG2	0.005–1.0 µg/ml	–	a	Concentrations higher than 0.05 µg/ml could not be analyzed due to inhibition of cell proliferation.
Micronucleus test	V79	Up to ~10^{-6} M	+	b	A doubling of the number of micronucleated cells was found at 10^{-7} M hypericin. Higher concentrations could not be analysed due to inhibition of cell proliferation.
Comet assay	HaCaT keratinocytes	Up to 1 µM	+	c	Pronounced phototoxicity was observed.
Comet assay	Human lymphocytes	0.005–1.0 µg/ml	+	a	Increase in DNA damage was dose-dependent and light intensity-dependent. No cytotoxicity reported.

a) Feruszova et al. (2016); b) Kersten et al. (1999); c) Traynor et al. (2005)

5.3.4 *Chronic toxicity and carcinogenicity*

No chronic toxicity or carcinogenicity studies were identified.

5.3.5 *Reproductive and developmental toxicity*

In the SCF evaluation, only one pilot study was available (Gonzalez et al., 1998). EMA considered 3 *in vitro* studies in rats and 4 in mice and concluded that the data on reproductive toxicity were contradictory. No differences were observed between mice exposed to *Hypericum* extract (108 mg/kg bw) and controls in a study of reproductive toxicity. However, isolated hypericin seemed to have teratogenic properties. EMA concluded that for safety reasons the oral use of *Hypericum* during pregnancy and lactation should not be recommended (EMA, 2009a). Avila et al. (2018) published a systematic review of rodent studies on the reproductive and developmental toxicity of St John's wort (extracts). Ten rodent studies that met a priori inclusion criteria were identified up to 10 November 2017. No gross abnormalities were observed, and general assessments of maternal and postnatal health in rodents reported to be exposed to St John's wort (extracts) were unremarkable in the majority of trials. Beyond this general statement, this systematic review of animal studies was unable to draw reliable conclusions. Below, the studies described in SCF (2002), EMA (2009a) and Avila et al. (2018) and newly identified studies (Capasso et al., 2005; da Conceição et al., 2010; Nakamura et al., 2013; Vieira et al., 2013a; Vieira et al., 2013b; Campos et al., 2017a; Campos et al., 2017b; Kahyaoğlu et al., 2018) are summarized.

In vitro

Sperm cells were exposed to 0.06 or 0.6 mg/ml of St John's wort (no further details provided) in medium for 7 days. At both concentrations, a statistically significant denaturation of sperm DNA (sperm with intact DNA 79% at low concentration and 0.5% at high concentration, versus 92-95% in controls) and sperm viability (18% viable sperm at low concentration and 7.5% at high concentration versus 53–54% in controls) was observed. Sperm exposed to the high concentration for 7 days showed point mutations in the BRCA1 exon 11 gene. In addition, hamster oocytes were incubated with 0.06 or 0.6 mg/ml St John's wort for 1 hour, and sperm was added thereafter. After 3 hours, the percentage of penetrated oocytes was determined. At the high concentration, there was no penetration of Hamster oocytes, whereas at the low concentration penetration did not differ from control (Ondrizek et al., 1999).

Chan et al. (2001) studied the influence of hypericin (0, 14.2, 28.4, 71.0 and 142.0 ng/ml) on rat embryos explanted on gestational day (GD) 9.5 and cultured *in vitro* for 48 hours. High concentrations of hypericin (71.0 and 142.0 ng/ml) resulted in a significantly lower total morphological score and number of somites compared with control. There was a negative linear trend in total morphological score, yolk sac diameter and number of somites with increasing concentration of hypericin. No statistical difference was observed between exposed and non-exposed rat embryos in crown–rump length. The no-observed-adverse-effect-concentration (NOAEC) in this study was 28.4 ng/ml.

The effect of St John's wort extract (1–300 µg/ml of dry hydromethanolic extract standardized to 0.3% hypericin; corresponding to 0.003–0.9 µg/ml hypericin) on male Wistar rat and human isolated vas deferens contractility as well as the possible mechanism was studied by Capasso et al. (2005). In addition, the St John's components hypericin, hyperforin, quercitrin, rutin and kaempferol were tested. St John's wort extract inhibited rat vas deferens contraction with a statistically significant inhibitory effect achieved irrespective of the stimulus at a concentration of 30 µM. Hyperforin (10^{-8}–10^{-4} M) significantly inhibited phenylephrine-induced contractions, with statistically significant inhibition at concentrations of $3x10^{-7}$ and higher. Hypericin, rutin and quercitrin increased contractions, and kaempferol was inactive. The phenylephrine-induced contraction of human vas deferens was inhibited by St John's wort extract or hyperforin with IC_{50} concentrations of 13.9±2.0 µg/ml and 0.45±0.04 µM, respectively.

The effects of 25–250 µg/ml St John's wort extract (10:1 extract, standardized to 0.3% hypericin; corresponding to 0.075–0.75 µg/ml hypericin) and 7.5 and 75 ng/ml hypericin (>85%) on *in vitro* placental calcium (Ca^{2+}) transport using the human placental choriocarcinoma cell line JEG-3 were examined by da Conceição et al. (2010). After 48-hour incubation, a significant decrease in the viability of the cells was observed with 150 and 250 µg/ml St John's wort extract (no information on effects of hypericin on cell viability was provided). At 75 µg/ml, cells showed morphological alterations, although there was a higher percentage of viable cells compared with controls. The results did not show effects of St John's wort extract and hypericin on placental differentiation. However, St John's wort extract and hypericin affected the intracellular Ca^{2+} levels by changing the protein expression of the Ca^{2+} transport system. There was no significant difference in this respect between the different concentrations.

The embryotoxic effect of increasing concentrations up to 10 µM hyperforin (not further specified) was studied using mouse embryonic stem (ES) cells (an embryonic tissue cell model) and NIH/3T3 fibroblasts (an adult tissue cell model) (Nakamura et al., 2013). Hyperforin statistically significantly caused cell death in both cell types in a dose-dependent manner after a 7-day incubation period at high concentrations (approx. >1 µM). IC_{50} values were 2.38 and 5.89 µM for NIH/3T3 fibroblasts and ES cells, respectively. After a 24- or 72-hour incubation period with 10 µM hyperforin, viable ES cells were 37.38±1.42% and 16.46±3.13%, respectively. Further experiments on ES cells indicated that at high concentrations hyperforin inhibited ES cell proliferation rather than inducing apoptosis. After a 72-hour incubation period, 10 µM hyperforin resulted in 6.52±0.25% viable NIH/3T3 cells. Further experiments on NIH/3T3 cells suggested that hyperforin could both induce apoptotic cell death in this cell type, as indicated by increased activity of caspases, and inhibit cell proliferation. The ES cell differentiation system was affected by 10 µM hyperforin, leading to a decreased differentiation into mesodermal and endodermal lineages.

In vivo

Mice
In a pilot study where mice were dosed with 0.75 mg dried St John's wort plant material/g food (136 mg/kg bw per day, no detailed information on the preparation) from day 14 before mating throughout gestation, a reduced litter size and a reduced body size at birth were observed (no information provided on the size of the effects). The study was published only as an abstract (Gonzalez et al., 1998, as cited in SCF, 2002).

Gonzalez et al. (1999) studied the impact of antenatal St John's wort exposure on cognition in mice. Female mice were given 182 mg/kg bw per day St John's wort (no details provided on the preparation) orally for 2 weeks before mating and throughout gestation. This dose has antidepressant efficacy in the maternal mice. Prenatal exposure to a therapeutic dose of St Johns; wort did not have a major impact on selected cognitive tasks in mice offspring (Gonzalez et al., 1999, as cited in EMA, 2009a). No further details are provided by EMA, but this abstract by Gonzalez et al. (1999) might describe the same study as the publications listed below by Rayburn et al. (2000, 2001a, 2001b).

Three publications by Rayburn et al. on reproductive toxicity studies in CD-1 mice are available (Rayburn et al., 2000, 2001a, 2001b). The same body weights are reported for male offspring in Rayburn et al. (2000) and Rayburn et al. (2001a). The same decrease in dose level for the dams is also reported in all three studies. In this report it is therefore assumed that these three publications describe the results from one study, although the authors do not comment on this in their papers.
Groups of 45 female CD-1 mice were provided with feed bars containing either St John's wort extract (standardized to 0.3% hypericin) at 0.75 mg/g (equivalent to 180 mg/kg bw per day) or placebo for two weeks before mating and throughout gestation (Rayburn et al., 2000). This dose of 180 mg/kg bw per day equals the human therapeutic dose of 900 mg extract, or 15 mg extract/kg bw per day, using a mouse–human surface conversion factor of 12. Feed bars were made with a mixture of food flour and either St John's wort extract in water or water only. Live births per litter, birth weights and sex ratios of the pups were determined. Litter size was reduced to a maximum of 8 pups (4 males and 4 females) on postnatal day (PND) 5. Behavioural testing of offspring was conducted at the same time during the infant, juvenile and adult periods. These included separation vocalization on PND3 and 5, negative geotaxis on PND3 and 5, homing on PND9, locomotor activity on PND13, 15, 21 and 65, exploratory behaviour on PND30 and 60, elevation plus maze on PND30 and 60, social play on PND18, forced swim on PND90, and male aggression on PND32–38, 65 and 95. In addition, the reproductive capability of two adult female and two adult male offspring from each litter was assessed for a maximum of 3 oestrus cycles.

Maternal body weight did not differ significantly between the groups. The daily dose of St John's wort extract was decreased from 182 mg/kg bw per day to 150 mg/kg bw per day on GD11 because feed intake per kg bw decreased. No statistically significant differences were observed between treated and control groups with respect to time to pregnancy,

duration of gestation, number of live pups, litter size or sex ratio. Birth weights of male offspring were slightly decreased in the St John's wort group compared with the placebo group (1.68 vs 1.75 g; P<0.01). There were no differences in body length or head circumference between the groups or in birth weights of the female offspring and maternal body weight gain during lactation. Offspring in the treatment group (n=52 males and 52 females) showed no statistically significant differences in early developmental tasks, locomotor activity or exploratory behaviour throughout development compared with the placebo group. Performances on a depression task (forced swim) and on anxiety tasks (elevated-plus-maze test as juveniles and adults) revealed no differences between the treatment and placebo groups. Reproductive abilities of the offspring were not affected, as no significant differences were observed in conception rates, litter sizes, sex ratio of pups or birth weights (Rayburn et al., 2000).

In another publication by the same group, results from the same study were described, although in this study a group size of 20 instead of 43 for the F0 females was reported (Rayburn et al., 2001b). Additional parameters described were physical maturation milestones included incisor eruption, eyes opening, vaginal patency and testis bifurcation. A temporary delay in eruption of the upper incisors was observed (details not provided). Female offspring of treated dams showed a lower body weight than control animals at PND30, 37 and 45. No external anomalies were observed in any of the offspring (Rayburn et al., 2001b).

In a third publication, Rayburn et al. (2001a) reported the effects of prenatal exposure of CD-1 mice on certain cognitive tasks in offspring. In this study a group size of 20 instead of 43 for the F0 females was reported. Offspring (one per gender from each litter) were tested in five neurobehaviour tasks: a tube runway on PND34–38, 45 and 80, a water straight runway between PNDs 60 and 64, a Morris spatial maze between PNDs 65 and 72, a Cincinnati decision maze between PNDs 97 and 108, or a passive avoidance task between PNDs 110 and 117. Generally, there were no differences between groups in performing the neurobehaviour tasks, except that female offspring in the St John's wort group were consistently slower (25–62% more time needed) than those in the placebo group in the Morris spatial maze in all 5 sessions, and the difference reached statistical significance in session 2 and 4 (Rayburn et al., 2001a).

Rats
Mated female Sprague-Dawley rats (n=4–5 per group) were exposed to diets containing 0, 180, 900, 1,800 or 4,500 mg/kg St John's wort extract (0.3% hypericin) from GD3 to PND21 (Cada et al., 2001). According to the authors this corresponds to approximately 0, 15, 75, 150 or 375 mg/kg bw per day (assuming that a 300 g pregnant/ lactating rat consumes 25 g of feed/day). Food consumption, body weights and whole and regional brain weights were recorded. In addition, the following behavioural tests were conducted: open field test on PND29-31, 47–49, 70–72, acoustic startle response test on PND61, complex maze test on PND87–91, Morris water maze test on PND97-100, and elevated-plus-maze activity test on PND95.

There were no differences between dose groups with respect to the number of dams that were not pregnant and the number of dams that had birthing difficulties. No differences in gestation duration, pregnancy weight, maternal weight gain or food consumption were observed between the groups. However, it should be noted that the group sizes (n=4–5) were small. Pups from the 15, 75 and 150 mg/kg bw per day groups had lower body weights than pups from control groups on PND56 ($p<0.05$), and pups from the 15 and 150 mg/kg bw per day groups had lower body weights on PND78 ($p<0.05$). Weights of whole brain and brain regions were not statistically significantly different between treatment and control groups. There were no signs of St John's wort-related behavioural alterations.

St John's wort extract (methanol extract, containing 0.3% total hypericin (unspecified)) was administered via gavage to adult female Wistar rats from 2 weeks before mating to 21 days after delivery at doses of 0 (vehicle), 100 and 1,000 mg/kg bw (n=3 per group) or from day of delivery to PND21 by Gregoretti et al. (2004, as cited in EMA, 2009a). Pregnant animals were weighed daily, and number of live pups per litter, birth weights and sex ratios were recorded. Two pups per litter (one male and one female, n=6 per group) from dams that were given St John's wort extract throughout the gestation and lactation period were killed on PND0 and two on PND21, and from dams that received St John's wort extract only during the lactation period and were euthanized on PND21 for microscopic examination of liver, kidney, lung, heart, brain and bowel. No significant differences between the treatment groups were evident regarding maternal body weight, gestation duration, number of live pups or weight of offspring at birth or during the postnatal period. However, it should be noted that the group sizes (n=3) were small. Microscopic analysis revealed signs of toxicity in the liver and kidneys of offspring of treated dams of both dose groups. The livers of pups of mothers that were treated before and during pregnancy and that were euthanized at PND0 showed focal hepatocyte damage with vacuolization of cells. These lesions were more evident at the highest dose, with hepatocyte hyaline degeneration, lobular fibrosis and disorganization of hepatocyte arrays. In the kidneys there was a reduction in glomerular size with disappearance of Bowman's space and hyaline tubular degeneration. Again, these lesions were more prominent at the higher dose. Pups of dams that received St John's wort extract before and during pregnancy and during the breastfeeding period and that were euthanized at PND21 showed the same lesions but more diffuse and serious. In pups of dams that received St John's wort extract only during the breastfeeding period the same, but more severe, lesions were observed. No signs of toxicity were found in the heart, lung, brain or small bowel. All-important in-life data regarding dams and offspring did not show significant differences between the treatment groups. EMA (2009a) noted that the results obtained in this study indicate that further histological studies should be performed in other animal species (not further substantiated) to better evaluate the safety of St John's wort extracts taken during pregnancy and breastfeeding (Gregoretti et al., 2004, as cited in EMA, 2009a). The LOAEL for offspring toxicity was 100 mg extract/kg bw per day.

A reproductive toxicity study with St John's wort extract in female Wistar rats was conducted by Borges et al. (2005). Groups of 15

inseminated rats received 0 or 36 mg/kg bw per day of dried extract of St John's wort (Jarsin) with 0.4% hypericin, corresponding to 0 or 0.14 mg hypericin/kg bw per day, in saline by gavage from GD9 to GD15. Maternal toxicity was evaluated through water and food intake, body weight gain, piloerection, locomotor activity, diarrhoea and mortality. On GD21, the rats were killed and the maternal reproductive tract was removed. The number of corpora lutea, number and weight of foetuses, number of resorptions in the uterine cornea and placental weight were recorded. The indices of implantation and resorption were calculated. No clinical signs of maternal toxicity were observed and none of the variables analysed showed statistically significant differences.

Vieira et al. (2013a) studied the effects of St John's wort on the behaviour of rats treated during gestation and evaluated 10 and 60 days after parturition. Groups of pregnant Wistar rats received 0, 36, 72 or 144 mg/kg bw St John's wort extract (not further specified). The dams were weighed daily during the treatment period. Ten days after parturition a hole-board test was performed to evaluate anxiety and a tail suspension test to evaluate depression. Sixty days after parturition, the same tests were performed as well as a forced swim test. No statistically significant differences in body weight were observed. The animals in the highest treatment group generally performed better in the tests than the controls. No parameters related to reproductive toxicity or offspring toxicity were tested in this study (Vieira et al., 2013a). It should be noted that PND10 is very early for these behavioural tests, because the animals are not yet very mobile at that age.

Groups of nine dams (Wistar rats) were treated daily, by gavage, with 0 or 100 mg/kg St John's wort extract (not further specified) during pregnancy and lactation (GD0–PND21) (Vieira et al., 2013b). Maternal body weight was measured weekly during gestation and lactation. Body weight and anogenital distance of male pups were recorded on PND0 and PND21. Male pups were studied for behavioural changes at PND35. For the evaluation of male reproductive development, 2 pups from each litter were used, one for sexual behaviour evaluation at PND90–100 and the other for testosterone plasma levels, sexual organ weights, sperm count and testis morphometry at PND90–100. Maternal body weight gain and body weight and anogenital distance of male pups at birth and on PND21 were unaffected by St John's wort treatment compared with control. In addition, no adverse effects were observed on behaviour (general activity, habituation memory) or reproductive parameters (histopathological and histological analysis of the testis, absolute and relative testis weight, plasma testosterone levels, sperm production, sperm number and transit time in epididymis, male sexual behaviour, and sexual incentive motivation) of male pups.

In other studies by the same group, the possible antinociceptive and anticonvulsant effects of prenatal *H. perforatum* exposure were studied in female Wistar rats and possible antidepressant and anxiolytic activity of prenatal *H. perforatum* exposure was studied in male Wistar rats. Wistar rats (numbers not provided) were given oral doses of St John's wort extract (hydro alcohol dry extract containing 0.3% hypericin) at 0 (vehicle), 36, 72 and 144 mg/kg bw per day in distilled water via gavage throughout pregnancy (Campos et al., 2017a; Campos et al., 2017b).

F1 females (90 days old) were subjected to different tests to evaluate antinociceptive and anticonvulsant activity (Campos et al., 2017a). Antinociceptive activity was measured in a hot plate test, writhing test and paw oedema test. Anticonvulsant activity was measured by administration of pentylenetetrazol or pilocarpine or by auricular electroshock. Treatment with St John's wort extract showed an antinociceptive and anticonvulsant effect in adult F1 female rats. F1 males (90 days old) were subjected to several tests to evaluate the antidepressant and anxiolytic effects of prenatal exposure to Hypericum extract (Campos et al., 2017b). In male offspring of mothers that received 144 mg/kg bw extract there was an effect on their behaviour in the behavioural depression and anxiety tests. No significant changes in body weight were found. The authors suggest that treatment with Hypericum interferes with neurodevelopment and neurofunction. No parameters related to reproductive toxicity and maternal toxicity were tested in these studies (Campos et al., 2017a; Campos et al., 2017b).

A study on the possible embryotoxic and teratogenic effects of St John's wort in pregnant rats was conducted by Kahyaoğlu et al. (2018). Groups of 18 female Wistar rats (4–5 months old) were given St John's wort extract in drinking water at levels corresponding to 0 (vehicle), 100 and 300 mg/kg bw per day from one week before mating until delivery of the pups. Drinking water was provided ad libitum. Maternal rats were weighed every week to adjust doses and killed immediately after delivery. The obtained offspring were morphologically examined and fixed for evaluation using hematoxylin, eosin and immunohistochemical staining. Exposure to St John's wort extract decreased the pregnancy rate in a dose-dependent manner; however, the total number of pregnancies was low in both the treatment and control groups (8 in control group, 6 in 100 mg/kg bw dose group, 3 in 300 mg/kg bw dose group). The total foetal number (106 in control group, 74 in low-dose group and 38 in high-dose group) was statistically significantly decreased ($p<0.014$), in relation to the number of pregnancies. In the pups, no structural extremity anomalies, facial anomalies or differences of eye openness were observed. There was a 19.9% reduction in the weight of the foetuses in the low-dose group and an 8.4% reduction in the high-dose group, so the reduction was not dose-related. Histological evaluation showed an inflammatory reaction in the liver of the offspring of St John's wort-treated groups. Focal necrosis in each lobe and deteriorating cell layout were detected in the 300 mg/kg bw group. Hydropic and vacuolar degeneration was also observed in the foetuses of rats in the high-dose group. In the kidney tissues of both St John's wort-treated groups, the diameter of glomeruli was decreased, the Bowman's spacee was absent and intense congestion was observed. Additionally, hydropic and hyaline degeneration was seen in kidney tubules. Staining to determine oxidative stress parameters showed low to high levels of damage to liver and kidney tissues in the treated foetuses. The LOAEL for offspring and reproductive toxicity was 100 mg extract/kg bw per day.

5.3.6 *Phototoxicity*

Previous evaluations by SCF and EMA addressed the phototoxic effects of hypericin. SCF concluded that there is ample evidence that St John's wort (extracts) enhances photosensitivity, both in animals and in humans. That is, exposure to hypericin increases the sensitivity of the

skin to subsequent exposure to light due to the formation of reactive oxygen species by light-excited hypericin. The NOAELs for enhanced photosensitivity in humans and animals after single dosing are 62 and 124 µg/kg bw, respectively. Upon repeated dosing, enhanced photosensitivity in humans is seen at lower dose levels (31 µg/kg bw per day) (SCF, 2002). In its assessment of the outcome of the clinical tests with herbal preparations containing St John's wort, EMA concluded that, although St John's wort extracts exert phototoxicity, being less potent than pure hypericin they can be considered safe when administered in the proposed dosage for medicinal use (EMA, 2009a). The community herbal monographs, though, include a reference to possible (allergic) skin reactions, and state that fair-skinned individuals may react to exposure to intense sunlight with sunburn-like symptoms (EMA, 2009b, 2009c).
In the following section, an overview of the relevant studies is given based on the summaries provided by SCF and EMA, complemented by additional studies that were identified in the literature search.

In vitro
Kersten et al. (1999) studied the photogenotoxicity and photocytotoxicity of hypericin (purity not specified) in a micronucleus test in Chinese hamster lung V79 cells. In the presence of UV irradiation (100/3.3 or 300/10 mJ UVA/UVB per cm^2), cell proliferation was clearly inhibited at concentrations from approximately 10^{-7} M hypericin onwards (see Section 5.3.3 for details) (Kersten et al., 1999).

Bernd et al. (1999) investigated the phototoxic activity of different concentrations of the methanolic extract of St John's wort (0.3% hypericin-like derivates considered as hypericin) using human keratinocytes (HaCaT cell line). A comparison was made with the well-known phototoxic agent psoralen. The extract showed absorbance maxima in the whole UV range and in parts of the visible range (around 225 nm, 270 nm and 315 nm). Using UVB irradiation no clear phototoxic effect of St John's wort extract was observed with concentrations up to 100 µg/ml, while using UVA irradiation a clear phototoxic effect (decrease in DNA synthesis) was found at concentrations ≥50 µg/ml. The inhibition of cell growth was both concentration- and light-dependent. Visible light also led to a phototoxic effect of the extract at high concentrations (~≥50 µg/ml) (Bernd et al., 1999, based on summary in EMA, 2009a).

The phototoxic and apoptosis-inducing capacity of pseudohypericin compared with hypericin was assessed by Schempp et al. (2002) using human leukemic lymphoma cells. Concentrations tested included 0, 50, 100, 200, 400 and 800 ng/ml. Both photo-activated hypericin and pseudohypericin resulted in a dose-dependent inhibition of cell proliferation, while neither had this effect when not photo-activated. The IC_{50} of hypericin (100 ng/ml) was lower than that for pseudohypericin (200 ng/ml). In addition, a dose-dependent increase of DNA fragmentation was observed after treatment with both photo-activated pseudohypericin and hypericin (Schempp et al., 2002, based on summary in EMA, 2009a).

Wilhelm et al. (2001) investigated the phototoxic potential of three St John's wort extracts from different sources, as well as that of hypericin,

quercitrin and rutin (three constituents of St John's wort) in a neutral red assay in the human keratinocyte cell line HaCaT. As reference compounds 5-methoxy psoralen, 8-methoxypsoralen, chlorpromazine (positives), coumarine and 7-hydroxy-coumarine (negatives) were included. The three extracts demonstrated cytotoxicity and phototoxicity in a dose- and UVA dose-dependent manner. It was noted that the cytotoxic effect differed greatly between the three extracts, whereas the phototoxic potential was comparable. Hypericin itself evoked severe phototoxic effects under UVA irradiation. Quercetin was found to be cytotoxic without UV irradiation, while rutin was phototoxic. Quercitrin was found to be effective in inhibiting the phototoxic activity of *H. perforatum* extracts, as it significantly increased the LC_{50} values when added to St John's wort extracts as compared with St John's wort extract alone under UVA irradiation (Wilhelm et al., 2001, based on summary in EMA, 2009a).

Schmitt et al. (2006a) examined the cytotoxicity of St John's wort extracts prepared in solvents ranging in polarity, fractions of one extract, and purified compounds in three cell lines. All extracts exhibited significant cytotoxicity. Light-sensitive toxicity (i.e. a statistically significantly lower survival of cells after exposure to light compared with cells not exposed to light) was mainly observed with 20 µM hypericin and with ethanol extracts sequentially extracted following removal of material extracted in either chloroform or hexane before. Light-sensitive toxicity was absent in other St John's wort extracts, despite the presence of more or less comparable concentrations of hypericin compounds in these extracts. From the results of this study it is not clear which compounds contribute most to the (photo)toxicity of the extracts (Schmitt et al., 2006a).

Schmitt et al. (2006b) investigated whether the phototoxicity of hypericin in HaCaT keratinocytes could be attenuated by St John's wort extracts and/or their pure constituents. When cells were exposed to 20 µM hypericin in the presence of an ethanol extract or a chloroform extract, less phototoxicity (25% and 50%, respectively) was observed than with hypericin (20 µM) alone. The ethanol extract used was an ethanol re-extraction of residue following a chloroform extraction containing 3.35 µM hypericin and 124.0 µM total flavonoids. The chloroform extract used did not contain detectable levels of hypericin or other flavonoids. Furthermore, when cells were exposed to 20 µM hypericin in the presence of the St John's wort constituents chlorogenic acid (10 µM) or pyropheophorbide (0.25 µM), less (24% and 40%, respectively) phototoxicity was observed than with 20 µM hypericin alone (Schmitt et al., 2006b).

Traynor et al. (2005) investigated the photoclastogenic ability of hypericin (purity not specified) upon irradiation with UVA in human HaCaT keratinocytes. Irradiation with 0.4 or 4 J/cm^2 UVA led to phototoxicity, with IC_{50} values of 0.36 and 0.06 µM, respectively. Without irradiation, the percentage of viable cells at the highest dose of hypericin tested (1 µM) was approximately 80% (see Section 5.3.3 for details) (Traynor et al., 2005).

Vandenbogaerde et al. (1998) compared the photocytotoxic effects of hypericin (purity unknown) and pseudohypericin (purity >99%, hypericin 0.44%) *in vitro*. Pseudohypericin had a much lower photobiological effect than hypericin in the presence of protein-containing medium, while in the absence of foetal calf serum (FCS) or bovine serum albumin (BSA) this was less pronounced. Further experiments indicated that the intracellular accumulation of pseudohypericin was inhibited by FCS while the cellular uptake of hypericin was not. Both compounds were found to be located near the nucleus in the cell. Hypericin could be extracted from BSA or FCS using organic solvents, while pseudohypericin had much lower yields, indicating irreversible interactions with serum constituents. The authors conclude that the photocytotoxicity of pseudohypericin is about 20 times lower than that of hypericin in the presence of serum, and that it is hypericin that is responsible for the phototoxic action of *Hypericum* (Vandenbogaerde et al., 1998).

The *in vitro* cellular uptake and the photocytotoxic properties of synthesized protohypericin (purity >98%, 1.01% hypericin) were investigated and compared with hypericin by Delaey et al. (1999). Protohypericin is photoconverted into hypericin when irradiated with white light, but not under filtered light (>645 nm) conditions. The photoconversion was slower when the concentration was higher, and occurred faster in dimethylsulfoxide than in phosphate buffer saline supplemented with 10% FCS, probably due to binding to albumin. Regarding cellular uptake, it was found that in HeLa cells the applied extracellular concentrations were lower than the average intracellular concentrations; for example, extracellular application of 0.25, 0.5 and 1 µM and 24-hour incubation resulted in 19.5, 24.0 and 98.6 µM protohypericin intracellularly, respectively. Twice as much hypericin accumulated compared with protohypericin using the same extracellular concentrations. Visualizing the subcellular distribution showed that both compounds concentrated mainly in the perinuclear region.
The phototoxicity of hypericin and protohypericin was investigated by a 24-hour incubation followed by white light irradiation for 1, 4 or 15 minutes. Concentrations inducing 50% photocytotoxicity reduced with increased irradiation time and were >9, 2.4 and 1.5 times, respectively, higher for protohypericin than hypericin (Delaey et al., 1999).

Delaey et al. (2000) studied the role of specific hypericin concentration/light condition combinations on its photocytotoxic effects under normoxic and hypoxic conditions *in vitro*. The concentrations of hypericin used were 300, 740 and 2,950 nM, combined with light fluences of 0.45, 1.35 and 4.05 J/cm^2. In one setting, the fluence rate was kept constant (4.5 mW/cm^2) while the irradiation time was varied: 1.7, 5 or 15 minutes. In the other setting, the irradiation time was kept constant (15 min) while the fluence rate was 0.5, 1.5 or 4.5 mW/cm^2. In addition, flasks made of different materials, i.e. glass flasks and polystyrene flasks, were used to grow the cells. The experiments indicate that the photocytotoxic effect of hypericin is completely oxygen-dependent. The photocytotoxic effect of hypericin decreased to a significant extent under hypoxic conditions compared with normoxic conditions. However, the higher the fluence rate or longer the irradiation time, the lower the decrease in photocytotoxic effect under hypoxic conditions. The type of material the flask was made from had a

significant influence on the results of the experiments under hypoxic conditions, as some photoactivity was still present in the case of polystyrene flasks, depending on the light conditions, while with glass flasks all photoactivity was lost (Delaey et al., 2000).

Theodossiou et al. (2004) studied the photo-induced toxicity of hypericin (purity 99.3%) on PAM 212 murine keratinocytes at different concentrations. In dark conditions, incubation with concentrations up to 250 µM did not result in significant cytotoxicity, but at 500 µM decreased cell survival was observed. The fluorescence intensity of cells after incubation with 5 µM increased with time, whereas incubation with 50 µM showed a decrease in intracellular fluorescence intensity with time. This suggests intracellular aggregation of hypericin. Another finding was that, at a concentration of 5 µM hypericin, the calculated light dose that resulted in 50% or 90% cell death decreased when the incubation time increased. At 50 µM hypericin, on the other hand, 1-hour incubation was just as effective in producing cell death as 7-hour incubation, although in both cases the irradiation doses to reach 50% or 90% cell death were lower than at 5 µM. The authors suggest that non-fluorescent hypericin aggregates have negligible phototoxic potential (Theodossiou et al., 2004).

Onoue et al. (2011) studied the *in vitro* photochemical and phototoxicological properties of major St John's wort components by performing a reactive oxygen species (ROS) assay. The photoreactive compounds were characterized. These included γ-amino-*n*-butyric acid (GABA), melatonin, hyperforin, (pseudo)hypericin, mangiferin, amentoflavone, chlorogenic acid, (-)-epicatechin, I3,II8-biapigenin, procyanidin B-2, hyperoside, isoquercitrin, kaempferol, luteolin, quercetin, quercitrin and rutin. Several constituents showed a photochemical reaction, but only hypericin, pseudohypericin and hyperforin showed a photo-irritant potential. The authors therefore conclude that hyperforin, pseudohypericin and hypericin might be responsible for the *in vitro* phototoxic potential of St John's wort (extracts) (Onoue et al., 2011).

In vitro ocular phototoxicity
Wahlman et al. (2003) studies the in vitro ocular phototoxicity of hypericin. They found that total accumulated protein leakage was positively correlated (r = 0.9) with variability in focal length. Lenses treated with hypericin and irradiated with UVB had an increase in focal length variability as compared with the lenses that were only UVB-irradiated. Lenses without UVB irradiation had much lower focal length variability than irradiated lenses. For non-hypericin-treated lenses, UVB-irradiated lenses had a larger variability (4.58 mm) than unirradiated lenses (1.78 mm). The lenses incubated in elevated glucose concentrations had a focal length variability (3.23 mm) equivalent to that of the unirradiated hypericin-treated lenses (3.54 mm). The authors conclude that photooxidative damage by hypericin results in changes in the optical properties of the lens, protein leakage and finally cataract formation. In contrast to this, high concentrations of glucose induced protein leakage but not changes in optical properties or the opacity associated with a cataract (Wahlman et al., 2003, as cited in EMA, 2009a).

Lens alpha-crystallin, isolated from calf lenses, was irradiated by Schey et al. (2000) in the presence of hypericin (5 x 10^{-5} M, 10 mM ammonium bicarbonate, pH 7.0) and in the presence and absence of light (>300 nm, 24 mW/cm^2). Hypericin-induced photosensitized photopolymerization was assessed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Further analysis of the oxidative changes occurring in alpha-crystallin using mass spectrometry showed specific oxidation of methionine, tryptophan and histidine residues, which increased with irradiation time. Hypericin did not damage the lens protein in the dark. Damage to alpha-crystallin could undermine the integrity of the lens directly by protein denaturation and indirectly by chaperone function disturbance. Therefore, in the presence of light, hypericin can induce changes in lens protein that could lead to the formation of cataracts. The authors concluded that appropriate precautions should be taken to protect the eye from intense sunlight while on this medication (Schey et al., 2000, as cited in EMA, 2009a).

He et al. (2004) assessed whether hypericin could be phototoxic to the eye. They exposed human lens epithelial cells to 0.1–10 µM hypericin and irradiated them with 4 J/cm^2 UVA or 0.9 J/cm^2 visible light. Neither hypericin exposure alone nor light exposure alone reduced cell viability. In contrast, cells exposed to hypericin in combination with UVA or visible light underwent necrosis and apoptosis. The ocular antioxidants lutein and N-acetyl cysteine did not prevent damage. Thus, ingested St John's wort extract is potentially phototoxic to the eye and could contribute to early cataractogenesis (He et al., 2004, based on summary in EMA, 2009a).

Wielgus et al. (2007) analysed whether hypericin might be phototoxic to the human retina by exposing human retinal epithelial cells to 0.1–10 µM hypericin. The detection of fluorescence emission from the cells confirmed hypericin uptake by human retinal pigment epithelial (hRPE) cells. Neither hypericin exposure alone nor visible light exposure alone reduced cell viability. However, when hypericin-exposed cells were irradiated with 0.7 J/cm^2 of visible light, there was loss of cell viability. The presence of hypericin in irradiated hRPE cells significantly changed the redox equilibrium of glutathione and decreased the activity of glutathione reductase. Increased lipid peroxidation correlated to hypericin concentration in hRPE cells and visible light radiation. The authors conclude that ingested St John's wort extract is potentially phototoxic to the retina and could contribute to retinal or early macular degeneration (Wielgus et al., 2007, based on summary in EMA, 2009a).

Ehrenshaft et al. (2013) found that hypericin could promote polymerization of bovine alpha-crystallin when the latter was exposed to UVA, accumulate in HLE cells in the cytoplasma, and that hypericin could colocalize with alpha-crystallin. In addition, UVA irradiation of hypericin-containing human lens epithelial cells resulted in decreased detection of alpha-crystallin and accumulation of *N*-formylkynurenine (NFK). Blocking wavelengths <400 nm decreased hypericin-mediated photosensitization but did not completely eliminate it. In the absence of light, hypericin did not result in cytotoxicity but cell viability decreased with increasing hypericin concentration: 79.3% survival rate at 2 µM and 69% at 10 µM. From these results the authors conclude that hypericin may cause irreversible, cataract-promoting changes to proteins of the human lens,

and because both visible and UV wavelengths can do this, blocking UV irradiation only protects to a limited extent against hypericin photosensitization (Ehrenshaft et al., 2013).

Animals
Studies describing phototoxic effects observed in animals after a single dose of St John's wort (Araya and Ford, 1981; Bourke, 2000, 2003; Fox et al., 2001) are described in Section 5.3.1 and a study describing phototoxic effects after short-term exposure (Kako et al., 1993) is described in Section 5.3.2.

5.3.7 *Human information*
SCF (2002) described one study (Brockmöller et al. 1997) with the St John's wort extract LI 160 in human volunteers, from which a NOAEL of 62 µg/kg bw for enhanced photosensitivity was identified for a single dose, and a LOAEL of 31 µg/kg bw per day for the same effect after repeated dosing. In addition, they reported one meta-analysis of clinical trials (Linde et al., 1996) in which several adverse effects, such as dry mouth, gastrointestinal effects and skin redness with pruritus, were observed, but it was not possible to derive a NOAEL for these effects. EMA (2009a) described several clinical trials and case reports and concluded that the observed adverse events are generally mild and that they occur with a lower frequency than reported for synthetic antidepressants. These studies as well as newly identified studies are described below.

Case reports

Reported in literature
For the present report, only case reports in which adverse events were associated with the oral use of St John's wort products in individuals who were not also taking selective serotonin reuptake inhibitors (SSRIs) or other herbal products were included. This referred to 14 males and 21 females aged between 21 and 76 years (all 35 case reports are listed in Annex 3). The St John's wort products used included extracts, tea and oil. Dose information was not always available, but the dose information that was reported was consistent with normal use of these products (no overdoses). The time to onset of the adverse events varied greatly. In some cases the adverse effect occurred a couple of hours after a single exposure, whereas in other cases it required years of constant/regular exposure.
The reported adverse effects were on the nervous system, skin (phototoxicity) and hair, urinary tract, reproductive system and digestive system. In almost all cases, adverse effects were reversible and disappeared (quickly) after withdrawal from St John's wort products with or without treatment with supporting medication. In one case, however, the patient was still suffering from gross hyperpigmentation seven years after withdrawal from the St John's wort product. In this case, also St John's wort oil was applied topically.

Reported in the Netherlands
The Netherlands pharmacovigilance centre, Lareb, published a report about the reported side effects of the use of products containing St John's wort, as a medicinal product and as a herbal preparation (Lareb,

2018). From June 1999 to September 2018, Lareb received 57 reports of adverse drug reactions and interactions, possibly related to St John's wort products, whether or not in combination with (other) human medicinal products (in total 94 side effects) (Lareb, 2018). Of these 57 reports, 4 related to a registered medicinal product; the remainder related to non-registered food supplements (n=50) or teas (n=3). Reported side effects included dizziness, diarrhoea, skin reactions and, most prevalent, psychiatric symptoms. Lareb concluded that side effects can be serious and that consumers need to be fully informed of the risk of these. Lareb therefore advised that warnings about possible undesirable side effects and interactions should be included on the packaging of all products containing St John's wort.

Clinical data
Clinical trials investigating the use of St John's wort extracts are listed in Annex 4. The results of these studies suggest that St John's wort extract has an incidence (see Annex 4) of adverse reactions similar to that of placebo (data not shown). The most common adverse effects are gastrointestinal symptoms, sleep disturbance, dizziness/confusion and tiredness/sedation. A potential serious adverse effect is photosensitivity, but this appears to occur rarely (see also phototoxicity).

Pregnancy and breastfeeding
Lee et al. (2003) studied the safety of products containing St John's wort during breastfeeding. From 33 women who were using St John's wort extracts during breastfeeding and contacted the Motherisk Program (Canada) with a concern related to this (group 1), information was collected via a questionnaire about maternal age, marital status, education, family income, obstetrical history, medication use, gestational age when infant was born, infant birth weight, age and weight at time of follow-up, health problems of the infant, duration of breastfeeding and age at introduction of formula, maternal and infant problems related to breastfeeding, decrease in milk production and concerns about infant's weight gain. The same information was collected from disease-matched controls – 101 mothers who did not take St John's wort as (group 2) – and a non-disease matched control group – 33 age- and parity-matched mothers (group 3). This information was collected when their infants were about 15–16 months old.
There were no significant differences between the three groups related to maternal and infant demographics, infant feeding methods or infant weight gain. The dose used by women in the St John's wort group (group 1) was 705±464 mg/day (225–2,150 mg/day) for a duration of 1.5±1.7 months, commencing at 4.2±3.6 months postpartum. Three of the 33 women commenced St John's wort extracts during pregnancy. The mean duration of infant exposure to St John's wort extract was 2.1±3.5 months. No adverse events were reported for the mothers in any of the groups. In the St John's wort group, two cases of colic, two cases of drowsiness and one case of lethargy were reported, while in both control groups only one infant was reported as having colic. It is not known whether these five adverse events were due to St John's wort extract exposure via breast milk. No medical intervention was needed for any of these infants. The median daily dose of St John's wort extract used by the women in group 1 who reported an adverse event for their infant was not

significantly different from that used by women not reporting an adverse event (450 mg/day versus 600 mg/day) (Lee et al., 2003).

Moretti et al. (2009) assessed the risk of major congenital malformations and other adverse pregnancy outcomes using a prospective, observational, controlled cohort design within the Motherisk Program. Women who used St John's wort extracts at any time during pregnancy were compared with a disease-matched group of women with depression who were using conventional pharmacologic treatments and with healthy women not exposed to any known teratogens.
Fifty-four pregnancies were followed in which the mothers took St John's wort during pregnancy: in 49 cases (76%) St John's wort extract was used at least during the first trimester, in 7 cases the use was continued into the second or third trimester, and in 5 cases it was used only during the second or third trimester. The reported average daily dose was 615 mg among those using tablets. In three cases St John's wort was taken as a tea, in one case as a tincture and in another case as granules. There were no differences in baseline characteristics between the different groups and pregnancy outcomes also showed no statistically significant differences. The rates of major malformations were similar across the three groups with two malformations observed in the St John's wort group (5%), two in the disease control group (4%) and zero in the healthy control group (0%). There were slightly more spontaneous abortions in the St John's wort group (11/54, 20.3%) than in the disease control (7/54, 12.5%) or healthy control (5/54, 8.9%) group but this did not reach statistical significance (Moretti et al., 2009).

Another study was undertaken by Kolding et al. (2015) into the association between maternal use of St John's wort extract during pregnancy and pregnancy outcome and malformations in the Danish National Birth Cohort. A total of 38 pregnant women who took St John's wort extract during pregnancy were included, as determined during an interview at week 17 of gestation. None of these women used other antidepressants during pregnancy. The control group consisted of 90,128 women, including women who had used other antidepressants during pregnancy. Gestational age, preterm birth (defined as birth before 37 completed weeks of gestation), birth weight, head circumference, length, malformations and Apgar scores were recorded at birth and compared between groups. Adjustments were made for maternal age, smoking and alcohol intake.
The pregnancy outcomes were similar between the two groups. Malformations (hypospadias, bilateral hip dislocation and heart septum defect) were found in 8.1% (3/37) of the infants from women who took St John's wort extract during pregnancy, and in 3.3% of the infants born in the control group. The incidences of malformations were not statistically significantly different between the two groups ($p=0.13$). Neither adjustments for potential confounders nor exclusion of women who used any kind of antidepressants in the control group influenced the results (Kolding et al., 2015).

Phototoxicity
SCF reports the study of Brockmöller et al. (1997), who conducted a single- and multiple-dose study with tablets (LI 160) containing 300 mg St John's wort extract (363 µg hypericin and 574 µg pseudohypericin)

per tablet in healthy male volunteers. In the single-dose study, each volunteer received 12 tablets (placebo or with St John's wort extract), resulting in a dose of 0, 900, 1,800 or 3,600 mg St John's wort extract with a washout period of 14 days between each dose. Skin irradiation by UV light was done 4 hours after intake, which corresponds to the T_{max}, and the skin reaction was measured at 5, 20 and 68 hours after irradiation.
There were no effects on the minimal erythema dose found after irradiation with solar-stimulated irradiation (UVA and UVB) when compared with placebo. Irradiation with only UVA resulted in a marginally significant increase in the tanning reaction, thus to a reduction in the minimal tanning dose (-16%). This was observed only at the highest dose of 3,600 mg St John's wort extract (equal to 4,356 µg hypericin and 6,888 µg pseudohypericin). There was also no correlation found between total hypericin (hypericin + pseudohypericin) plasma concentrations and photosensitivity. As previously mentioned, SCF derived a NOAEL for phototoxicity of 4,356 µg hypericin, corresponding to 62 µg/kg bw for a 70 kg person.
In the repeated-dose study, subjects received 2 tablets of 300 mg St John's wort extract each three times a day (1,800 mg per day) corresponding to a daily dose of 2,180 µg hypericin and 3,440 µg pseudohypericin during two weeks, with the last dose in the morning of day 15. Skin irradiation by UV light was done 4 hours after intake on day 1 and day 15 and the skin reaction was measured at 5 hours, 20 hours and 7 days after irradiation. Both the minimal erythema dose (-6%) and minimal tanning dose (-21%) were statistically significantly lower than pre-treatment. From this study, SCF identified a LOAEL of 2,180 µg hypericin for enhanced photosensitivity, corresponding to 31 µg/kg bw per day for a 70 kg person (Brockmöller et al., 1997, based on summary in SCF).

Köppel et al. (2008) investigated the effect of an ethanolic (60% v/v) St John's wort extract on photosensitivity. Each volunteer received 3 capsules (3.5–6:1/capsule, Esbericum ® capsule) twice daily for two weeks. The photoreaction and symptoms of skin reaction were evaluated 24 hours after irradiation, which was performed at days -2 and 14. The study revealed that in the presence of a stable plasma concentration of hypericin (6.72 ng/ml) the minimal erythema dose values did not change significantly (Köppel et al., 2008, based on summary in EMA, 2009a).

EMA (2009a) reported a prospective randomized study by Schempp et al. (2003), who investigated the effect of the St John's wort extract LI 160 on skin sensitivity to UVA and UVB, visible light and solar-simulated radiation. Seventy-two volunteers of skin types II and III were included and were divided into six groups of 12. In the single-dose study the volunteers (n=48) received 6 or 12 coated tablets (5,400 or 10,800 µg hypericin). In the steady-state study the volunteers (n=24) received an initial dose of 6 tablets (5,400 µg hypericin), and subsequently 3 x 1 tablet (2,700 µg hypericin) per day for seven days. Photo testing was performed on the forearms prior to medication and 6 hours after the last administration of St John's wort extract. After both single-dose and steady-state administration, no significant influence on the erythema index or melanin index could be detected, with the exception of a marginal influence on UVB-induced pigmentation ($p = 0.0471$) in the

single-dose study. The results do not provide evidence for a phototoxic potential of St John's wort extract LI 160 in humans when administered orally in typical clinical doses up to 1,800 mg extract daily for seven days (Schempp et al., 2003, based on summary in EMA, 2009a).

Schulz et al. (2006) investigated the effect of two different St John's wort extracts (STW 3, STW 3-VI) on photosensitivity with respect to minimal erythema dose (MED) after 14 days' treatment. Both open, multiple-dose, one-phase studies were conducted in 20 healthy men, receiving 1 tablet per day. MED values were determined prior to St John's wort extract administration (baseline) and after 14 days of treatment using an erythema tester emitting a light very similar to sunlight (main emission spectrum: 285–350 nm). Skin reactions with respect to MED were evaluated 12 h, 24 h (primary endpoint), 48 h and 7 days after irradiation. All volunteers reached a steady state of hypericin/pseudohypericin plasma concentration before study day 14, when the irradiation under treatment conditions took place. In all subjects MED was measurable under baseline and under St John's wort treatment conditions. With respect to the primary endpoint, in both studies, mean MED (24 h) were not significantly different between baseline and after 14 days of St John's wort treatment. There were no clinically relevant changes in the laboratory parameters, the vital signs, physical findings or other observations related to safety during the examinations. In one study (STW 3), two adverse events were reported, both described as hypersensitivity to light in mild intensity. Both studies showed that both St John's wort extracts were safe medications under steady state and prescribed conditions, without significant increases of photosensitivity (Schulz et al., 2006, based on summary in EMA, 2009a).

Hohmann et al. (2016) analysed the occurrence of adverse events in a clinical phase I trial of St John's wort treatment. Healthy human volunteers (n=12) were given 300 mg St John's wort extract (Jarsin®) orally once daily for two weeks, then 300 mg St John's wort extract orally three times a day for two weeks and finally 600 mg St John's wort extract three times a day for two weeks. During the last week of the trial 600 mg St John's wort extract three times a day was orally co-administered with 600 mg rifampicin (an antibiotic) once daily. Within 3 to 6 days of the increase in St John's wort extract dose to 600 mg three times a day, 5 of the 6 female volunteers developed ambient temperature-dependent allodynia and paraesthesia in sun-exposed areas, i.e. face and hands, and phototoxic erythroderma. The neuropathy was not influenced by co-administration of rifampicin. In one case the trial had to be stopped, while for the other females the symptoms were mild to moderate and resolved completely within 12 to 16 days of the end of the study. No such effects were observed in the male volunteers. The authors state that the high frequency of the phototoxic neuropathy and its gender-specific occurrence are noteworthy. They hypothesize that the latter phenomenon could be explained by the higher IL-10 levels after UV-light exposure in males compared with females, leading to less neuropathic pain (Hohmann et al., 2016).

5.3.8 *Interactions*

In 2013, RIVM conducted a literature search to provide an overview of the possible interactions between herbal preparations containing St John's wort and human medicinal products when used concomitantly at recommended doses (Tiesjema et al., 2013). In this report, only interactions that had been demonstrated in humans were included. More specifically, significant effects must have been found in at least two case studies, or in a clinical study with more than two people. Interactions that had been demonstrated only in animal studies and/or *in vitro* experiments were not included.

Herbal preparations containing St John's wort can induce cytochrome P450 enzymes, such as CYP3A4 and 2C19, via the activation of the pregnane X receptor (PXR) and stabilization of mRNA. CYP2E1 can be induced via stabilization of the mRNA. As a result, medicinal products that are being metabolized by these enzymes will have lower plasma levels due to a higher rate of biotransformation. In addition, transporters that are involved in the transport of medicinal products in the body can be induced by St John's wort preparations, as is the case for P-glycoprotein (P-gp). This will also lead to lower plasma levels because P-gp is involved in transporting medicinal products out of the body. A lower plasma level will often result in reduced effectiveness of the medicinal product, which can have severe consequences, as medicinal products that can be affected by these mechanisms include medicines prescribed for the treatment of fungal and viral infections, cancer (chemotherapy) and suppression of the immune system (in tissue transplants).

A third mechanism by which St John's wort preparations can interact with medicinal products is via non-selective inhibition of the reuptake of neurotransmitters such as serotonin, noradrenalin, dopamine and glutamate. This may result in higher concentrations of these neurotransmitters in the synaptic cleft, which leads to stronger and more prolonged activity of the neurotransmitter. When preparations containing St John's wort are concomitantly used with other reuptake inhibitors, like SSRIs, an additive effect may occur, resulting in serotonin syndrome.

The severity of side effects caused by an interaction depends on both the dose of the drug and the dietary herbal supplement (Tiesjema et al., 2013).

Because the focus of the current report was to investigate whether other restrictions on the use of St John's wort in food supplements and herbal teas might be needed besides warnings about interactions with medicines, no new literature search on interactions was performed.

The Dutch Medicines Evaluation Board (MEB) and the Dutch pharmacovigilance center Lareb reported recently on cases of serious drug interactions with St John's wort products.

In 2016, MEB published a news item that St John's wort products can decrease the effectiveness of 'morning-after pills' containing levonorgestrel.[8] Lareb, in its previously mentioned report, reported six cases of adverse events labelled as an interaction between a St John's

[8] https://www.cbg-meb.nl/actueel/nieuws/2016/08/09/aangepast-advies-voor-gebruiksters-morning-afterpil-die-ook-andere-medicatie-gebruiken.

wort-containing product and co-medication (Lareb, 2018). In one case, the use of St John's wort-containing tea concomitantly with the contraceptive pill was reported to have resulted in an unintended pregnancy. In another case, symptoms of serotonergic syndrome were reported after a possible interaction between two non-registered products, one containing St John's wort and the other 5-hydroxytryptophan. The other four cases were not specified in the report.

Hyperforin is hypothesized to be the main responsible constituent for the interactions known with St John's wort products (Linde, 2009).

5.3.9 *Summary on toxicological information*

Acute toxicity

Oral LD_{50} values for St John's wort extract LI 160 of ≥5,000 mg/kg bw were reported, indicating that this extract is of low acute oral toxicity (EMA, 2009a). Acute oral exposure to ground St John's wort followed by exposure to sunlight led to phototoxicity in sheep, calves and steers (Bourke & White, 2004; Bourke, 2000, based on citation by SCF, 2002; 2003; Araya and Ford, 1981, as cited in SCF, 2002).

Short-term and sub-chronic toxicity

A LOAEL of 50 mg/kg bw per day was derived from a 28-day study in mice with hyperforin ethylene diammonium salt (corresponding to approximately 45 mg/kg bw hyperforin). At this dose level, increased liver enzyme activity and granulovacuolar hepatosis were observed (Negreş et al., 2016). In male rats exposed orally to 5,000 mg/kg bw St John's wort meal for 178 days, a decreased average body weight gain was observed. In rats and dogs exposed to up to 2,700 mg/kg bw St John's wort extract (LI 160) for 26 weeks, minor reversible symptoms (weight loss, minor pathological changes in liver and kidney – no further details available) were observed (Garret et al., 1982, as cited in SCF, 2002). In sheep fed freshly cut St John's wort at doses ranging from 4 to 16 g plant/kg bw for 14 days and exposed to daylight, severe ocular and dermal adverse effects were observed, as well as haemolytic anaemia and liver and kidney damage (Kako et al., 1993, as cited in SCF, 2002).

Genotoxicity

Several *in vitro* and *in vivo* genotoxicity studies on hypericin, hypericin irradiated with UV and St John's wort extracts are available. Except for the study by Feruszová et al. (2016), these studies were not reported to be performed according to OECD or other relevant guidelines.
Positive results were obtained for St John's wort extract in a bacterial reverse mutation assay. This effect could be assigned to the quercetin present in the extract (Poginsky et al., 1988). St John's wort extract tested negative in a gene mutation assay in V79 cells and in a UDS test in primary rat hepatocytes (Okpanyi et al., 1990). In a chromosomal aberration test in Wistar rats and in a mouse spot test, St John's wort extract gave negative results (Okpanyi et al., 1990; Peron et al., 2013). Hypericin gave negative results in bacterial reverse mutation assays, *in vitro* chromosomal aberration assays, an *in vitro* and *in vivo* micronucleus test and in *in vitro* comet assays (Feruszová et al., 2016; Kersten et al., 1999; Miadokova et al., 2010; Traynor et al., 2005; Turek et al., 1997).

Hypericin irradiated with UV gave negative results in a bacterial reverse mutation assay and in a chromosomal activation assay in HepG2 cells (Feruszova et al., 2016). In a micronucleus test, hypericin irradiated with UV caused a twofold increase in micronucleated cells (Kersten et al., 1999). In the last two tests, cytotoxicity limited the number of doses that could be analysed. In two *in vitro* comet assays, hypericin irradiated with UV gave positive results (Feruszova et al., 2016; Traynor et al., 2005).
From these results it can be concluded that hypericin is not genotoxic, whereas hypericin irradiated with UV may cause genotoxicity. Due to limitations in the available genotoxicity data and data on the chemical composition of St John's wort extract, it is not possible to adequately evaluate the genotoxicity of St John's wort extract.

Chronic toxicity and carcinogenicity
No chronic toxicity or carcinogenicity studies were identified.

Reproductive and developmental toxicity
In vitro studies showed some adverse effects of St John's wort extracts, hypericin and hyperforin on reproductive parameters. Several studies in mice and rats are available, but none was performed according to OECD or other relevant guidelines, and in most studies only selected parameters were investigated.
No indications for maternal toxicity were reported. Most studies did not provide indications for reproductive toxicity, although a reduction in litter size was reported in a pilot study on mice (Gonzalez et al., 1998, as cited in SCF, 2002) and a reduction in the number of pregnancies, and consequently the number of live pups, but not litter size, was observed in a study on rats (Kahyaoğlu et al., 2018).
Indications for offspring toxicity were observed in some studies in mice and rats. In some studies, statistically significant effects on birth weight or postnatal body weight were observed, whereas in other studies no effects on body weight were reported. In one of the studies in mice a temporary delay in the eruption of the upper incisors was also observed (Rayburn et al., 2001b). In two studies in rats, signs of liver and kidney toxicity were observed in the offspring (Gregoretti et al., 2004, as cited in EMA, 2009a; Kahyaoğlu et al., 2018).
Three studies investigated the effects of St John's wort (extract) use during pregnancy or lactation on the pregnancy outcome and/or on the infant's health (Lee et al., 2003; Moretti et al., 2009; Kolding et al., 2015). No statistically significant differences were found between treated and placebo groups of women, although there were slightly more spontaneous abortions and malformations reported and there were more adverse events reported in (the infants of) mothers who used St John's wort extracts.
Overall, the available data indicate that there is a possibility of reproductive, foetal and offspring toxicity when products containing St John's wort are used during pregnancy and lactation.

Phototoxicity
The *in vitro* studies on phototoxicity do not include validated studies for the assessment of phototoxicity. Their relevance is therefore questionable (EMA, 2012). Nevertheless, the results from different assays and with different extracts/compounds and cell lines point in the same direction. St

John's wort extracts show phototoxic activity *in vitro* when irradiated with UVA but also when using visible light. When tested alone, different constituents of St John's wort extracts exert phototoxicity at lower concentrations than the extract as a whole. Acute oral exposure to ground St John's wort followed by exposure to sunlight led to phototoxicity in sheep, calves and steers (Bourke & White, 2004; Bourke, 2000, based on citation by SCF, 2002; Araya and Ford, 1981, as cited in SCF, 2002).

The *in vitro* concentrations at which phototoxicity was observed (e.g. 1.5 µg/ml hypericin) are rather high compared with those in skin blister fluid level after oral administration in humans (5.3 ng/ml hypericin after a single dose of 5,400 µg hypericin) or the C_{max} after oral administration in humans (e.g. 91 ng/ml after a single dose of 4,356 µg hypericin) (Brockmöller et al., 1997; Bernd et al., 1999; Schempp et al., 1999). A single hypericin dose of 4,356 µg, or 62 µg/kg bw per day for a 70 kg individual, was identified as a NOAEL for phototoxicity in humans by the SCF (2002). When repeated doses of St John's wort extract (3 x 600 mg per day (in total 1,800 mg per day); equal to 2,180 µg hypericin per day and 3,340 µg pseudohypericin per day) were given for two weeks, statistically significant decreases in minimal erythema dose and minimal tanning dose were observed, indicating an enhanced photosensitivity. A hypericin dose of 2,180 µg, i.e. 31 µg/kg bw per day for an individual of 70 kg, was identified by SCF (2002) as a LOAEL for enhanced photosensitivity. In general in the clinical studies, no phototoxic reaction was observed when using St John's wort extracts at clinical doses (generally 500–900 mg extract). On the other hand, in one study where adverse events from a clinical phase I trial were analysed, phototoxic neuropathy was observed (only in women) at repeated high doses (1,800 mg extract per day) (Hohmann et al., 2016). It therefore seems that after repeated daily dosing with high doses, accumulation of hypericin (and/or other St John's wort constituents) takes places, leading to enhanced photosensitivity.
The LOAEL for hypericin of 2,180 µg, i.e. 31 µg/kg bw per day for an individual of 70 kg, rather than the NOAEL (of 62 µg/kg bw after single use) is used in the current risk assessment as the reference value for phototoxicity, as herbal preparations containing St John's wort are used for a longer period of time.
In addition, ocular phototoxicity of hypericin when irradiated with UVA or visible light was found in several *in vitro* studies. As there are no *in vitro* models that specifically assess ocular phototoxicity and the predictive value of skin assays is unknown, no conclusions can be drawn with respect to the risk of ocular phototoxicity. In sheep, severe ocular phototoxicity was observed after consumption of St John's wort for 14 days and exposure to daylight (Kako et al., 1993, as cited in SCF, 2002).

Human information
Thirty-five case reports were found in the literature of adverse events associated with the oral use of St John's wort products. Reported adverse effects were on the nervous system, skin (phototoxicity) and hair, urinary tract, reproductive system and digestive system. When dose information was reported, this was consistent with normal use of St John's wort products (no overdoses). In almost all cases, the adverse effects were reversible and disappeared after withdrawal from St John's

wort products (for references see section 5.3.7). Similar cases were reported after the use of St John's wort as a food supplement by Lareb (2018) in the Netherlands.
Adverse events that were reported in (clinical) trials with human volunteers using St John's wort extracts (gastrointestinal symptoms, sleep disturbance, dizziness/confusion and tiredness/sedation) generally occurred at a similar incidence as after placebo use. Photosensitivity was reported after St John's wort extract use but occurred rarely. However, it should be noted that in clinical studies not every possible effect will be observed. Changes within the body, such as alterations in serum enzyme levels, can be noted as indicators of toxicity, but mutations or other changes in the DNA as indicator for genotoxicity or alterations leading to reproduction or developmental toxicity cannot be noted. This is especially relevant for St John's wort products, because no conclusions can be drawn from animal data (for chronic toxicity, carcinogenicity and genotoxicity). The non-clinical data may indicate a possible concern (for reproduction and developmental toxicity) (for references see section 5.3.7).

5.4 Derivation of toxicological reference value

It is not possible to establish a health-based guidance value (HBGV) for St John's wort extract or for its main constituents, hypericin and hyperforin, due to limited data and unresolved concerns on several endpoints.

The genotoxicity of St John's wort extract cannot be adequately addressed. From the available genotoxicity data it can be concluded that hypericin is not genotoxic, whereas hypericin irradiated with UV may cause genotoxicity.
No studies of reproductive and developmental toxicity performed according to OECD or other relevant guidelines are available. However, the available data indicate that there is a possibility of reproductive, foetal and offspring toxicity when St John's wort extract is used during pregnancy and lactation. The short-term studies on St John's wort do not allow a NOAEL to be derived. No chronic toxicity or carcinogenicity data are available for St John's wort (extracts) and its constituents. The clinical studies cannot be used as a basis for an HBGV either, because these cover only a limited set of toxicological endpoints.

In humans, a LOAEL of 31 µg hypericin/kg bw per day was identified by SCF (2002) for enhanced photosensitivity after repeated dosing. This LOAEL can be used for assessing photosensitivity, but it cannot be used as a point of departure for establishing an HBGV due to the limited data available and unresolved concerns on several endpoints.

6 Risk assessment

6.1 Risk assessment

Safety of a herbal preparation can be presumed when "available data would allow concluding that exposure to known levels of the botanical ingredient has occurred in large population groups for many years without reported adverse effects" (EFSA, 2009). Since it is already established that herbal preparations containing St John's wort can cause serious interactions with medicinal products at recommended dose levels, the presumption of safety does not apply in this case (Tiesjema et al., 2013).

No HBGV could be derived for St John's wort preparations or its main constituents, hypericin and hyperforin and therefore, no safe use level for food supplements containing St John's wort can be determined. A LOAEL of 31 µg hypericin/kg bw per day was derived for enhanced photosensitivity in humans (see Section 5.4). Based on the reported hypericin content of some food supplements containing St John's wort that are available in the Netherlands, the estimated exposure to hypericin by users ranges from 1.4 to 41 µg/kg bw per day for a 70 kg person (see Section 4.1). The LOAEL of 31 µg hypericin/kg bw for enhanced phototoxicity is within this exposure range and therefore phototoxicity can occur when using food supplements with St John's wort.

In addition, there are indications for genotoxicity and reproductive and developmental toxicity. Chronic toxicity/carcinogenicity data are lacking for St John's wort (extracts) and its constituents. Owing to omissions in the toxicological profile, no firm conclusions can be drawn on these aspects. Moreover, effects are difficult to determine because details on the composition of herbal preparations containing St John's wort are often lacking and the composition can vary greatly.

Indicative of the occurrence of adverse effects are also the reports of adverse effects received by Lareb in which dizziness, diarrhoea, skin reactions and psychiatric symptoms were mentioned (Lareb, 2018).

Furthermore, the estimated exposure to hypericin is around or higher than the therapeutic dose of 0.13 – 0.40 mg hypericins per day (approximately 1.9 – 5.7 µg/kg bw for a 70 kg person) for the single registered medicinal product currently available in the Netherlands, indicating that a pharmacological effect can be expected for these food supplements. Medicines undergo a pre-market assessment of the quality, safety and efficacy of the product, whereas for food supplements there is no such assessment (Jeurissen, de Wit & Tiesjema, 2020). Due to these differences in requirements between the two legal frameworks it can be questioned if this is a desirable situation from a safety perspective.

The same concerns may apply to herbal teas containing St John's wort. In 2002, SCF estimated the daily exposure to hypericin by consuming

these teas to be in the same range as for food supplements and medicinal products (1.5 mg per day, equal to 21 µg/kg bw per day for a 70 kg person) (SCF, 2002). However, more information on the hypericin content of teas containing St John's wort currently on the market would be needed for a more reliable exposure estimate.

Contamination with pyrrolizidine alkaloids (genotoxic carcinogens) may also occur during the harvesting of the flower tops of St John's wort. According to the Herbal Preparations Decree, herbal preparations may maximally contain 1 µg/kg toxic pyrrolizidine alkaloids. In time, this will be overruled by the amendment of Regulation (EC) 1881/2006 on contaminants (EC, 2006), which will specify maximum levels of pyrrolizidine alkaloids in several products, including herbal preparations.

6.2 Sensitive/vulnerable groups

Because of the interactions of St John's wort products with many medicinal products, people that concomitantly use medicines or other (plant) food supplements are a high-risk group. Unborn children and infants might also be at higher risk, because there is a possibility of foetal and offspring toxicity when St John's wort products are used during pregnancy and lactation.

6.3 Uncertainties

6.3.1 *Exposure*

The composition of the St John's wort extracts/plant material used in food supplements is not always known and may differ between different brands and between batches. This hampers exposure assessment. Furthermore, the estimated exposure specified in this report is based on normal or recommended use. More than recommended use will lead to higher exposure to hypericin and other constituents. In addition, to be able to perform a reliable exposure assessment for herbal teas containing St John's wort, more information on the hypericin content of the teas currently available on the market would be needed.

6.3.2 *Toxicity*

As described above, the toxicological data for St John's wort extracts or its main constituents are limited. It is therefore not possible to reach a final conclusion on the genotoxicity and the reproductive and developmental toxicity of St John's wort extract. Moreover, no statements about safety after long-term use or regarding carcinogenicity can be made, as there are no studies available investigating chronic (lifetime) exposure.
Finally, it is still a matter of debate what the precise active components of St John's wort extract are. This may cause additional omissions in the toxicological profile of St John's wort preparations.

7 Conclusion and recommendations

Food supplements
The use of food supplements containing St John's wort can cause adverse effects because:

- serious drug interactions with a wide variety of human medicinal products can occur at recommended dose levels;
- the estimated exposure to hypericin could result in enhanced photosensitivity in humans;
- a pharmacological effect can be expected for these food supplements with doses around or higher than the therapeutic dose of St John's wort extract;
- case reports of adverse events associated with oral use of St John's wort products at recommended use levels are described in the literature and reported by Lareb.

In addition, there are indications for genotoxicity and reproductive and developmental toxicity. Chronic toxicity/carcinogenicity data are lacking. Owing to omissions in the toxicological profile of St John's wort extract and its constituents, no firm conclusions can be drawn on these aspects.

Details on the composition of these food supplements are often lacking and the composition can vary greatly. Therefore, the precise effects are difficult to determine.

Herbal tea
For herbal teas made from St John's wort the same concerns apply. More information on the hypericin content of teas made from St John's wort currently on the market would be needed for a more reliable exposure estimate and to draw more firm conclusions.

Given these concerns, RIVM advises consumers to be cautious with the use of herbal preparations containing St John's wort, and to not use these supplements and herbal teas in combination with medicines. RIVM considers that these concerns cannot be covered with obliging warning phrases. Therefore, RIVM advises VWS to consider to restrict the use of St John's wort in herbal preparations by law. Also, it is advised to consider which St John's wort product should be regarded as medicines and consequently would require a premarket assessment on safety, efficacy and quality.

Acknowledgements

The authors are grateful to Gerrit Wolterink, Wim Mennes, Jan van Benthem and Anton Rietveld (RIVM) and Birgitte Tiesjema (CBG) for their critical review of (parts of) this document.
The authors would also like to thank Chodidjah Rafioeddin for her contribution to the first draft of this report.

References

Agrosi M, Mischiatti S, Harrasser PC, Savio D (2000) Oral bioavailability of active principles from herbal products in humans. A study on Hypericum perforatum extracts using the soft gelatin capsule technology. Phytomedicine 7(6): 455–62.

Ang CY, Hu L, Heinze TM, Freeman JP, Kozak K, Luo W, Liu FF, Mattia A, DiNovi M (2004) Instability of St. John's wort (Hypericum perforatum L.) and degradation of hyperforin in aqueous solutions and functional beverage. J. Agric. Food Chem. 52: 6156–64.

Araya OS, Ford EJH (1981) An investigation of the type of photosensitization caused by the ingestion of St John's wort (*Hypericum perforatum*) by calves. J. Comp. Path. 91: 135–41 (as cited in SCF, 2002).

Avila C, Whitten D, Evans S (2018) The safety of St John's wort (Hypericum perforatum) in pregnancy and lactation: A systematic review of rodent studies. Phytother. Res. 32: 1488–500.

Bernd A, Simon S, Ramirez Bosca A, Kippenberger S, Diaz Alperi J, Miquel J, Villalba Garcia JF, Pamies Mira D, Kaufmann R (1999) Phototoxic effects of Hypericum extract in cultures of human keratinocytes compared with those of psoralen. Photochem. Photobiol. 69: 218–21.

Biber A, Fischer H, Römer A, Chatterjee SS (1998) Oral bioavailability of hyperforin from hypericum extracts in rats and human volunteers. Pharmacopsychiatry 31: 36–43.

Bilia AR, Bergonzi MC, Morgenni F, Mazzi G, Vincieri FF (2001) Evaluation of chemical stability of St. John's wort commercial extract and some preparations. Int. J. Pharm. 213: 199–208.

Borges LV, do Carmo Cancino JC, Peters VM, Las Casas L, de Oliveira Guerra M (2005) Development of pregnancy in rats treated with Hypericum perforatum. Phytother. Res. 19: 8857.

Bourke CA (2000) Sunlight-associated hyperthermia as a consistent and rapidly developing clinical sign in sheep intoxicated by St John's wort (Hypericum perforatum). Aust. Vet. J. 78: 483–8 (as cited in SCF, 2002).

Bourke CA (2003) The effect of shade, shearing and wool type in the protection of Merino sheep from Hypericum perforatum (St John's wort) poisoning. Aust. Vet. J. 81(8): 494–8.

Bourke CA, White JG (2004) Reassessment of the toxicity of Hypericum perforatum (St John's wort) for cattle. Aust. Vet. J. 82: 707–12.

Brockmöller J, Reum T, Bauer S, Kerb R, Hübner WD, Roots I (1997) Hypericin and pseudohypericin: pharmacokinetics and effects on photosensitivity in humans. Pharmacopsychiatry 30: 94–101.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (2014) List of Substances of the Competent Federal Government and Federal State Authorities – Category 'Plants and plant parts'. pp. 9–141. doi: 10.1007/978-3-319-10732-5_3.

Butterweck V, Schmidt M (2007) St. John's wort: role of active compounds for its mechanism of action and efficacy. Wien. Med. Wochenschr 157: 356–61.

Cada AM, Hansen DK, LaBorde JB, Furguson SA (2001) Minimal effects from development exposure to St. John's wort (Hypericum perforatum) in Sprague-Dawley rats. Nutritional Neuroscience 4: 135–41.

Campos LV, Guerra MO, Peters VM, De Cássia da Silveira e Sá R (2017a) Effect of the extract of Hypericum perforatum on neurodevelopment of regions related to pain control and convulsion. Journal of Medicinal Plant Research 11: 107–17.

Campos, LV, Vieira VA, Silva LR, Jasmin J, Guerra MO, Peters VM, De Cássia da Silveira e Sá R (2017b) Rats treated with Hypericum perforatum during pregnancy generate offspring with behavioural changes in adulthood. Revista Brasileira de Farmacognosia 27: 361–8.

Capasso R, Borrelli F, Montanaro V, Altieri V, Capasso F, Izzo AA (2005) Effects of the antidepressant St. John's wort (Hypericum perforatum) on rat and human vas deferens contractility. Journal of Urology 173: 2194–7.

Cervo L, Rozio M, Ekalle-Soppo CB, Guiso G, Morazzoni P, Caccia S (2002) Role of hyperforin in the antidepressant-like activity of Hypericum perforatum extracts. Psychopharmacology (Berl) 164(4): 423–8.

Chan LYS, Chiu PY, Lau TK (2001) A study of hypericin-induced teratogenicity during organogenesis using a whole rat embryo culture model. Fertility and Sterility 76: 1073–4.

Cui Y, Ang CYW, Beger RD, Heinze TM, Hu L, Leakey J (2004) In vitro metabolism of hyperforin in rat liver microsomal systems. Drug Metab. Dispos. 32(1): 28–34.

CVMP (1998) Committee for Veterinary Medicinal Products. Hyperici oleum. Summary report. EMA/MRL/359/908-FINAL July 1998. Available via: https://www.ema.europa.eu/en/documents/mrl-report/hyperici-oleum-summary-report-committee-veterinary-medicinal-products_en.pdf (accessed June 2020).

CVMP (1999) Committee for Veterinary Medicinal Products. Hypericum perforatum (use in veterinary medicine). Summary report. EMA/MRL/690/99-FINAL August 1999. Available via: https://www.ema.europa.eu/en/documents/mrl-report/hypericum-perforatum-use-veterinary-homeopathy-summary-report-committee-veterinary-medicinal_en.pdf (accessed June 2020).

Da Conceição AO, Takser L, Lafon J (2010) Effect of St. John's wort standardized extract and hypericin on in vitro placental calcium transport. J. Med. Food 13: 934–42.

Delaey EM, Kamuhabwa AR, Vandenbogaerde AL, de Witte PA (1999) Photocytotoxicity of protohypericin after photoconversion to hypericin. Planta Medica 65(8): 719–22.

Delaey E, Vandenbogaerde A, Merlevede W, de Witte P (2000) Photocytotoxicity of hypericin in normoxic and hypoxic conditions. Journal of Photochemistry and Photobiology B: Biology 56(1): 19–24.

Delaey EM, Obermuëller R, Zupkó I, de Vos D, Falk H, de Witte PAM (2001) In vitro study of the photocytotoxicity of some hypericin analogs on different cell lines. Photochemistry and Photobiology 74(2): 164–71.

De Wit-Bos L, Jeurissen SMF, Mennes WC, Rorije E, Wolterink G (2019). RIVM Template for safety assessment of plant food supplements. RIVM letter report 2019-0114. Available via www.rivm.nl.

Dostalek M, Pistovcakova J, Jurica J, Tomandl J, Linhart I, Sulcová A, Hadasova E (2005) Effect of St John's wort (Hypericum perforatum) on cytochrome P-450 activity in perfused rat liver. Life Sciences 78: 239–44.

Dostalek M, Pistovcakova J, Jurica J, Sulcova A, Tomandl J (2011) The effect of St John's wort (Hypericum perforatum) on cytochrome p450 1a2 activity in perfused rat liver. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 155(3): 253–7.

EC (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364/5 20.12.2006 Available via: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN (accessed June 2020).

EC (2008) Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. Available via: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R1331&qid=1551260338570&from=NL (accessed June 2020).

Ehrenshaft M, Roberts JE, Mason RP (2013) Hypericin-mediated photooxidative damage of alpha-crystallin in human lens epithelial cells. Free Radic. Biol. Med. 60: 347–54.

EMA (2009a) Assessment report on Hypericum perforatum L., Herba. Doc. Ref.: EMA/HMPC/101303/2008. Available via: https://www.ema.europa.eu/documents/herbal-report/assessment-report-hypericum-perforatum-l-herba_en.pdf (accessed June 2020).
EMA (2009b) Community herbal monograph on Hypericum perforatum L., Herba (traditional use). Doc. Ref.: EMEA/HMPC/745582/2009. Available via: https://www.ema.europa.eu/documents/herbal-monograph/final-community-herbal-monograph-hypericum-perforatum-l-herba-traditional-use_en.pdf (accessed June 2020).

EMA (2009c) Community herbal monograph on Hypericum perforatum L., Herba (well-established medicinal use). Doc. Ref.: EMA/HMPC/101304/2008. Available via: https://www.ema.europa.eu/documents/herbal-monograph/final-community-herbal-monograph-hypericum-perforatum-l-herba-well-established-medicinal-use_en.pdf (accessed June 2020).

EMA (2010) Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products. EMA/HMPC/CHMP/CVMP/287539/2005 Rev.1 11, March 2010. Available via: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-declaration-herbal-substances-herbal-preparations-herbal-medicinal-products/traditional-herbal-medicinal-products-spc_en.pdf (accessed June 2020).

EMA (2012) ICH guideline S10. Guidance on photosafety evaluation of pharmaceuticals. EMA/CHMP/ICH/752211/2012. Available via: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-1.pdf (accessed June 2020).

European Pharmacopoeia (2017). St. John's wort. European Pharmacopoeia Version 9.0.

Feruszová J, Imreová P, Bodnárová K, Ševčovičová A, Kyzek S, Chalupa I et al. (2016) Photoactivated hypericin is not genotoxic. General Physiology and Biophysics 35: 223–30.

Fox E, Murphy RF, McCully CL, Adamson PC (2001) Plasma pharmacokinetics and cerebrospinal fluid penetration of hypericin in nonhuman primates. Cancer Chemother. Pharmacol. 47: 41–4.

Franklin M, Chi J, McGavin C, Hockney R, Reed A, Campling G, Whale RWR, Cowen PJ (1999) Neuroendocrine evidence for dopaminergic actions of hypericum extract (LI 160) in healthy volunteers. Biological Psychiatry 46(4): 581–4.

Garret BJ, Cheeke PR, Miranda CL, Goeger DE, Buhler DR (1982) Consumption of poisonous plants (Scenecio jacobea, Symphytum officinale, Pteridium aquilinum, Hypericum perforatum) by rats: chronic toxicity, mineral metabolism and hepatic drug-metabolizing enzymes. Toxicol. Lett. 10: 183–8.

Godtel-Armbrust U, Metzger A, Kroll U, Kelber O, Wojnowski L (2007) Variability in PXR-mediated induction of CYP3A4 by commercial preparations and dry extracts of St. John's wort. Naunyn Schmiedebergs Arch Pharmacol 375(6): 377–82.

Gonzalez CL, Stewart JD, Rayburn WF, Christensen HD (1998) Establishment of a relevant dose of antenatal hypericum (St John's wort) for neurobehavioural development study. Neurotoxicol. Teratol. 20: 369, NBTS 59 (as cited in SCF, 2002).

Gonzalez CL, Christensen HD, Rayburn WF, Coleman FH (1999) Impact of antenatal Hypericum (St. John's wort) exposure on cognition in mice. Neurobehavioral Teratology Society abstracts 328 (as cited in EMA, 2009a).

Gregoretti B, Stebel M, Candussio L, Crivellato E, Bartoli F, Decorti G (2004) Toxicity of Hypericum perforatum (St. John's wort) administered during pregnancy and lactation in rats. Toxicol. Appl. Pharmacol. 200: 201–5 (as cited in EMA, 2009a).

Gry J, Hallas-Møller T, Pedersen E, Pilegaard K, Strube M (2011) Drug List. Assessment of plants, fungi and parts thereof used in supplements and herbal teas [Drogelisten. Vurdering af planter, svampe og dele heraf anvendt i kosttilskud og urtete] (In Danish) Available via: https://www.food.dtu.dk/resultat?qt=NetmesterSearch&fr=1&sw=drogelisten#tabs.

Hatanaka J, Shinme Y, Kuriyama K, Uchida A, Kou K, Uchida S, Yamada S, Onoue S (2011) In vitro and in vivo characterization of new formulations of St. John's wort extract with improved pharmacokinetics and anti-nociceptive effect. Drug Metab. Pharmacokinet. 26(6): 551–8.

He YY, Chignell CF, Miller DS, Andley UP, Roberts JE (2004) Phototoxicity in human lens epithelial cells promoted by St. John's wort. Photochem. Photobiol. 80: 583–6.

Hellum BH, Hu Z, Nilsen OG (2007) The induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. Basic Clin. Pharmacol. Toxicol. 100(1): 23–30.

Hennessy M, Kelleher D, Spiers JP, Barry M, Kavanagh P, Back D, Mulcahy F, Feely J (2002) St. John's wort increases expression of P-glycoprotein: Implications for drug interactions. British Journal of Clinical Pharmacology 53(1): 75–82.

Hohmann N, Maus A, Carls A, Haefeli WE, Mikus G (2016) St. John's wort treatment in women bears risks beyond pharmacokinetic drug interactions. Arch. Toxicol. 90: 1013–15.

Hokkanen J, Tolonen A, Mattila S, Turpeinen M (2011) Metabolism of hyperforin, the active constituent of St. John's wort, in human liver microsomes. Eur. J. Pharm. Sci 42(3): 273–84.

Jacobson JM, Feinman L, Liebes L, Ostrow N, Koslowski V, Tobia A, Cabana BE, Lee DH, Spritzler J, Prince AM (2001) Pharmacokinetics, safety, and antiviral effects of hypericin, a derivative of St. John's wort plant, in patients with chronic hepatitis C virus infection. Antimicrobial Agents and Chemotherapy 45(2): 517-24.

Jeurissen S, Beukers M, Tiesjema B (2015) Intake assessment of selected botanicals via plant food supplements. Unpublished memo.

Jeurissen SMF, Buurma-Rethans EJM, Beukers MH, Jansen-van der Vliet M, Van Rossum CTM, Sprong RC (2018) Consumption of plant food supplements in the Netherlands. Food Funct. 9: 179–90. doi: 10.1039/c6fo01174h.

Jeurissen S, de Wit-Bos L, Tiesjema B (2020) 'Ask patients for their use of herbal products' ['Vraag patiënten naar gebruik kruidenproduct'] (in Dutch). Pharmaceutisch Weekblad 35, jaargang 155, 28 augustus 2020.

Juergenliemk G, Boje K, Huewel S, Lohmann C, Galla HJ, Nahrstedt A (2003) In vitro studies indicate that miquelianin (quercetin 3-O-beta-D-glucuronopyranoside) is able to reach the CNS from the small intestine. Planta Med. 69: 1013–17.

Kahyaoğlu F, Gökçimen A, Demirci B (2018). Investigation of the embryotoxic and teratogenic effect of hypericum perforatum in pregnant rats. Turk Jinekoloji ve Obstetrik Dernegi Dergisi 15: 87–90.

Kako MD, al Sultan II, Saleem AN (1993) Studies of sheep experimentally poisoned with Hypericum perforatum. Veterinary and Human Toxicology 35(4): 298–300.

Kamuhabwa AR, Augustijns P, de Witte PA (1999). In vitro transport and uptake of protohypericin and hypericin in the Caco-2 model. Int J Pharm. 188: 81–6.

Keller JH, Karas M, Müller WE, Vomer DA, Eckert GP, Tawab MA, Blume HH, Dingermann T, Schubert-Zsilavecz M (2003) Determination of hyperforin in mouse brain by high-performance liquid chromatography/tandem mass spectrometry. Anal. Chem. 75(22): 6084–8.

Kerb R, Brockmöller J, Staffeldt B, Ploch M, Roots I (1996) Single-dose and steady-state pharmacokinetics of hypericin and pseudohypericin. Antimicrobial Agents and Chemotherapy 40(9): 2087–93.

Kersten B, Zhang J, Brendler-Schwaab SY, Kasper P, Müller L (1999) The application of the micronucleus test in Chinese hamster V79 cells to detect drug-induced photogenotoxicity. Mutat. Res. 445: 55–71.

Klier CM, Schäfer MR, Schmid-Siegel B, Lenz G, Mannel M (2002) St. John's wort (Hypericum Perforatum): is it safe during breastfeeding? Pharmacopsychiatry 35(1): 29–30.

Klier CM, Schmid-Siegel B, Schäfer MR, Lenz G, Saria A, Lee A, Zernig G (2006) St. John's wort (Hypericum perforatum) and breastfeeding: plasma and breast milk concentrations of hyperforin for 5 mothers and 2 infants. J. Clin. Psychiatry 67(2): 305–9.

Kolding L, Pedersen LH, Henriksen TB, Olsen J, Grzeskowiak LE (2015) Hypericum perforatum use during pregnancy and pregnancy outcomes. Reproductive Toxicology 58: 234–7.

Koninklijk Besluit (1997) Royal Decree of 29 August 1997 on the manufacture and trade of foods composed of or containing plants or plant preparations [Koninklijk Besluit van 29 Augustus 1997 betreffende de fabricage van en de handel in voedingsmiddelen die uit planten of uit plantenbereidingen samengesteld zijn of deze bevatten] (in Dutch) (Stbl. 21.XI.1997). Available via: https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/consolidated_version_rd_29_august_1997_v10-02-2017_nl_0.pdf (accessed June 2020).

Köppel H, Naser B, Schulz HU, Kibbel T, Schürer M, Liske E (2008) Investigation of the effect on phytosensitivity following repeated oral dosing of Hypericum extract in 20 healthy male and female volunteers. Zeitschrift für Phytotherapie 29: P16.

Lareb (2018) Overview of reports on St. John's wort (Hypericum perforatum). Netherlands Pharmacovigilance Centre, Lareb, 20 December.

Letsyo E, Jerz G, Winterhalter P, Lindigkeit R, Beuerle T (2017) Incidence of pyrrolizidine alkaloids in herbal medicines from German retail markets: Risk assessments and implications to consumers. Phytother. Res. 31: 1903–9.

Lee A, Minhas R, Matsuda N, Lam M, Ito S (2003) The safety of St. John's wort (Hypericum perforatum) during breastfeeding. J. Clin. Psychiatry 64: 966–8.

Leuschner J (1996) Preclinical toxicological profile of Hypericum extract LI 160. Abstracts of the 2nd International Conference on Phytomedicine, Munich. SL 80 (as cited in EMA, 2009a).

Li JJ, Cona MM, Feng Y, Chen F, Zhang G, Fu X, Himmelreich U, Oyen R, Verbruggen A, Ni Y (2012) A single-dose toxicity study on non-radioactive iodinated hypericin for a targeted anticancer therapy in mice. Acta Pharmacologica Sinica 33: 1549–56.

Linde K (2009) St. John's wort – an overview. Forschende Komplementärmedizin 16: 146–55.

Linde K, Ramirez G, Mulrow CD, Pauls A, Weidenhammer W, Melchart D (1996) St John's wort for depression – an overview and meta-analysis of randomized clinical trials. Brit. Med. J. 313: 253-8 (as cited in SCF, 2002).

MEB (2020) Medicines Evaluation Board. Medicines Information Bank. [Geneesmiddeleninformatiebank] (in Dutch) Available via: https://www.geneesmiddeleninformatiebank.nl/nl/ (accessed July 2020).

Miadokova E, Chalupa I, Vlckova V, Sevcovicova A, Nadova S, Kopaskova M et al. (2010) Genotoxicity and antigenotoxicity evaluation of non-photoactivated hypericin. Phytotherapy Research 24: 90–5.

Moretti ME, Maxson A, Hanna F, Koren G (2009) Evaluating the safety of St. John's wort in human pregnancy. Reproductive Toxicology 28: 96–9.

Mulder PPJ, López Sánchez P, These A, Preiss-Weigert A and Castellari M (2015) Occurrence of pyrrolizidine alkaloids in food. EFSA supporting publication 2015:EN-859, 116 pp. Available via: https://www.efsa.europa.eu/en/supporting/pub/en-859 (accessed June 2020).

Mullaicharam AR, Halligudi N (2018) St John's wort (Hypericum perforatum L.): A review of its chemistry, pharmacology and clinical properties. International Journal of Research in Phytochemical and Pharmacological Sciences 1: 5–11.

Nakamura K, Aizawa K, Yamauchi J, Tanoue A (2013) Hyperforin inhibits cell proliferation and differentiation in mouse embryonic stem cells. Cell Proliferation 46: 529–37.

Negreş S, Scutari C, Ionică FE, Gonciar V, Velescu BS, Şeremet OC, Zanfirescu A, Zbârcea CE, Ştefănescu E, Ciobotaru E, Chiriţă C (2016) Influence of hyperforin on the morphology of internal organs and biochemical parameters, in experimental model in mice. Rom. J. Morphol. Embryol. 57: 663–73.

NVWA (2019a) Important safety warning on St. John's wort from various producers [Belangrijke veiligheidswaarschuwing Sint Janskruid diverse producenten] (in Dutch) Available via: https://www.nvwa.nl/documenten/waarschuwingen/2019/03/01/belangrijke-veiligheidswaarschuwing-sint-janskruid-diverse (Accessed June 2020).

NVWA (2019b) Plant toxins in St John's wort. [Plantengifstoffen in sint-janskruid. Inspectieresultaten 2018-2019] (in Dutch) Available via: https://www.nvwa.nl/documenten/consument/eten-drinken-roken/supplementen-en-preparaten/publicaties/plantengifstoffen-in-sint-janskruid-inspectieresultaten-2018-2019 (accessed June 2020).

Okpanyi SN, Lidzba H, Scholl BC, Miltenburger HG (1990) Genotoxizität eines standardisierten Hypericum-Extracktes. [Investigations into the genotoxicity of a standardized extract of Hypericum perforatum] (In German). Arzneimittel-Forschung/Drug Research 40: 851–5.

Ondrizek RR, Chan PJ, Patton WC, King A (1999) An alternative medicine study of herbal effects on the penetration of zona-free hamster oocytes and the integrity of sperm deoxyribonucleic acid. Fertil. Steril. 71: 517–22.

Onoue S, Seto Y, Ochi M, Inoue R, Ito H, Hatano T, Yamada S (2011) In vitro photochemical and phototoxicological characterization of major constituents in St. John's wort (Hypericum perforatum) extracts. Phytochemistry 72(14–15): 1814–20.

Pace, N (1942) The etiology of hypericism, a photosensitivity produced by St John's wort. Am. J. Physiol 136: 650–6 (as cited in SCF, 2002).

Paulke A, Nöldner M, Schubert-Zsilavecz M, Wurglics M (2008) St. John's wort flavonoids and their metabolites show antidepressant activity and accumulate in brain after multiple oral doses. Pharmazie. 63: 296–302.

Peron AP, Mariucci RG, de Almeida IV, Düsman E, Mantovani MS, Vicentini VE (2013) Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of a natural antidepressant, Hypericum perforatum L. (St. John's wort), on vegetal and animal test systems. BMC Complement Altern. Med. 13: 97, 9 pp.

Poginsky B, Westendorf J, Prosenc N, Kuppe M, Marquardt H (1988) Johanniskraut (Hypericum perforatum L. Genotoxizität bedingt durch den Quercetingehalt [Hypericum perforatum L. Genotoxicity depends on quercetin content] (in German). Deutsche Apotheker Zeitung 128: 1364–6.

Pu XY, Liang JP, Wang XH, Xu T, Hua LY, Shang RF, Liu Y, Xing YM (2009) Anti-influenza A virus effect of Hypericum perforatum L. extract. Virologica Sinica 24: 19–27.

Rayburn WF, Dix Christensen HD, Gonzalez CL (2000) Effect of antenatal exposure to Saint John's wort (Hypericum) on neurobehaviour of developing mice. American Journal of Obstetrics and Gynecology 183: 1225–31.

Rayburn WF, Gonzalez CL, Dix Christensen H, Harkin TL, Kupiec TC (2001a) Impact of hypericum (St. John's wort) given prenatally on cognition of mice offspring. Neurotoxicology and Teratology 23: 629–37.

Rayburn WF, Gonzalez CL, Dix Christensen H, Stewart JD (2001b) Effect of prenatally administered hypericum (St John's wort) on growth and physical maturation of mouse offspring. American Journal of Obstetrics and Gynecology 184: 191–5.

Sattler S, Schaefer U, Schneider W, Hoelzl J, Lehr CM (1997) Binding, uptake, and transport of hypericin by Caco-2 cell monolayers. J. Pharm. Sci. 86: 1120–6.

SCF (2002) 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. Available via: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out113_en.pdf (accessed June 2020).

Schempp CM, Winghofer B, Langheinrich M, Schöpf E, Simon JC (1999) Hypericin levels in human serum and interstitial skin blister fluid after oral single-dose and steady-state administration of Hypericum perforatum extract (St. John's wort). Skin Pharmacology and Applied Skin Physiology 12(5): 299–304.

Schempp CM, Simon-Haarhaus B, Simon JC (2002) Phototoxic and apoptosis-inducing capacity of pseudohypericin. Planta Med. 68: 171–3.

Schempp CM, Windeck T, Hezel S, Simon JC (2003) Effect of oral administration of Hypericum perforatum extract (St. John's wort) on skin erythema and pigmentation induced by UVB, UVA, visible light and solar-simulated radiation. Phytother. Res. 17: 141–6.

Schey KL, Patat S, Chignell CF, Datillo M, Wang RH, Roberts JE (2000) Photooxidation of lens alpha-crystallin by hypericin (active ingredient in St. John's wort). Photochem. Photobiol. 72: 200–3.

Schimmer O, Krüger A, Paulini H, Häfele F (1994) An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie 49: 448–51 (as cited in EMA, 2009a).

Schmitt LA, Liu Y, Murphy PA, Birt DF (2006a) Evaluation of the light-sensitive cytotoxicity of Hypericum perforatum extracts, fractions, and pure compounds. J. Agric. Food Chem. 54: 2881–90.

Schmitt LA, Liu Y, Murphy PA, Petrich JW, Dixon PM, Birt DF (2006b). Reduction in hypericin-induced phototoxicity by Hypericum perforatum extracts and pure compounds. J. Photochem .Photobiol. B, Biol. 85: 118–30.

Schulz HU, Schürer M, Bässler D, Weiser D (2005) Investigation of the bioavailability of hypericin, pseudohypericin, hyperforin and the flavonoids quercetin and isorhamnetin following single and multiple oral dosing of a hypericum extract containing tablet. Arzneimittelforschung 55(1): 15–22.

Schulz HU, Schürer M, Bässler D, Weiser D (2006) Investigation of the effect on photosensitivity following multiple oral dosing of two different Hypericum extracts in healthy men. Arzneimittelforschung 56: 212–21.

Silva SM, Martinho A, Moreno I, Silvestre S, Breitenfeld Granadeiro L, Alves G, Duarte AP, Domingues F, Gallardo E (2016) Effects of Hypericum perforatum extract and its main bioactive compounds on the cytotoxicity and expression of CYP1A2 and CYP2D6 in hepatic cells. Life Sciences 144: 30–6.

Staffeldt B, Kerb R, Brockmöller J, Ploch M, Roots I (1994) Pharmacokinetics of hypericin and pseudohypericin after oral intake of the hypericum perforatum extract LI 160 in healthy volunteers. Journal of Geriatric Psychiatry and Neurology 7: 47–53.

Theodossiou T, Spiro MD, Jacobson J, Hothersall JS, MacRobert AJ (2004) Evidence for intracellular aggregation of hypericin and the impact on its photocytotoxicity in PAM 212 murine keratinocytes. Photochemistry and Photobiology 80(3): 438–43.

Tiesjema B, De Wit L, Brandon E, Jeurissen S, Kupper N, Noorlander C, Van Kranen K (2013). Interactions between herbs and medicines: St. John's wort. [Interacties tussen kruiden en geneesmiddelen. Sint Janskruid] (in Dutch) RIVM briefrapport 090425001/2013. Available via: https://www.rivm.nl/bibliotheek/rapporten/090425001.pdf (accessed June 2020).

Traynor, NJ, Beattie PE, Ibbotson SH, Moseley H, Ferguson J, Woods JA (2005) Photogenotoxicity of hypericin in HaCaT keratinocytes: Implications for St. John's wort supplements and high dose UVA-1 therapy. Toxicology Letters 158: 220–4.

Turek B, Bárta I, Šmerák P, Kováčová E, Semkíková M, Šestáková H (1997) Mutagenní aktivity některých látek rostlinného původu. [Mutagenic activity of substances of plant origin] (in Czech). Potrav. Vědy 15: 271–88.

Vandenbogaerde AL, Kamuhabwa A, Delaey E, Himpens BE, Merlevede WJ, de Witte PA (1998). Photocytotoxic effect of pseudohypericin versus hypericin. Journal of Photochemistry and Photobiology B: Biology 45(2–3): 87–94.

Vandenbogaerde A, Zanoli P, Puia G, Truzzi C, Kamuhabwa A, de Witte P, Merlevede W, Baraldi M (2000) Evidence that total extract of Hypericum perforatum affects exploratory behavior and exerts anxiolytic effects in rats. Pharmacology Biochemistry and Behavior 65: 627–33.

Vieira M L, Hamada RY, Gonzaga NI, Bacchi AD, Barbieri M, Moreira EG, De Fátima Paccola Mesquita S, Gerardin DCC (2013a). Could maternal exposure to the antidepressants fluoxetine and St. John's wort induce long-term reproductive effects on male rats? Reproductive Toxicology 35: 102–7.

Vieira VA, Camposa LV, Silva LR, Guerraa MO, Peters VM, De Cássia S. Sá S (2013b) Evaluation of postpartum behaviour in rats treated with Hypericum perforatum during gestation. Revista Brasileira de Farmacognosia 23: 796–801.

Wahlman J, Hirst M, Roberts JE, Prickett CD, Trevithick JR (2003) Focal length variability and protein leakage as tools for measuring photooxidative damage to the lens. Photochem. Photobiol. 78: 88–92.

Wang LS, Zhu B, El-Aty AMA, Zhou G, Li Z, Wu J, Chen GL, Liu J, Tang ZR, An W, Li Q, Wang D, Zhou HH (2004) The influence of St John's wort on CYP2C19 activity with respect to genotype. J. Clin. Pharmacol. 44(6): 577–81.

Wenk M, Todesco L, Krähenbühl S (2004) Effect of St John's wort on the activities of CYP1A2, CYP3A4, CYP2D6, N-acetyltransferase 2, and xanthine oxidase in healthy males and females. British Journal of Clinical Pharmacology 57(4): 495–9.

WHO (2004) Herba Hyperici. WHO monographs on selected medicinal plants. Volume 2. World Health Organization. pp 149–71. Available via: https://apps.who.int/iris/bitstream/handle/10665/42052/9241545372.pdf;jsessionid=BE6B4F772AA8C5096175F0FA4539AA0E?sequence=2 (accessed June 2020).

Wielgus AR, Chignell CF, Miller DS, Van Houten B, Meyer J, Hu DN, Roberts JE (2007) Phototoxicity in human retinal epithelial cells promoted by hypericin, a component of St. John's wort. Photochem. Photobiol. 83: 706–13.

Wilhelm KP, Biel S, Siegers CP (2001) Role of flavonoids in controlling the phototoxicity of Hypericum perforatum extracts. Phytomedicine 8: 306–9.

Yang JF, Liu YR, Huang CC, Ueng YF (2018) The time-dependent effects of St John's wort on cytochrome P450, uridine diphosphate-glucuronosyltransferase, glutathione S-transferase, and NAD(P)H-quinone oxidoreductase in mice. J. Food Drug Anal. 26(1): 422–31.

Annex 1 Search strategy St John's wort

Embase
20181101

Query	Results	No.
#13	#9 OR #10 OR #11 OR #12	282
#12	(#1 OR #2 OR #3) AND #8	89
#11	#2 AND #3 AND (#4 OR #5 OR #6)	113
#10	#3 AND #7	49
#9	(#1 OR #2 OR #3) AND (#4 OR #5 OR #6) AND #7	110
#8	'physical disease'/exp/mj/dm_co,dm_si OR 'mental disease'/exp/mj/dm_co,dm_si	1,278,832
#7	toxic*:ti OR intoxic*:ti OR toxin*:ti OR poison*:ti OR genotox*:ti OR neurotox*:ti OR hepatotox*:ti OR cytotox*:ti OR immunotox*:ti OR mutagen*:ti OR carcinogen*:ti OR phototox*:ti OR embryotox*:ti OR risk*:ti OR safe*:ti OR photocytotox*:ti	1,260,489
#6	'risk'/exp	2,189,795
#5	'toxicokinetics'/exp/mj OR 'pharmacokinetics'/exp/mj OR 'metabolism'/exp/mj	1,412,193
#4	'toxic substance'/exp OR 'toxicity and intoxication'/exp OR 'exposure'/exp	2,234,235
#3	'hypericum perforatum extract'/exp/mj/dd_ae,dd_to OR 'hypericin'/exp/mj/dd_ae,dd_to OR 'hyperforin'/exp/dd_ae,dd_to	379
#2	'john s wort':ti OR 'hypericum perforatum':ti OR 'johnswort':ti OR 'johns wort':ti OR ((john* NEAR/2 wort):ti) OR 'hyperforin':ti OR 'hypericin':ti	2,768
#1	'hypericum perforatum'/exp/mj OR 'hypericum perforatum extract'/exp/mj OR 'hyperforin'/exp/mj OR 'hypericin'/exp/mj	2,660

PubMed
20181105

((("hypericum perforatum"[ti] OR "johns wort"[ti] OR "john's wort"[ti] OR "hyperin"[ti] OR "hypericin"[ti])))
AND
Search ((toxic*[Title]) OR intoxic*[Title] OR toxin*[Title] OR poison*[Title] OR genotox*[Title] OR neurotox*[Title] OR hepatotox*[Title] OR cytotox*[Title] OR immunotox*[Title] OR mutagen*[Title] OR carcinogen*[Title] OR phototox*[Title] OR embryotox*[Title] OR risk*[Title] OR safe*[Title] OR photocytotox*[Title] OR acute[Title]))

Scopus
20181105

(TITLE (hypericum-perforatum OR john*-wort OR hypericin OR hyperin)) AND (TITLE (*toxic* OR *toxin* OR poison* OR mutagen* OR carcinogen* OR risk* OR safe* OR acute))

Toxcenter
20181106

(hypericum perforatum OR john?-wort OR hypericin OR hyperin)/TI
AND (?toxic? OR ?toxin? OR poison? OR mutagen? OR carcinogen? OR risk? OR safe? OR acute)
AND (person# OR human? OR volunteer# OR man OR men OR woman OR women OR boy# OR girl# OR child? OR infant# OR worker# OR employee# OR case OR cases) OR (rat OR rats OR mouse OR mice OR dog# OR hamster# OR pig# OR monkey# OR rabbit# or mammal#)

Annex 2 Intake assessment of St John's wort via plant food supplements

Introduction[9]

In 2014, a specific plant food supplement (PFS) consumption survey was performed among 739 PFS users in eight different age and gender subgroups of the Dutch population. This work was conducted within projects 5.4.2B and 9.4.33. The first results, including the prevalence of PFS users in the Dutch population and a ranked overview of the herbs that were reported to be used in ≥5 PFS in the study, are described in a research paper (Jeurissen et al., 2015).

Several botanicals, including St John's wort, were selected for a more detailed study on their intake. In agreement with the VWS and NVWA, the 10 most frequently reported botanicals of the PFS consumption survey were studied. Botanicals for which factsheets on herb–drug interactions had been published within project 9.4.25 (Tiesjema et al., 2013; NVWA, 2015) were also investigated. In addition, isoflavones, black cohosh and red yeast rice were studied because of the potential health risks associated with the consumption of these botanicals. An overview of the selected herbs can be found in Table 1.

Method

An excel sheet was created with data on all PFS containing St John's wort that had been reported to be used in the PFS consumption survey. These data included details on the age and gender of the respondent who reported the use of the PFS, the name and the brand of the PFS, the frequency, amount and duration of PFS use, and the reported concomitant use of medicines.

Names and brands of PFS were corrected where necessary to match the actual names of the products used. Where possible, information on the concentration of the botanical and/or its active constituents was collected on the internet and added to the excel sheet. For the medicines that were reported to be used concomitantly, the ATC (Anatomical Therapeutic Chemical) codes were included in the excel file. Medicines for which the ATC code could not be verified were not included.

The distribution of use between the different age and gender groups was determined. In cases where a respondent used multiple PFS with the same botanical, the respondent was counted multiple times.

For each PFS, the daily dosage was calculated. This represents the amount consumed on consumption days, and is not corrected for long-term intake.

In addition, duration of use was determined and expressed in months. If PFS were not used on a daily basis, the duration was corrected. For example, if a PFS was reported to be used 4 times a week during 7 months, the corrected duration of use was 4 months.

[9] This Annex is based on Jeurissen et al. (2015).

Results

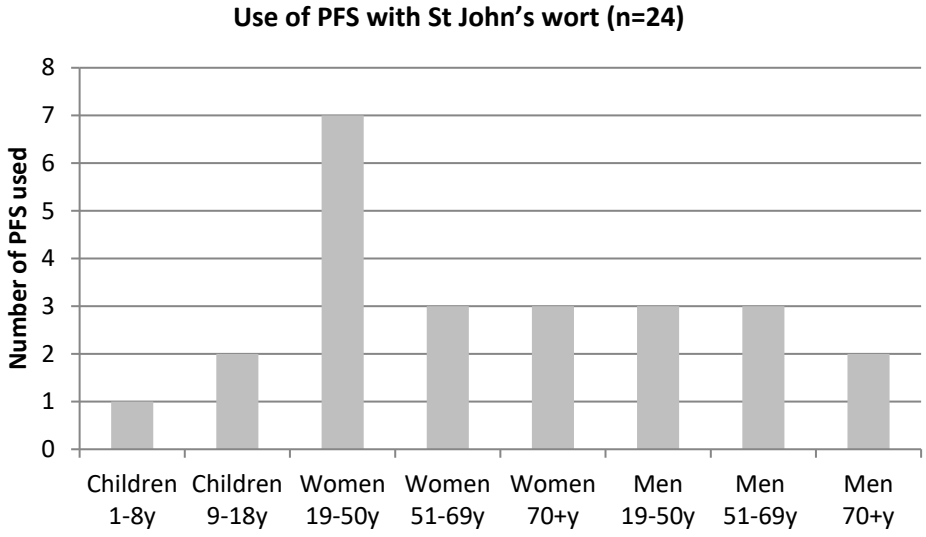


Figure A2.1. Use of different plant food supplements (PFS) containing St John's wort in the Dutch population expressed per gender and age group.

Table A2.1. Reported plant food supplements (PFS) containing St John's wort used per individual, including daily dose and duration of use.

Gender, age	PFS name	Brand	Daily dose based on reported use	Duration of use (months)
Child, 9–18y	Ansemilla	Bloem	20 drops[a]	3.5
F, 19–50y	Axium multi + echinacea	Axium	0	6
M, 19–50y	Brain Mood	Ortholon	0.6 mg hypericin[b]	2
F, 19–50y	Famosan overgang balans	A.Vogel	2,000 mg[c]	1.5
F, 19–50y	Famosan overgang balans	A.Vogel	2,000 mg[c]	3
M, 51–69y	Hyperiforce forte sint-janskruid	A.Vogel	66 mg extract[d]	6
M, 51–69y	Lavandula complex	Bonusan	90 mg extract	3
F, 19–50y	Melissa Complex	Bonusan	unknown	1
F, 19–50y	Menstrual Care	Care for Women	75 mg extract	2.5
F, 70+ y	Monarda Complex	A.Vogel	1,000 mg extract[e]	12
F, 51–69y	No sweat	Onbekend	unknown	2
Child, 9–18y	Posivrouw Mentale Veerkracht	Lucovitaal	112.5 mg extract	1
F, 19–50y	Sint Janskruid	Divers	unknown	12
F, 70+ y	Sint Janskruid	Dr de Hoog	unknown	1
M, 70+ y	Sint Janskruid	Etos	1.8 mg hypericin[b]	12
F, 51–69y	Sint Janskruid	Hema	0.45 mg hypericin[b]	12
M, 19–50y	Sint Janskruid	Hema	0.9 mg hypericin[b]	3
Child, 1–8y	Sint Janskruid	Huismerk	unknown	12
F, 51–69y	Sint Janskruid	Kruidvat	1[b]	12
F, 19–50y	Sint Janskruid	Vitotaal	>0.52 mg hypericin[c]	1
F, 70+ y	St Janskruid	Kneipp	unknown	5
M, 51–69y	Super Sint Janskruid	Optimax	2 mg hypericin[b]	6
M, 19–50y	Tai-Ginseng	Emonta bv	22.5 mg extract	3
M, 70+ y	Tai-Ginseng	Emonta bv	45 mg extract	12

[a] Contains, among other ingredients, 7.7% St John's wort extract.
[b] The food supplement contains St John's wort extract containing 0.3% hypericin.
[c] The food supplements contains the active ingredients of 2,000 mg St John's wort.
[d] Extract from in total 205-264 mg St John's wort
[e] Plant extract, containing St John's wort, among other ingredients.

Table A2.2. Medicines (ATC code plus name) reported to be used by users of PFS containing St John's wort.

A10AD01 - Insulin (Human)
A02BC01 - Omeprazole
A06AD15 - Macrogol
A10AB05 - Insulin Aspart
A10BA02 - Metformin
A12AA04 - Calcium Carbonate (2x)
B01AA07 - Acenocoumarol
B01AC08 - Carbasalate Calcium or N02BA15 - Carbasalate Calcium
C01BC04 - Flecainide
C07AB02 - Metoprolol (2x)
C09AA02 - Enalapril (2x)
C09CA04 - Irbesartan
H03AA01 - Levothyroxine Sodium (2x)
M01AB05 - Diclofenac (3x)
M01AX05 - Glucosamine
N02BE01 - Paracetamol (2x)
N03AX11 - Topiramate
N05AH04 - Quetiapine
N05AN01 - Lithium
N05BE01 - Buspirone
N06AB10 - Escitalopram
N06AX16 - Venlafaxine (2x)
N06BA04 - Methylphenidate
R03AK06 - Salmeterol and fluticasone
R03BA05 - Fluticasone
R03DC03 - Montelukast
R06AX27 - Desloratadine

References

Jeurissen S, Beukers M, Tiesjema B (2015) Intake assessment of selected botanicals via plant food supplements. Unpublished memo.

Jeurissen SMF, Buurma-Rethans EJM, Beukers MH, Jansen-van der Vliet M, Van Rossum CTM, Sprong RC (2018) Consumption of plant food supplements in the Netherlands. Food Funct. 9: 179–90. doi: 10.1039/c6fo01174h.

Tiesjema B, De Wit L, Brandon E, Jeurissen S, Kupper N, Noorlander C, Van Kranen K (2013). Interactions between herbs and medicines: St. John's wort. [Interacties tussen kruiden en geneesmiddelen. Sint Janskruid] (in Dutch) RIVM briefrapport 090425001/2013. Available via: https://www.rivm.nl/bibliotheek/rapporten/090425001.pdf (accessed June 2020).

NVWA (2015) Voedingssupplementen en kruidenpreparaten. Risico's gelijktijdig gebruik van kruidenpreparaten met geneesmiddelen. Dossier op NVWA website. Available via: https://www.nvwa.nl/onderwerpen/voedingssupplementen-en-kruidenpreparaten/risico-s-gelijktijdig-gebruik-van-kruidenpreparaten-met-geneesmiddelen (accessed June 2020).

Annex 3 Case studies

Table A3.1 Overview of reported cases of adverse events after St John's wort (SJW) use in literature. Only case reports in which adverse events were associated with the oral use of St John's wort in individuals that were not also taking selective serotonin reuptake inhibitors (SSRIs) or other herbal products were included.

Ref.	**Gender age**	**Presenting symptoms**	**Daily SJW dose**	**Time to onset of adverse effects**	**Co-medications**	**Alcohol use**	**Medical history**	**Medical examination, blood tests, serology**	**Treatment**
Assalian (2000)	M, 49	Erectile dysfunction	2 tablets of 0.9 mg SJW extract twice daily	1 week	No	No	Recurrent depression	No abnormalities	Sildenafil when necessary
Bhopal (2001)	M, 42	Diminution in libido, more depressive symptoms	NR	9 months	No	No	Anxiety, depression, obsessive-compulsive disorder	NR	Replacement with citalopram, libido returned
Bove (1998)	F, 35	Stinging pain on face and dorsum of both hands, arms and legs upon sun exposure	500 mg/day	4 weeks	NR	NR	NR	No skin burns. Light brushing, gust of air and cold were painful. No motor or other sensory disturbances. Diagnosis: subacute toxic neuropathy	Discontinuation of SJW led to disappearance symptoms
Brown (2000)	F, 33	Extreme anxiety, nausea	NR	2 days	No	No	NR	BP 195/110 mmHg, HR 122 beats/min	Alprazolam, which helped. Another

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
									4 episodes in a period of 2 weeks
Cotterill (2001)	F, 45	Large blisters on legs after laser treatment of multiple solar keratoses. Inflammation of face after laser treatment of facial telangiectasia	SJW	NR	NR	NR	NR	NR	After stopping use of SJW no reaction to laser treatment
Crowe & McKeating (2002)	F, 21	None	1,000 mg 3 times daily (last weeks); tablet contains 500 mg (0.3% hypericin)	3 months	No	No	Depression	Delayed emergence after surgery under anaesthesia. White blood cells $12.1x10^9$, sodium 138 nM, potassium 4.4 mM, PCO_2 46.5 mmHg	NR
Fahmi et al. (2002)	F, 28	Acute mania characterized by marked hyperactivity,	18 g/day	2 weeks	No	No	NR	Hyperactive, disorganized, pressured in speech, paranoid	Hospitalized, treated with olanzapine, sodium

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
		aggressive behaviour, mood elevation and irritability, overspending, overeating, poor sleep						delusions. Physical examination unremarkable	valproate. Manic symptoms resolved over 3–4 weeks
Ferrara et al. (2017)	M, 25	Psychotic symptoms	4 g herbal infusion with *Hypericum* 4 times a day	<3 months	No	No	Former drug-induced psychotic episode	Delusions, no anxiety or sleep disturbances. No abnormalities	Risperidone, paliperidone
Golsch et al. (1997)	F, 61	Itching and oedematous erythema on face, neck and chest	40 mg SJW hyper-phorate tablets (0.05 mg hypericin), 3x2 per day	~3 years	Diclofenac, acetylsalicylic acid	No	Depression	Recurring elevated itching erythematous lesions in light-exposed areas. No abnormalities. Systemic photoprovocation test with orally given SJW was positive	Withdrawal from SJW
Gurok et al. (2014)	F, 47	Smiling/ laughing without reason, disorganized speech, bizarre behaviour,	600 ml SJW extract Daily	7 days	No	No	NR	Anxiety, hallucinations, insomnia and delusions. Physical and neurological examinations unremarkable.	Haloperidol, olanzapine. Withdrawal from SJW. Symptoms disappeared

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
		delusion, hallucination, social/ emotional withdrawal, self-neglect						No abnormalities in blood tests	
Guzelcan et al. (2001)	F, 67	Confusion, delusions	3x300 mg extract/ day	3 weeks	Levothyroxine	No	NR	Hallucinations, anxiety, paranoid and bizarre delusions, disoriented. Physical examination unremarkable. Small infarct in putamen. No abnormalities in blood tests, T_4 and TSH levels normal. Diagnosis: delirium	Haloperidol
Guzelcan et al. (2001)	F, 23	Insomnia, delusions, hyperactivity, mania	1 or 2 tablets of combina-tion prepara-tion with a.o. hypericin (350 µg) and	10 weeks	No	8 units over week-end	No	Hallucinations, hyperactivity, megalomania. Physical and neurological examination unremarkable.	Olanzapine. Withdrawal from combination preparation

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
			valerian extract (125 mg) per day, then for 3 days 3 tablets 3x/day					No abnormalities in blood tests	
Holme & Roberts (2000)	M, 44	Erythematous eruption on all skin, burning discomfort	333 mg capsules *Hypericum*	4 days	Dothiepin for 2 years	No	Depression	Bright red, hot, dry skin, fine scaling on both light-exposed and non-exposed skin	Systemic steroid. Eruption cleared completely
Imbernón-Moya et al. (2016)	F, 37	Pruritic rash on trunk	900 mg/day SJW orally	1 week	No	No	No	Several erosive crusted patches with erythematous edges on lower neck and upper back. No abnormalities in blood tests. Negative serology. Diagnosis: pemphigus foliaceus	Azathioprine and discontinuance of SJW
Irefin & Sprung (2001)	F, 23	Hysteroscopy with general anaesthesia	NR	6 months	No	No	Depression	Shortly after induction anaesthesia BP 60/20 mmHg, HR	Fluid, ephedrine and epinephrine. Patient

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
								60 bpm. No bronchospasm, cyanosis, skin flushing or dysrhythmia	remained steady during rest of anaesthesia
Karalapillai & Bellomo (2007)	F, 16	Seizures, confusion	Up to 15 tablets of 300 µg (not specified) + additional 50 tablets prior to admission	2 weeks	NR	NR	NR	CT scan brain and CSF examination unremarkable. Electrolyte levels normal, tox screens negative. EEG confirmed generalized epileptic activity	Withdrawal of SJW
Khalifa (2015)	F, 40	Tingling sensation in hands when washing with cold water	SJW 500 mg twice daily	4 weeks, after started working long hours in sun	NR	NR	Depression	No skin erythema or skin burning. Cold tuning fork on hands produced tingling sensation and discomfort. Light touch did not provoke pain. No motor or other sensory disturbances, normal peripheral reflexes	SJW use discontinued. Symptoms disappeared within 3 weeks

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
Laird & Webb (2001)	F, 76	Delirium, psychosis	75 mg tablet (0.3% standardized hypericin)	3 weeks	NR	NR	NR	Disoriented, dishevelled. BP 180/90 mmHg. Integument tanned but otherwise normal. Diagnosis: Alzheimer's with acute delirium and psychotic factors	Risperidone, donepezil. Withdrawal of SJW. Continuous and gradual improvement over 7 days
Lal & Iskandar (2000)	F, 26	Acute psychosis, paranoid delusions	Herbal tea of SJW, 1 or 2 times a week for 3 months, then daily for 2 months	5 months	No. Other herbal products on occasion	NR	Schizophrenia	NR	Olanzapine
Lal & Iskandar (2000)	M, 34	Abrupt recurrence of persecutory delusions, bizarre behaviour	SJW daily	2–3 months	NR	NR	Paranoid psychosis	Schizophrenic episode	Risperidone
Lane-Brown (2000)	F, 52	Erythemato-bullous dermatosis developed after sun exposure on	SJW oil topically and orally three times daily	2 weeks	Hydroxychloroquinone and corticosteroid cream	NR	Cutaneous lupus erythema-tosus	Negative serology (for lupus antibodies)	Withdrawal of SJW and avoidance of sunlight. Prednisone treatment

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
		two successive days							settled the rash. Gross hyperpigmentation persisted even after seven years
Lane-Brown (2000)	M, 63	Follicular erythema, urticarial oedema, burning pain after UVB treatment for psoriasis	SJW, 6 pills daily	NR	No	NR	Psoriasis	NR	Rash resolved over 10 days after withdrawal of SJW and UVB treatment
Moses & Mallinger (2000)	F, 70	Missing sleep, careless, overspending	3x300 mg tablets, later reduced to 2x300 mg tablets	Couple of weeks	Stopped all previous medication before SJW use	NR	Ménière's, left internal capsule infarct, depression	NR	Reduction of SJW dose to 1 tablet per day
Moses & Mallinger (2000)	M, 53	Overactive, careless, visual illusions, mood elevation	900 mg, later reduced to 300 mg	2 months	NR	NR	Bipolar II disorder	NR	Withdrawal of SJW use

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
Nanayakkara et al. (2005)	F, 51	Fatigue, suicidal and homicidal thoughts, insomnia, dry mouth	150 mg SJW extract daily (hypericin 0.3%)	3 weeks	NR	A bit	NR	No abnormalities in physical examination. No abnormalities in blood tests	Disappeared after withdrawal. Second time this had happened to her
Nierenberg et al. (1999)	M, 20	Extreme agitation, irritability, pressured speech, pacing, anxiety, insomnia	Two tablets of 150 SJW herb extract plus 0.2% *Hypericum* 3 times daily	3 days	No	2 beers daily	Major depressive episode of bipolar disorder	No abnormalities in physical examination. No abnormalities in blood tests	Lithium and clonazepam. Discontinuance of SJW
Nierenberg et al. (1999)	F, 51	Hallucination, disorganized speech, uncontrollable giggling, hypersexuality, hypermotoric behaviour	300 mg standardized extract of SJW, 3 times daily	A few days	Lithium	NR	Psychotic mania	No abnormalities in physical examination. No abnormalities in blood tests	Lithium, haloperidol
O'Breasil & Argouarch (1998)	M, 76	Hypomanic, physically overactive, euphoric, marked	NR	6 weeks	Verapamil, ticlopidine	NR	CVA, depression	Hypomania	Valproate. Withdrawal of SJW use. Patient stabilized

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
		increased irritability							
O'Breasil & Argouarch (1998)	M, 28	Irritability, anger, mood lability, grandiosity, sleep disturbance	NR	3 months	NR	Alcohol abuse	Symptoms of PTSS	Agitated, pressure of speech, irritable, grandiose delusions, impaired concentration, limited sight. Diagnosis: bipolar disorder, acute manic episode	Lithium carbonate. Mood stabilized over 2 weeks
Parker et al. (2001)	M, 40	Flushing, diaphoresis, agitation, weakness of legs, dry mouth, tightness in chest, inability to focus	450 mg SJW daily	10 days	Clonazepam	Not excessive	Anxiety disorder, depression, mania. Adverse reactions to SSRIs	BP 172/120 mmHg, HR 94/minute, RR 22/minute. Confused, disoriented. Neurological examination and electrocardiogram normal	NR
Parker et al. (2001)	F, 24	Hair loss on scalp and eyebrows	300 mg SJW extract, 3 times daily	5 months	Olanzapine	NR	Schizophrenia	Normal physical examination. Normal blood tests. Microscopic examination of	NR

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
								hair revealed mixed telogen and normal anagen morphology	
Patel et al. (2002)	M, 41	Confused, disoriented	SJW according to dosing instruction	7 days	No	NR	NR	HR 115 beats/minute, BP 210/140 mmHg, RR 16/minute. Tangential speech, confused. Remainder physical exam unremarkable. No abnormalities in blood tests	Phentolamine, labetalol
Sultana et al. (2000)*	F, 33	Rash, particularly on cheek and chin	SJW	14 days	No	No	Pre-menstrual dysphoric disorder, major depressive disorder	Erythematous macules and papules with severe pruritus	Hydrocortisone cream, Benadryl. Withdrawal from SJW. Symptoms resolved within a week
Yildirim & Canan (2013)	M, 35	Trembling that gradually increased, fear of dying	SJW extract, one glass	2 hours	No	No	No	Palpitations, sweating, chest discomfort, shortness of breath, nausea,	Alprazolam

Ref.	Gender age	Presenting symptoms	Daily SJW dose	Time to onset of adverse effects	Co-medications	Alcohol use	Medical history	Medical examination, blood tests, serology	Treatment
								blurred vision, feelings of derealisation. Tachycardia (HR 110 bpm), RR 22 breaths/min. Reported a similar episode after drinking glass with SJW extract two months before	
Zullino & Borgeat (2003)	M, 56	Hypertension	SJW standardized dry extract, 250 mg twice a day	5 weeks	No	No	No	BP 145–160/95–110	Discontinuance of SJW extract. Blood pressure returned to normal

BP = blood pressure
CSF = cerebrospinal fluid
CT = computed tomography
EEG = electroencephalography
HR = heart rate
NR = not reported
RR = respiratory rate
SJW = St John's wort
SSRI = selective serotonin re-uptake inhibitor
TSH = thyroid-stimulating hormone
UVB = ultraviolet B

References

Assalian P (2000) Sildenafil for St. John Wort-induced sexual dysfunction. Journal of Sex and Marital Therapy 26: 357-8.

Bhopal JS (2001) St John's wort-induced sexual dysfunction. Canadian Journal of Psychiatry 46: 456-7.

Bove GM (1998) Acute neuropathy after exposure to sun in a patient treated with St John's Wort. The Lancet 352: 1121-2.

Brown TM (2000) Acute St. John's wort toxicity. American Journal of Emergency Medicine. 18: 231-2.

Cotterill (2001) Severe phototoxic reaction to laser treatment in a patient taking St John's wort. J. Cosmetic & Laser Ther. 3: 159-60.

Crowe S, McKeating K (2002) Delayed emergence and St. John's wort. Anesthesiology 96: 1025-7.

Fahmi M, Huang C, Schweitzer I (2002) A case of mania induced by hypericum. World J. Biol. Psychiatry 3: 58-9.

Ferrara M, Mungai F, Starace F (2017) St John's wort (Hypericum perforatum)-induced psychosis: a case report. J. Med. Case Rep. 11: 137-40.

Golsch S, Vocks E, Rakoski J, Brockow K, Ring J (1997) Reversible erhöhung der photosensitivität im UV-B-bereich durch Johanniskrautextrakt präparate. Hautarzt 48: 249-52.

Gurok MG, Canan F, Kiliç F (2014) Psychotic epidose induced by St. John's wort (Hypericum perforatum): a case report. Journal of Mood Disorders 4:38-40.

Güzelcan Y, Becker HE, Assies J (2001a) Een delier tijdens gebruik van sint-janskruid (hypericum perforatum). Tijdschrift voor Psychiatrie 43: 793-7.

Güzelcan Y, Scholte WF, Assies J, Becker HE (2001b) Manie tijdens her gebruik van een combinatiepreparaat met sint-janskruid (Hypericum perforatum). Ned. Tijdschr. Geneeskd. 145: 1943-5.

Holme SA, Roberts DL (2000) Erythroderma associated with St John's wort. British Journal of Dermatology 143: 1127-8.

Imbernón-Moya A, Burgos F, Vargas-Laguna E, Fernández-Cogolludo E, Aguilar-Mártinez A, Gallego-Valdés MA (2016) Pemphigus foliaceus associated with Hypericum perforatum. JAAD Case Reports 2: 326-8.

Irefin S, Sprung J (2000) A possible cause of cardiovascular collapse during anesthesia: long-term use of St. John's Wort. J. Clin. Anesth. 12: 498-9.

Karalapillai DC, Bellomo R (2007) Convulsion associated with an overdose of St John's wort. Medical Journal of Australia 186: 213-4.

Khalifa S (2015) Photodynamic sensory neuropathy in a patient treated with St John’s wort. Prim. Care Companion CNS Disord. 17: 3 pp.

Laird RD, Webb M (2001) Psychotic episode during use of St. John's wort. Journal of Herbal Pharmacotherapy 1: 81-7.

Lal S, Iskandar H (2000) St. John's wort and schizophrenia. Cmaj 163: 262-3.

Lane-Brown MM (2000) Photosensitivity associated with herbal preparations of St John’s wort (Hypericum perforatum). MJA 172:302.

Moses EL, Mallinger AG (2000) St. John's Wort: Three cases of possible mania induction. J. Clin. Psychopharmacol. 20: 115-7.

Nanayakkara PWB, Meijboom M, Schouten JA (2005) Suïcidale en agressieve gedachten bij gebruik van hypericumpreparaat (sint-janskruid). Ned. Tijdschr. Geneeskd. 149: 1347-9.

Nierenberg AA, Burt T, Matthews J, Weiss AP (1999) Mania associated with St. John's wort. Biol. Psychiatry 46: 1707-8.

O'Breasail AM, Argouarch S (1998) Hypomania and St John's Wort. Can. J. Psychiatry 43: 746-7.

Parker V, Wong AHC, Boon HS, Seeman MV (2001) Adverse reactions to St John's Wort. Can. J. Psychiatry 46: 77-9.

Patel S, Robinson R, Burk M (2002) Hypertensive crisis associated with St. John's Wort. American Journal of Medicine 112: 507-8.

Sultana D, Peindl KS, Wisner KL (2000) Rash associated with St. John's Wort treatment in premenstrual dysphoric disorder. Archives of Women's Mental Health 3: 99-101.

Yildirim O, Canan F (2013) A case of panic attack induced by St John's wort. Prim. Care Companion CNS Disord. 15: 2 pp.

Zullino D, Borgeat F (2003) Hypertension induced by St. John’s Wort. Pharmacopsychiatry 36: 32.

Annex 4 Clinical trials

Table A4.1. Overview of clinical trials with St John's wort extract.

Study	**Extract**	**Daily dose**	**Duration**	**Study population**	**No. of participants**	**Adverse events**		
Lecrubier et al. (2002)	WS 5570; 3%–6% hyperforin, 0.12%–0.28% hypericin	3 x 300 mg extract 1.08–2.52 mg Hypericin	6 weeks	Outpatients aged 18-65 years with mild to moderate major depression	375 patients, 186 assigned to SJW (18 dropouts, 2 because of AEs)	*Total*		*57/186 (30.6%)*
						Nausea		9 (4.8%)
						Headache		3 (1.6%)
						Dizziness		4 (2.2%)
						Abdominal pain		2 (1.1%)
						Insomnia		3 (1.6%)
Anghelescu et al. (2006)	WS 5570; Extract not specified	3 x 300 mg or 3 x 600 mg	16 weeks	Outpatients aged 18-70 years suffering from moderate or severe depression	133 patients, 86 patients assigned to SJW (15 dropouts, 3 because of AEs) 33 patients (900 mg/day); 38 patients (1,800 mg/day)	*Total 19/71 (26.8%)* No AEs judged to be study-related, except 1 allergic reaction to sunlight (reason for withdrawal). Incidence 0.006 AEs/day		
Kasper et al. (2006)	WS 5570 Extract not specified	600 mg or 2 x 600 mg	6 weeks	Patients 18-65 years with mild or moderate depression	332 patients, 123 patients (600 mg) (12 dropouts, 2 due to AEs); 127 patients (1,200 mg) (19 dropouts, 4 due to AEs)		**600 mg**	**1,200 mg**
						Total	*49/123 (39.8%)*	*50/127 (39.4%)*
						Ear and labyrinth disorders	3 (2.4%)	2 (1.6%)
						Eye disorders	0 (0.0%)	1 (0.8%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events		
						Gastro-intestinal disorders	24 (19.5%)	30 (23.6%)
						General disorders and administration site conditions	2 (1.6%)	2 (1.6%)
						Infections and infestations	7 (5.7%)	4 (3.2%)
						Injury, poisoning and procedural complications	1 (0.8%)	1 (0.8%)
						Investigation	1 (0.8%)	0 (0.0%)
						Metabolism and nutrition disorders	1 (0.8%)	1 (0.8%)
						Musculo-skeletal and connective tissue disorder	1 (0.8%)	2 (1.6%)
						Nervous system disorder	6 (4.9%)	6 (4.7%)
						Psychiatric disorders	2 (1.6%)	2 (1.6%)
						Renal and urinary disorders	1 (0.8%)	0 (0.0%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events		
						Reproductive system and breast disorders	1 (0.8%)	2 (1.6%)
						Respiratory, thoracic and mediastinal disorders	4 (3.3%)	5 (3.9%)
						Skin and subcutaneous disorders	4 (3.3%)	2 (1.6%)
						Vascular disorders	1 (0.8%)	1 (0.8%)
Szegedi et al. (2005)	WS 5570; 3–6% hyperforin, 0.12–0.28% hypericin	3 x 300 mg, initial non-responders dose increased to 1,800 mg (3 x 600 mg)	6 weeks	Outpatients 18-70 years with moderate or severe episodes of unipolar major depression	251 patients, 125 assigned to SJW (17 dropouts, 4 due to AEs)	Incidence 900 mg: 0.029 AEs/day Incidence 1,800 mg: 0.039 AEs/day		
						Total		*69/125 (55.2%)*
						Gastrointestinal disorders (total)		42 (33.6%)
						Nervous system disorders (total)		29 (23.2%)
						Upper abdominal pain		12 (9.6%)
						Diarrhoea		12 (9.6%)
						Dry mouth		16 (12.8%)
						Nausea		9 (7.2%)
						Fatigue		14 (11.2%)
						Dizziness		9 (7.2%)
						Headache		13 (10.4%)
						Sleep disorder		5 (4.0%)
						Increased sweating		9 (7.2%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events		
Schüle et al. (2004)	WS 5570; Extract not specified	600 mg 900 mg 1,200 mg	Subject took part 4 times in the trial, in ran-domized order, to receive placebo/ SJW at different dosages on 4 different days	Healthy male volunteers 26-41 years	12 subjects	No AEs reported		
Kalb et al. (2001)	WS 5572; 5% hyperforin	3 x 300 mg	6 weeks	Outpatients 18-65 years with mild or moderate major depressive disorder	72 patients, 37 assigned to SJW	*Total: 3/37 (8.1%)* Not drug-related (sinusitis, bronchitis, common cold)		
Laakmann et al. (1998)	WS 5573; 0.5% hyperforin WS 5572; 5% hyperforin	3 x 300 mg	42 days	Outpatients with mild to moderate depression	147 patients, 49 assigned to SJW		**WS 5573**	**WS 5572**
						Total	*14/49 (28.6%)*	*17/49 (34.7%)*
						Bronchitis	3	1
						Influenza-like symptoms	2	0
						Cough	2	0
						Infection	1	0

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
Rychlik et al. (2001)	WS 5572; 5% hyperforin	600 mg 2 x 600 mg		Patients with mild to moderate depressive episodes	2,166 patients assigned to SJW (86 dropouts, 4 due to AEs), 1,385 patients (600 mg) 781 patients (1,200 mg)	*Total 17* AE frequency <1% (possibly drug-related: skin irritation, pruritus, allergic exanthema, nervousness, restlessness, gastrointestinal disorders, diarrhoea, insomnia)	
Schrader et al. (1998)	Ze 117; 0.5 mg hypericin	2 x 250 mg 1 mg hypericin	6 weeks	Patients with mild to moderate depression	162 patients >18 years, 81 assigned to SJW	*Total*	*6/81 (7.4%)*
						Abdominal pain	2 (2.5%)
						Diarrhoea	1 (1.2%)
						Melancholia	1 (1.2%)
						Acute deterioration	1 (1.2%)
						Dry mouth	1 (1.2%)
						GI disturbances	6/125 (4.8%)
Woelk (2000)	Ze 117; 0.2% hypericin	2 x 250 mg 1 mg hypericin	6 weeks	Patients >18 years with mild to moderate depression	324 patients, 157 assigned to SJW (15 dropouts, 4 due to AEs)	*Total*	*62/157 (39%)*
						Dry mouth	13 (8%)
						Headache	3 (2%)
						Sweating	2 (1%)
						Asthenia	2 (1%)
						Nausea	1 (<1%)
Brattström et al. (2009)	Ze 117; 0.2% hypericin	2 x 250 mg 1 mg hypericin	1 year	Patients ≥18 years with mild to moderate depression	440 patients (299 dropouts before a year, 25 (5.7%) due to AEs)	*Total*	*30/440 (7%)*
						Skin rash	4 (0.9%)
						Abdominal pain/ gastro-intestinal disorder	4 (0.9%)
						Urticaria/pruritus	3 (0.7%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
						Insomnia	3 (0.7%)
						Gastritis	2 (0.5%)
						Dry mouth	2 (0.5%)
						Nocturia / dysuria	2 (0.5%)
						Flatulence	1 (0.2%)
						Depression	1 (0.2%)
						Dizziness	1 (0.2%)
						Twitching	1 (0.2%)
						Skin discolouration	1 (0.2%)
						Photosensitivity	1 (0.2%)
						Tachycardia	1 (0.2%)
						Vasodilatation	1 (0.2%)
						Weight gain	1 (0.2%)
						Alopecia	1 (0.2%)
Friede et al. (1998)	Ze 117	2 x 250 mg	15 days	Healthy volunteers	19 subjects (no dropouts)	No significant difference between treatment and placebo in AEs (headache, tension, tachycardia, fatigue, imbalance, loss of concentration, depression, agitation, visual disturbance, restlessness)	
Friede et al. (2001)	Ze 117; Extract not specified	2 x 250 mg	6 weeks	Outpatients with mild to moderate depressive episodes	240 patients, 126 assigned to SJW (1 dropout)	*Total* *Possibly/probably related to drug*	*18/125 (14%)* *10/125 (8%)*
						GI disturbance	6 (4.8%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
Yechiam et al. (2019)	Ze 117; 0.1–0.3% total hypericins (unspecified), <1% hyperforin	250 mg 0.5 mg hypericin 500 mg 1 mg hypericin	single dose	Healthy volunteers 18-40 years	82 subjects, 42 subjects (250 mg), 40 subjects (500 mg)	No AEs, except: parched throat (500 mg)	
Bjerkenstedt et al. (2005)	LI 160 Extract not specified	3 x 300 mg	4–6 weeks	Outpatients 18-70 years with mild to moderate depression	174 subjects, 57 assigned to SJW (19 dropouts)	*Total*	*20/57 (35.1%)*
						Body as a whole	13 (22.8%)
						Gastrointestinal system disorders	6 (10.5%)
						Autonomic nervous system disorders	10 (17.5%)
						Central and peripheral nervous system disorders	10 (17.5%)
						Skin and appendages disorders	9 (15.8%)
						Psychiatric disorders	2 (3.5%)
						Others	5 (8.8%)
Fava et al. (2005)	LI 160 0.12–0.28% hypericin	3 x 300 mg 1.08–2.52 mg hypericin	12 weeks	Patients 18-65 years with major depressive episode	135 patients, 45 assigned to SJW (18 dropouts)	Headache	19/45 (42%)
						Dry mouth	10 (22%)
						Nausea	9 (20%)
						Gastrointestinal upset	9 (20%)
						Sleepiness	8 (18%)
						Insomnia	7 (16%)
						Cold symptoms	6 (13%)
						Flu	5 (11%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
						Upper respiratory tract infection	5 (11%)
						Muscle pain/aches	5 (11%)
Shelton et al. (2001)	LI 160 Extract not specified	900 mg (at least 4 weeks) 1,200 mg (8 weeks in event of no response at lower dose)	12 weeks	Patients with major depressive disorder	200 patients, 95 assigned to SJW, (19 dropouts, 1 due to AEs)	In ≥10% of patients: abdominal discomfort and headaches. Headaches (39/95, 41%) statistically significantly different from placebo	
Davidson et al. (2002)	LI 160 0.12–0.28% hypericin	900-1500 mg	8 weeks (patients could continue for another 18 weeks at max. 1,800 mg)	Outpatients ≥18 years with major depressive disorder	340 patients, 113 assigned to SJW (31 dropouts, 2 due to AEs), 112 included in safety analysis	Diarrhoea	23 (21%)
						Nausea	21 (19%)
						Anorgasmia	28 (25%)
						Forgetfulness	28 (25%)
						Frequent urination	30 (27%)
						Sweating	20 (18%)
						Swelling	21 (19%)
Wheatley (1997)	LI 160 240–320 µg total hypericins (unspecified)	3 x 300 mg	6 weeks	Patients 20-65 years with mild to moderate depression	156 patients, 87 assigned to SJW (20 dropouts, 6 due to AEs)	*Total*	*32/87 (37%)*
						Headache	6 (7%)
						Nausea/vomiting	6 (7%)
						Dry mouth	4 (5%)
						Constipation	4 (5%)
						Sleepiness	2 (2%)
						Pruritus	2 (2%)
						Dizziness	1 (1%)
						Drowsiness	1 (1%)
						Lethargy	1 (1%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
Vorbach et al. (1997)	LI 160	3 x 600 mg	6 weeks	Patients 18-70 years with severe episode of major depressive disorder	209 patients, 107 assigned to SJW (9 dropouts, 1 due to AEs)	*Total*	*25/107 (23%)*
						Restlessness	6 (5.6%)
						Dizziness	5 (4.7%)
						Gastric symptoms	5 (4.7%)
						Tiredness/sedation	5 (4.7%)
						Dry mouth	3 (2.8%)
						Tremor	2 (1.9%)
						Allergic skin reaction	1 (0.9%)
Barnes et al. (2006)	LI 160; Extract not specified	1 x 300 mg, 2 x 300 mg	13 weeks	Smokers 18-65 years	28 subjects, (12 dropouts, 1 due to AEs) 15 (300 mg) 13 (600 mg)	2 AEs suspected to be treatment-related	
Canning et al. (2010)	LI 160; 0.18% hypericin 3.38% hyperforin	2 x 450 mg 1.62 mg hypericin 30.42 mg hyperforin	2 menstrual cycles	Women 18-45 years diagnosed with mild premenstrual syndrome	36 subjects, (4 dropouts)	*Total*	*15*
						Digestive (stomach cramps, abdominal pain, nausea, diarrhoea, dizziness)	4
						Vasomotor (hot flushes, increased sweating)	2
						Respiratory (cold, sinus ache, sore throat, swollen glands, viral infection, laryngitis)	3
						Headache/migraine	1
						Spots	1
						Vaginal discharge	1
						Menstrual flooding	1

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
						Forgetfulness	1
						Chest pain	1 (other cause present)
Franklin et al. (1999)	LI 160; 0.3% total hypericin (unspecified)	9 x 300 mg 8.1 mg total hypericin	Single dose	Healthy male volunteers 22-49 years	12 subjects	Total 2/12 (17%): gastrointestinal side effects (flatulence)	
Hübner & Arnoldt (2000)	LI 160	3 x 300 mg	12 months	Patients 18-75 years with mild to moderate depression	313 patients (79 dropouts)	*Total 35/313 (11%)* 7 of them treatment-related Infection of upper respiratory tract, headache, gastrointestinal complaints (2.23% of 313). Sweating, diarrhoea, fatigue, calf cramps, itching, hallucination, papulosis (1x)	
Hubner & Kirste (2002)	LI 160; 900 µg hypericin	300–1,800 mg	4–6 weeks	Children 1-12 years with mild to moderate depressive symptoms	101 patients (9 dropouts) 15 (300 mg) 26 (600 mg) 25 (900 mg) 3 (1,800 mg) 16 (increased dose, end dose unknown) 7 (decreased dose, end dose unknown)	No AEs	
Kobak et al. (2005)	LI 160	Increasing dose by choice: 2 x 300 mg	12 weeks	Outpatients 18-65 years with social phobia	40 patients, 20 assigned to SJW (3 dropouts)	*Total*	*14/20 (70%)*
						Gastrointestinal upset	5 (25%)
						Upper respiratory infection	4 (20%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
		2 x 450 mg 2 x 600 mg 2 x 900 mg				Dizziness	4 (20%)
						Insomnia and fatigue	3 (15%)
Müller et al. (2004)	LI 160	2 x 300 mg	6 weeks	Out patients 18-65 years with (undifferentiated) somatization disorder and somatoform autonomic dysfunction	184 patients (9 dropouts), 87 assigned to SJW	*Total*	*21/87 (24.1%)*
						Body as a whole	8 (9.2%)
						Central and peripheral nervous system disorders	5 (5.7%)
						Psychiatric disorders	4 (4.6%)
						Respiratory system disorders	4 (4.6%)
						Musculoskeletal system disorders	2 (2.3%)
						Gastro-intestinal system disorders	1 (1.1%)
						Others	7 (8%)
Woelk et al. (1994)	LI 160	3 x 300 mg	4 weeks	Patients <20-90 years with mild to severe depression	3,250 patients	*Total*	*79/3250 (2.43%)*
						Nausea	6 (0.18%)
						Abdominal pain	5 (0.15%)
						Loss of appetite	3 (0.09%)
						Diarrhoea	2 (0.06%)
						Gastrointestinal symptoms	2 (0.06%)
						Allergy	6 (0.18%)
						Skin rash	6 (0.18%)
						Pruritus	5 (0.15%)
						Fatigue	13 (0.40%)
						Anxiety	8 (0.26%)
						Dizziness	5 (0.15%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
						Other side effects	18 (0.55%)
Taylor et al. (2000)	LI 160; standardized to 0.12–0.28% total hypericin (hypericin+ pseudo-hypericin)	2 x 300 mg, which could be increased to 6 x 300 mg	12 weeks	Patients 18-65 years with obsessive-compulsive disorder	60 subjects, 30 assigned to SJW (2 dropouts)	*Total*	*19/28 (63.3%)*
						Headache	6 (21.4%)
						Gastrointestinal symptoms	6 (21.4%)
						Fatigue	4 (14.3%)
						Agitation	4 (14.3%)
						Sleep disturbance	3 (10.7%)
van Gurp et al. (2002)	*Hypericum* extract; 0.3% *Hypericum*	3 x 300 mg, initial non-responders' dose increased to 1,800 mg (3 x 600 mg)	12 weeks	Patients 18-65 years with major depression	87 patients, 44 assigned to SJW (15 dropouts, 3 due to AEs)	Sleep disturbance	23/42 (54.8%)
						Anxiety	18 (42.9%)
						Sexual problems	5 (11.9%)
						Headaches	18 (42.9%)
						Dizziness	5 (11.9%)
						Tremor	8 (19.1%)
						Sweating	7 (16.7%)
						Dry mouth	16 (38.1%)
						Muscle spasms	5 (11.9%)
						Muscle or joint stiffness	8 (19.1%)
						Urinary problems	7 (16.7%)
						Difficulty digesting	8 (19.1%)
						Nausea or vomiting	4 (9.5%)
						Diarrhoea	10 (23.8%)
						Lack of appetite	10 (23.8%)
						Heart palpitations	4 (9.5%)
						Fatigue	19 (45.2%)
						Pain	5 (11.9%)
						Blurred vision	6 (14.3%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events		
Lenoir et al. (1999)	Hyperiforce	3 x 1 tablet, standardized to 0.17, 0.33 or 1 mg total hypericin (unspecified)	6 weeks	Outpatients 19-94 years with mild to moderate depression	348 patients, 119 (1 mg total hypericin/ day) 115 (0.33 mg total hypericin/ day) 114 (0.17 mg total hypericin/ day)	No difference in occurrence of AEs between the three treatment groups. AEs probably/possibly related to study medication: skin (3), nerves (7), psyche (2), gastrointestinal tract (4), organism as a whole (2)		
Gastpar et al. (2006)	STW3-VI; Standardized to 900 mg *H. perforatum*	3 x 300 mg	6 weeks	Outpatients 18-74 years with moderate depression	388 patients, 131 assigned to SJW (28 dropouts)	*Total*		*39/131 (29.8%)*
						Gastrointestinal disorders		6 (4.6%)
						Ear and labyrinth disorders		1 (0.8%)
						Skin and subcutaneous tissue disorders		1 (0.8%)
Abdali et al. (2010)	Hypiran, *H. perforatum* extract	3 x 20 drops, 0.2 mg/ml hypericin	8 weeks	Women 45-55 years experiencing hot flushes	100 subjects, 50 assigned to SJW (5 dropouts)		**4th week**	**8th week**
						Total	*11/42 (26.2%)*	*4/42 (9.5%)*
						Headache	2 (4.8%)	1 (2.4%)
						Abdominal pain	2 (4.8%)	3 (7.1%)
						Lethargy	7 (16.7%)	4 (9.5%)
Al-Alkoum et al. (2012)	*H. perforatum* extract, standardized to 0.3% hypericin	3 x 300 mg, 2.7 mg hypericin	12 weeks	Perimenopausal women 40-65 years experiencing hot flushes	47 subjects, 22 assigned to SJW (2 dropouts)	*Total*		*11/20 (55%)*
						Constipation		5 (25%)
						Somnolence (lethargy)		4 (20%)
						Loss of appetite		1 (5%)
						Dizziness		1 (5%)
						Nausea (sickness)		1 (5%)
Asgari et al. (2010)	*H. perforatum* extract	3 x 150 mg, 480 µg hypericin	4 weeks	Men 18-50 years with	50 subjects, 25 assigned to SJW (3 dropouts)	*Total*		*6/22 (27%)*
						Headache		3 (14%)
						Constipation		2 (9%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
				premature ejaculation		Photosensitivity	1 (4.5%)
Cloudwell et al. (2011)	hypericin	Gradually increasing dosage: 0.05-0.50 mg/kg bw	3 months	Patients ≥18 years with recurrent malignant gliomas	42 subjects, 17 completed	*Total*	*31/42 (73.8%)*
						Skin and subcutaneous tissue disorders (photo-sensitivity reaction, erythema, skin burning sensation)	30 (71.4%)
						Nervous system disorders (convulsion, hyperesthesia, paresthesia)	35 (83.3%)
						Gastrointestinal side effects (abdominal distension, vomiting, diarrhoea)	10 (23.8%)
Ellis et al. (2001)	*H. perforatum* extract, standardized to 0.3% hypericin, 3–5% hyperforin	900 mg, 1,800 mg	Single dose	Healthy volunteers 18-54 years	12 subjects	No AEs reported	
Gulick et al. (1999)	hypericin	0.5 mg/kg bw	8 weeks	HIV-infected patients	3 subjects (3 dropouts due to AE)	Phototoxicity of grade 3: 3 of 3 (100%)	
Hicks et al. (2004)	*H. perforatum* extract,	2 x 300 mg, 1,800 µg hypericin	Two menstrual cycles	Normally menstruating women with	169 subjects, 87 assigned to SJW (26 dropouts, 5 due to AEs)	Nausea	8 (13%)
						Diarrhoea	6 (10%)
						Flatulence	5 (8%)
						Headache	13 (21%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events		
	standardized to 900 µg hypericin			recurrent premenstrual symptoms		Skin rash	3 (5%)	
						Dizziness/confusion	4 (7%)	
						Tiredness/sedation	5 (8%)	
						Nausea, worsening of premenstrual symptoms	1 (1%)	
						Bloatedness, breast tenderness, rashes, sensitivity in right eye	1 (1%)	
						Headache, pain over eyes	1 (1%)	
						Tiredness, forgetfulness, woolly head	1 (1%)	
						Nausea, diarrhoea, dry mouth	1 (1%)	
Jacobson et al. (2001)	hypericin	0.05 or 0.1 mg/kg bw	8 weeks	Patients 18-70 years with chronic HCV infection	19 patients, 12 assigned to 0.05 mg/kg, 7 to 0.1 mg/kg		**0.05 mg/kg**	**0.1 mg/kg**
						Photosensitivity	7/12 (58%)	7/7 (100%)
						Headache, dizziness, amaurosis fugax	-	1 (14%)
						Dry mouth and angular cheilosis	-	1 (14%)
Lawvere et al. (2006)	*H. perforatum* extract, standardized to 0.3% hypericin, ≤4% hyperforin	2 x 450 mg, 2.7 mg hypericin	12 weeks	Smokers 18-65 years	37 subjects (13 dropouts)	*Total*	*10/37 (27%)*	
						Change in bowel movements	3 (8.1%)	
						Sensitivity to light	2 (5.4%)	
						Constipation	2 (5.4%)	

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events		
						Abdominal pain	1 (2.7%)	
						Dizziness	1 (2.7%)	
						Fatigue/tiredness	1 (2.7%)	
Mueller (1998)	HYP 811	425–850 mg	6 weeks	Patients ≥17 years with depressive mood disorder	607 patients (59 dropouts)	Side effects reported by 3 patients, included allergic reactions, increased skin sensitivity and nausea		
Randløv et al. (2006)	PM235, standardized to either 0.12% or 0.18% hypericin	3 x 270 mg, 0.972 mg or 1.458 mg hypericin	6 weeks	Patients 25-70 years with mild or moderately severe depressed episodes or with dysthymia	150 patients, 50 assigned to SJW low hypericin content (7 dropouts), 50 assigned to SJW high hypericin content (5 dropouts)		**0.12% hypericin**	**0.18% hypericin**
						Total	*16/50 (32%)*	*10/50 (20%)*
						AEs included mostly headache and gastrointestinal symptoms		
Rapaport et al. (2011)	*H. perforatum* extract	3 x 270 mg	12 weeks	Patients with minor depression	81 patients, 29 assigned to SJW (7 dropouts; in total 26 patients available for safety evaluation)	Gastrointestinal (diarrhoea, constipation, dry mouth, nausea/vomiting)		16 (61.5%)
						Heart (palpitations, dizziness upon standing, chest pain)		8 (30.8%)
						Skin (rash, increased perspiration, itching, dry skin)		5 (19.2%)
						Genital/Urinary (difficulty urinating, painful/frequent urination, menstrual irregularity)		4 (15.4%)

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
						Nervous system (headache, tremors, poor coordination, dizziness)	11 (42.3%)
						Eyes/ears (blurred vision, ringing in ears)	6 (23.1%)
						Sleep (difficulty sleeping, oversleeping)	16 (61.5%)
						Sexual function (loss of sexual desire, anorgasmia, erectile dysfunction)	7 (26.9%)
						Anxiety	5 (19.2%)
						Poor concentration	7 (26.9%)
						General malaise	2 (7.7%)
						Restlessness	8 (30.8%)
						Fatigue	3 (11.5%)
						Decreased energy	5 (19.2%)
Sardella et al. (2008)	*H. perforatum* extract, standardized to 0.31% hypericin, 3.0% hyperforin	3 x 300 mg, 2.79 mg hypericin	12 weeks	Patients diagnosis of burning mouth syndrome (BMS)	43 patients, 21 assigned to SJW (2 dropouts, 1 due to AE)	One subject in SJW group developed headache	
Philipp et al. (1999)	STEI 300, standardized to 0.2%–0.3% hypericin and pseudo-hypericin, 2%–3% hyperforin	3 x 350 mg, 2.1–3.15 mg hypericin	8 weeks	Patients 18-65 years with moderate depression	263 patients, 106 assigned to SJW	Incidence: 0.5 AEs/patient (22%) Nausea most frequently reported AE	

Study	Extract	Daily dose	Duration	Study population	No. of participants	Adverse events	
Gastpar et al. (2005)	STW3 (Laif 600)	612 mg	12 or 24 weeks	Patients 18-70 years with moderate depressive disorder	241 patients, 123 assigned to SJW (21 dropouts), 102 received for 12 weeks, 81 for 24 weeks (33 dropouts)	*Total*	*74/123 (60.2%)*
						Digestive tract	9 (7.3%)
						Central and peripheral nervous system	1 (0.8%)
						Skin and integumentary system	2 (1.6%)
						Diseases of liver and hepatic duct	1 (0.8%)
						Fatigue	1 (0.8%)
Weber et al. (2008)	*H. perforatum* extract, standardized to 0.3% hypericin	3 x 300 mg Hypericin 2.7 mg	8 weeks	Children 6–17 years with ADHD	54 children, 27 assigned to SJW (1 dropout)	Nausea/vomiting	26 (96%)
						Headache	15 (56%)
						Sunburn	4 (15%)

ADHD = attention-deficit/hyperactivity disorder; AEs = adverse effects; SJW = St John's wort

References

Abdali K, Khajehei M, Tabatabaee HR (2010) Effect of St John's wort on severity, frequency, and duration of hot flashes in premenopausal, perimenopausal and postmenopausal women: A randomized, double-blind, placebo-controlled study. Menopause: The Journal of the North American Menopause Society 17: 326-31.

Al-Alkoum M, Maunsell E, Verreault R, Provencher L, Otis H, Dodin S (2012) Effects of Hypericum perforatum (St. John's wort) on hot flashes and quality of life in perimenopausal women: a randomized pilot trial. Menopause: The Journal of the North American Menopause Society 16: 307-14.

Anghelescu IG, Kohnen R, Szegedi A, Klement S, Kieser M (2006) Comparison of Hypericum extract WS® 5570 and paroxetine in ongoing treatment after recovery from an episode of moderate to severe depression: results from a randomized multicenter study. Pharmacopsychiatry 39: 213-9.

Asgari SA, Falahatkar S, Sharifi SHH, Enshaei A, Jalili MF, Allahkhah A (2010) Safety and efficacy of the herbal drug hypericum perforatum for the treatment of premature ejaculation. UroToday International Journal 3: 7 pp.

Barnes J, Barber N, Wheatley D, Williamson EM (2006) A pilot randomised, open, uncontrolled, clinical study of two dosages of St John's Wort (Hypericum perforatum) herb extract (LI-160) as an aid to motivational/behavioural support in smoking cessation. Planta Medica 72: 378-82.

Bjerkenstedt L, Edman GV, Alken RG, Mannel M (2005) Hypericum extract LI 160 and gluoxetine in mild to moderate depression. A randomized, placebo-controlled multi-center study in outpatients. Eur. Arch. Psychiatry Clin. Neurosci. 255: 40-7.

Brattström A (2009) Long-term effects of St. John's wort (Hypericum perforatum) treatment: A 1-year safety study in mild to moderate depression. Phytomedicine 16: 277-83.

Canning S, Waterman M, Orsi N, Ayres J, Simpson N, Dye L (2010) The efficacy of hypericum perforatum (st john's wort) for the treatment of premenstrual syndrome: A randomized, double-blind, placebo-controlled trial. CNS Drugs 24: 207-25.

Couldwell WT, Surnock AA, Tobia AJ, Cabana BE, Stillerman CB, Forsyth PA, Appley AJ, Spence AM, Hinton DR, Chen TC (2011) A phase 1/2 study of orally administered synthetic hypericin for treatment of recurrent malignant gliomas. Cancer 117: 4905-15.

Davidson JRT, Gadde KM, Fairbank JA, Ranga Rama Krishnan K, Califf RM, Binanay C, et al (2002) Effect of Hypericum perforatum (St John's wort) in major depressive disorder: A randomized controlled trial. JAMA 287: 1807-14.

Ellis KA, Stough C, Vitetta L, Heinrich K, Nathan PJ (2001) An investigation into the acute nootropic effects of hypericum perforatum L. (St. John's Wort) in healthy human volunteers. Behavioural Pharmacology 12: 173-82.

Fava M, Alpert J, Nierenberg AA, Mischoulon D, Otto MW, Zajecka J, Murck H, Rosenbaum JF (2005) A double-blind, randomized trial of St John's wort, fluoxetine, and placebo in major depressive disorder. J. Clin. Psychopharmacol. 25: 441-7.

Franklin M, Chi J, McGavin C, Hockney R, Reed A, Campling G, Whale RWR, Cowen PJ (1999) Neuroendocrine evidence for dopaminergic actions of Hypericum extract (LI 160) in healthy volunteers. Biol. Psychiatry 46: 581-4.

Friede M, Hasenfuss I, Wustenberg P (1998) Verkehrs-und Arbeitssicherheit unter Johanniskraut. Fortschr. Med. 116: 44-5.

Friede M, Henneicke von Zepelin HH, Freudenstein J (2001) Differential therapy of mild to moderate depressive episodes (ICD-10 F 32.0; F 32.1) with St. John's wort. Pharmacopsychiatry 34(suppl 1): S38-S41.

Gastpar M, Singer A, Zeller K (2005) Efficacy and tolerability of Hypericum extract STW3 in long-term treatment with a once-daily dosage in comparison with sertraline. Pharmacopsychiatry 38: 78-86.

Gastpar M, Singer A, Zeller K (2006) Comparative efficacy and safety of a once-daily dosage of Hypericum extract STW3-VI and citalopram in patients with moderate depression: A double-blind, randomised, multicentre, placebo-controlled study. Pharmacopsychiatry 39: 66-75.

Gulick RM, McAuliffe V, Holden-Wiltse J, Crumpacker C, Liebes L, Stein DS, Meehan P, Hussey S, Forcht J, Valentine FT (1999) Phase I studies of hypericin, the active compound in St. John's wort, as an antiretroviral agent in HIV-infected adults: AIDS clinical trials group protocols 150 and 258. Annals of Internal Medicine 130: 510-4.

Hicks SM, Walker AF, Gallagher J, Middleton RW, Wright J (2004) The significance of "nonsignificance" in randomized controlled studies: A discussion inspired by a double-blinded study on St. John's Wort (Hypericum perforatum L.) for premenstrual symptoms. Journal of Alternative and Complementary Medicine 10: 925-32.

Hübner WD, Arnoldt KH (2000) St John’s wort: A one year treatment study. [Johanniskraut-Einjahresstudie. Offene multizentrische Therapiestudie mit Johanniskraut-Extrakt LI 160 bei Patienten mit depressiven Erkrankungen] (in German). Zeitschrift fur Phytotherapie 21: 306-10.

Hübner WD, Kirste T (2002) Symptoms of depression and psychovegetative disturbances in children under 12 years. [Depressive symptome und psychovegetative Störungen bei kindern unter 12 Jahren. Ergebnisse einer Beobachtungsstudie mit Johanniskraut (Hypericum perforatum)] (in German). Schweiz. Zschr. GanzheitsMedizin 14: 318-24.

Jacobson JM, Feinman L, Liebes L, Ostrow N, Koslowski V, Tobia A, Cabana BE, Lee DH, Spritzler J, Prince AM (2001) Pharmacokinetics, safety, and antiviral effects of hypericin, a derivative of St. John's wort plant, in patients with chronic hepatitis C virus infection. Antimicrobial Agents and Chemotherapy 45: 517-24.

Kalb R, Trautmann-Sponsel RD, Kieser M (2001) Efficacy and tolerability of Hypericum extract WD 5572 versus placebo in mildly to moderately depressed patients. Pharmacopsychiatry 34: 96-103.

Kasper S, Anghelescu IG, Szegedi A, Dienel A, Kieser M (2006) Superior efficacy of St John's wort extract WS®5570 compared to placebo in patients with major depression: A randomized, double-blind, placebo-controlled, multi-center trial [ISRCTN77277298]. BMC Medicine 4: 13 pp.

Kobak KA, Taylor LVH, Warner G, Futterer R (2005) St. John's wort versus placebo in social phobia: Results from a placebo-controlled pilot study. J. Clin. Psychopharmacol. 25: 51-8.

Laakmann G, Schüle C, Baghai T, Kieser M (1998) St. John’s wort in mild to moderate depression: The relevance of hyperforin for the clinical efficacy. Pharmacopsychiat 31(suppl): 54-9.

Lawvere S, Mahoney MC, Cummings KM, Kepner JL, Hyland A, Lawrence DD, Murphy JM (2006) A Phase II study of St. John's Wort for smoking cessation. Complementary Therapies in Medicine 14: 175-84.

Lecrubier Y, Clerc G, Didi R, Kieser M (2002) Efficacy of St. John's wort extract WS 5570 in major depression: A double-blind, placebo-controlled trial. American Journal of Psychiatry 159: 1361-6.

Lenoir S, Degenring FH, Saller R (1999) A double-blind randomized trial to investigate three different concentrations of a standardized fresh plant extract obtained from the shoot tips of Hypericum perforatum L. Phytomedicine 6: 141-6.

Mueller BM (1998) St. John's wort for depressive disorders: Results of an outpatient study with the hypericum preparation HYP 811. Advances in Therapy 15: 109-16.

Müller T, Mannel M, Murck H, Rahlfs VW (2004) Treatment of somatoform disorders with St. John's wort: A randomized, double-blind and placebo-controlled trial. Psychosomatic Medicine 66: 538-47.

Philipp M, Kohnen R, Hiller KO (1999) Hypericum extract versus imipramine or placebo in patients with moderate depression: randomized multicentre study of treatment for eight weeks. BMJ 319: 1534-9.

Randløv C, Mehlsen J, Thomsen CF, Hedman C, Von Fircks H, Winther K (2006) The efficacy of St. John's Wort in patients with minor depressive symptoms or dysthymia - A double-blind placebo-controlled study. Phytomedicine 13: 215-21.
Rapaport MH, Nierenberg AA, Howland R, Dording C, Schettler PJ, Mischoulon D (2011) The treatment of minor depression with St. John's Wort or citalopram: Failure to show benefit over placebo. Journal of Psychiatric Research 45: 931-41.

Rychlik R, Siedentop H, von den Driesch V, Kasper S (2001) Efficacy and tolerability of extract of St John's wort (Herba hypericin) at a dosage of 600 and 1,200 mg daily in patients with mild to moderate depression. [Johanniskrautextrakt WS 5572 bei leichten bis mittelschweren Depressionen. Wirksamkeit und Verträglichkeit von 600 und 1200 mg Wirkstoff täglich.] (in German). Fortschritte der Medizin 119: 119-28.

Sardella A, Lodi G, Demarosi F, Tarozzi M, Canegallo L, Carrassi A (2008) Hypericum perforatum extract in burning mouth syndrome: A randomized placebo-controlled study. J. Oral Pathol. Med. 37: 395-401.

Schrader E, Meier B, Brattström A (1998) Hypericum treatment of mild-moderate depression in a placebo-controlled study. A prospective, double-blind, randomized, placebo-controlled, multicenter study. Human Psychopharmacology 13: 163-9.

Schüle C, Baghai T, Sauer N, Laakmann G (2004) Endocrinological effects of high-dose Hypericum perforatum extract WS 5570 in healthy subjects. Neuropsychobiology 49: 58-63.

Shelton RC, Keller MB, Gelenberg A, Dunner DL, Hirschfeld R, Thase ME, Russell J, Lydiard RB, Crits-Christoph P, Gallop R, Todd L, Hellerstein D, Goodnick P, Keitner G, Stahl SM, Halbreich U (2001) Effectiveness of St John's wort in major depression: A randomized controlled trial. JAMA 285: 1978-86.

Szegedi A, Kohnen R, Dienel A, Kieser M (2005) Acute treatment of moderate to severe depression with hypericum extract WS 5570 (St John's wort): Randomised controlled double blind non-inferiority trial versus paroxetine. BMJ 330: 503-6.

Taylor LH, Kobak KA (2000) An open-label trial of St. John's wort (Hypericum perforatum) in obsessive-compulsive disorder. J. Clin. Psychiatry 61: 575-8.

Van Gurp G, Meterissian GB, Haiek LN, McCusker J, Bellavance F (2002) St. John's wort or sertraline? Randomized controlled trial in primary care. Can. Fam. Physician 48: 905-12.

Vorbach EU, Arnoldt KH, Hubner WD (1997) Efficacy and tolerability of St. John's wort extract LI 160 versus imipramine in patients with severe depressive episodes according to ICD-10. Pharmacopsychiatry 30(suppl): 81-5.

Weber W, Vander Stoep A, McCarty RL, Weiss NS, Biederman J, McClellan J (2008) Hypericum perforatum (St John's Wort) for attention-deficit/hyperactivity disorder in children and adolescents: A randomized controlled trial. JAMA 299: 2633-41.

Wheatley D (1997) LI 160, an extract of St. John's wort, versus amitriptyline in mildly to moderately depressed outpatients - A controlled 6-week clinical trial. Pharmacopsychiatry 30(suppl): 77-80.

Woelk H, Burkard G, Grünwald J (1994) Benefits and risks of the hypericum extract LI 160: Drug monitoring study with 3250 patients. J. Geriatr. Psychiatry Neurol. 7(suppl. 1): S34-8.

Woelk H (2000) Comparison of St John's wort and imipramine for treating depression: Randomised controlled trial. BMJ 321: 536-9.

Yechiam E, Ben-Eliezer D, Ashby NJS, Bar-Shaked M (2019) The acute effect of Hypericum perforatum on short-term memory in healthy adults. Psychopharmacology 236: 613-23.

RIVM
Committed to *health and sustainability* -