EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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EMA/HMPC/369801/2009
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Chelidonium majus* L., herba

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Draft

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Chelidonium majus* L. Dried whole or cut aerial parts of the plant
Herbal preparation(s)	**Internal Use** a) Chelidonii herba: comminuted b) Chelidonii tincture: 1:10 ethanol 45% (V/V) c) Chelidonii extractum fluidum: 1:1 ethanol 25% (V/V) d) Chelidonii extractum siccum (concentration not specified) e) *Chelidonium majus* mother tincture (M.T. (ø)) **External Use** a) Eye-drops: (preparation not specified) b) Ointment: (concentration not specified)
Pharmaceutical forms	Herbal preparation in solid or liquid dosage form or as a herbal tea for oral use. Herbal preparation in solid or liquid dosage form for external use.
Rapporteur	
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Note: This Assessment Report is published to support the release for public consultation of the draft Community herbal monograph on *Chelidonium majus* L. . It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Chelidonium majus L.

The European Pharmacopoeia (01/2008:1861) monograph Chelidonii herba provides the following definition: dried, whole or cut aerial parts of *Chelidonium majus* L. collected during flowering. Content: minimum 0.6 per cent of total alkaloids, expressed as chelidonine ($C_{20}H_{19}NO_5$; Mr 353.4) (dried drug).

Chelidonium majus L. is known under the synonyms:

English: greater celandine, devil's milk, rock poppy, tetterwort, swallow-wort

Italian: celidonia, erba di porri, cinerognola, erba maistra, erba nocca

German: Schöllkraut, Schellkraut, Schwalbenkraut, Goldwurz, Blutkraut, Gelbes Millkraut

French: chélidoine, herbe d'éclaire, herbe de l'hirondelle, felougue, herbes de Sainte Claire, herbe hirondalle

Dutch: stinkende gouwe

Spanish: celidonia

Portugal: celidonia

Polish: ziele glistnika, złotnik, jaskółcze ziele, glistnik pospolity

There are over 20 different *Chelidonium* alkaloids identified. Here are the different groups of chemical molecules who are present in the herb of *Chelidonium majus* (*Barnes et al, 2007; Bruneton, 1999*)

- **Benzylisoquinoline type (0.01-1%):** with at least three subgroups,
  - Benzophenanthridines: chelerythrine, chelidonine, sanguinarine, isochelidonine
  - Protoberberines: berberine, coptisine, dihydrocoptisine, stylopine
  - Protopine
- **Acids:** chelidonic, malic, citric, caffeic (0.4%) ferulic (0.02%), p-coumaric (0.06%), gentisic and p-hydroxybenzoic acids
- **Hydroxycinnamic acid derivates:** (-)-2-(E)-caffeoyl-D-glyceric acid, (-)-4-(E)-caffeoyl-L-threonic acid, (-)-(2)-(E)-coffeoyl threonic acid lactone, (+)-(E)-caffeoyl-L-malic acid
- **Others:** a saponine, carotenoids, a phytocytostatin (chelidocystatin), sparteine and flavonoids.

Chelidonium majus L. belongs to the family of the *Papaveracecae* and is widely distributed across the world. It can be found in Europe, Asia, Northwest Africa and North America. It is a typical ruderal plant that grows on nitrogenous ground.

Historically *Chelidonium majus* was already used in the middle ages. It was mentioned by Plinius and Dioscorides. The name Chelidon (χελιδον) in Greek means swallow because the plant begins to flower when the swallows return.

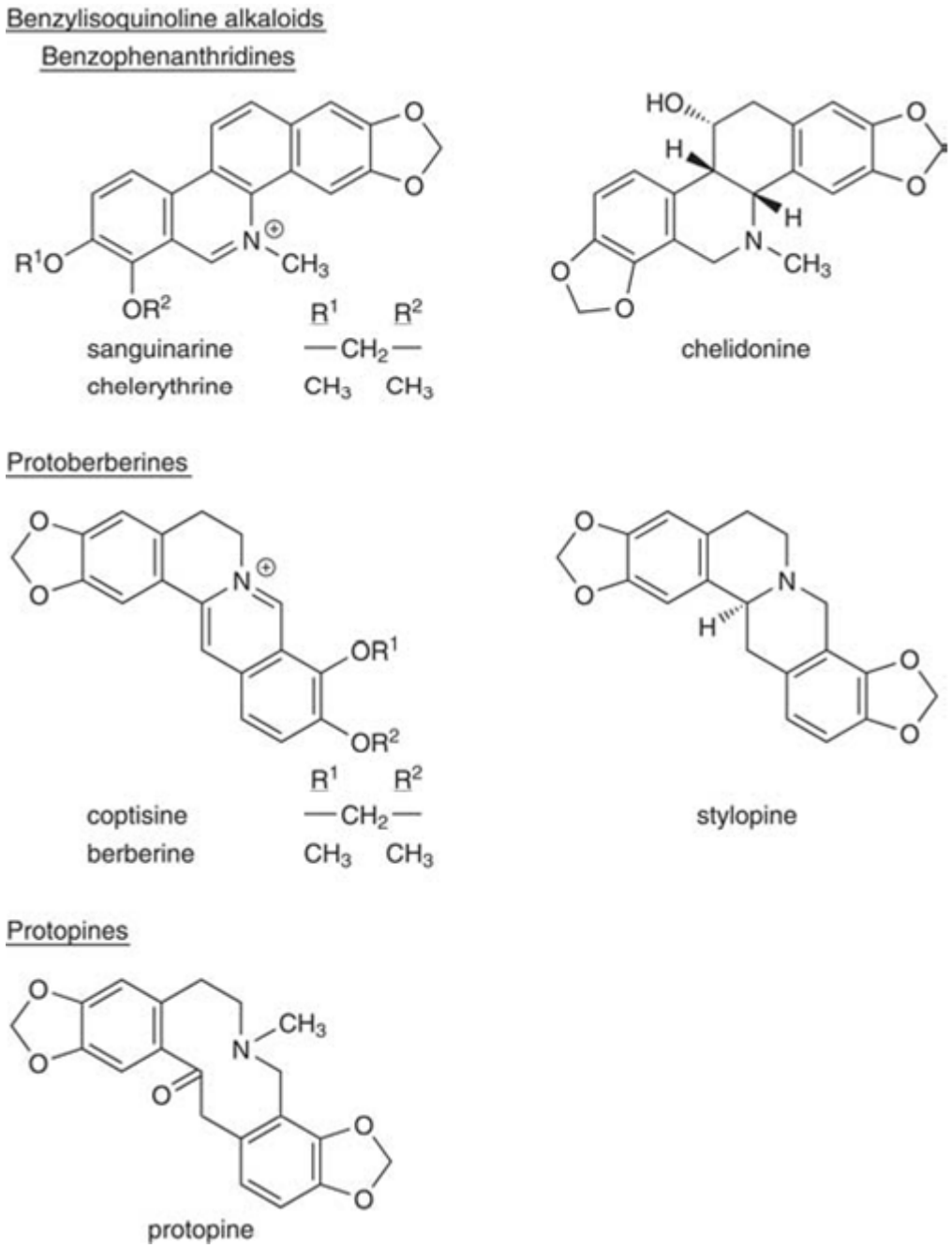

Figure 1. Alkaloids of *Chelidonium majus* (*Barnes et al, 2007*)

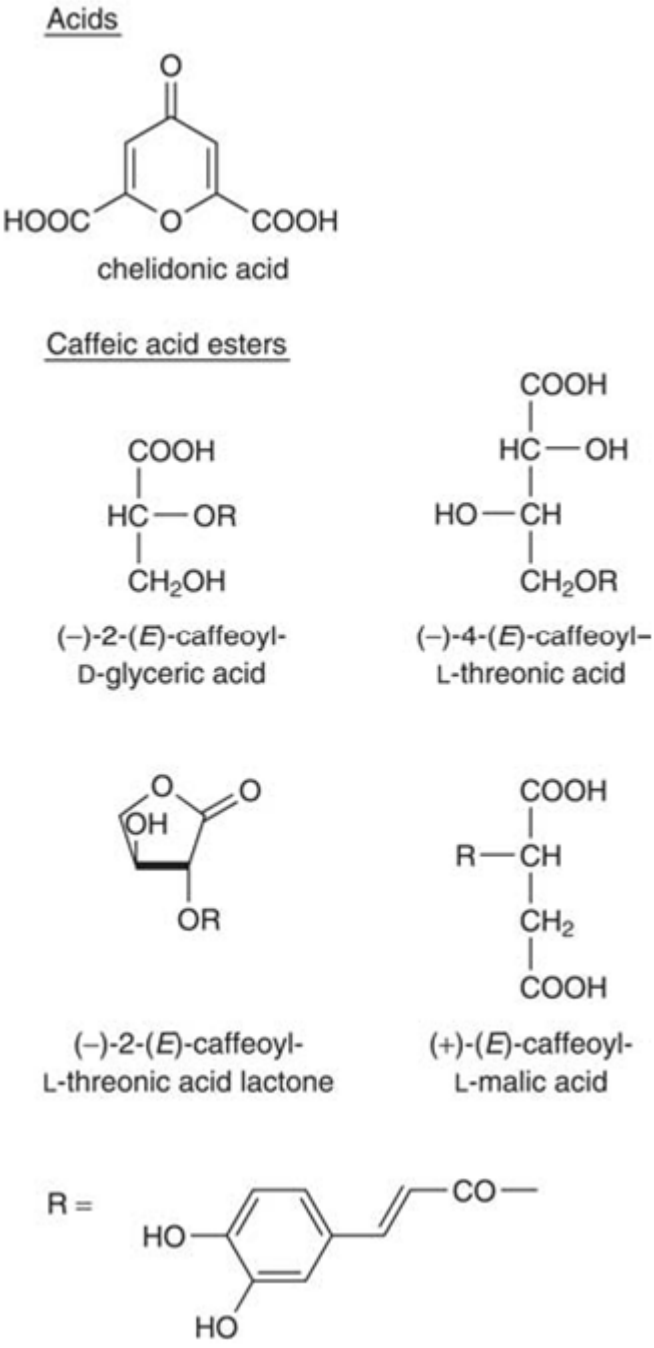

Figure 2. Acids of *Chelidonium majus* (*Barnes et al, 2007*)

- Herbal preparation(s)

Internal use

- Chelidonii herba comminuted: 1.2-3.6 g as a tea infusion (ESCOP, 2003)
- Chelidonii tincture (ethanol 45% V/V): 2-4 ml of a 1:5 preparation daily or 2-4 ml of a 1:10 preparation three times a daily (*Barnes et al, 2007*)
- Chelidonii extractum fluidum (ethanol 25% V/V): 1-2 ml of a 1:2 preparation daily or 1-2 ml of a 1:1 preparation three times daily (*Barnes et al, 2007*)
- Chelidonii extractum siccum (concentration not specified): 100-200 mg three times daily (*Van Hellemont, 1985; Delfosse 1998*)
- *Chelidonium majus* mother tincture (M.T. (ø)): 15 drops three times daily (*van Hellemont, 1985; Delfosse 1998*)

External use

- Eye-drops: one drop three times daily (Kommission C, 1992)

  Chelidonium, volatile grade of dilution D4 to D30

  Chelidonium Rh, volatile grade of dilution D4 to D30

  Chelidonium Flos (ethanol), volatile grade of dilution D4 to D30

  Chelidonium Flos Rh, volatile grade of dilution D4 to D30
- Ointment,: apply 1-2 times daily on rash (Kommission C, 1992)

  Chelidonium majus M.T. (ø)

  Chelidonium e radice ferm 34b, volatile grade of dilution D3 to D30

  Chelidonium ex herba cum radice ferm, volatile grade of dilution D3 to D30

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

A few herbal preparations in combination with *Chelidonium majus* are described in the *Deutschen* **Receptformeln:** Tinctura Cholagoga (DRF) (a combination of *Chelidonium majus* tincture with *Silybum marianum* tincture and *Strychnos nux-vomica* tincture) and Tinctura Cholagoga Fortis (DRF) (a combination of *Chelidonium majus* tincture, *Silybum marianum* tincture, *Atropa belladonna* tincture and Mint oil). (*van Hellemont, 1985; Weiss & Fintelman, 1999*)

Iberogast®, also known as STW 5, is a prokinetic liquid formulation of nine herbs indicated for Irritable bowel syndrome and dyspepsia. It contains a fixed combination of hydroethanolic herbal extracts from bitter candy tuft (*Iberis amara totalis*), peppermint leaves (*Menthae piperitae folium*), chamomile flower (*Matricariae flos*), liquorice root (*Liquiritiae radix*), angelica root (*Angelicae radix*), caraway fruit (*Carvi fructus*), milk thistle fruit (*Silybi mariani fructus*), lemon balm leaves (*Melissae folium*) and greater celandine herbs (Chelidonii herba). Each of which is reported to have multiple pharmacological properties relevant in gastrointestinal pathophysiology. (*Wegener & Wagner 2006*)

Iberogast® The composition of 100 ml liquid is the following:

Extracts from:

Iberis amara (fresh whole plant)	(1:1.5-2.5)	15.0 ml
Extraction solvent: ethanol 50% (V/V)		
Angelicae radix	(1:2.5-3.5)	10.0 ml
Matricariae flos	(1:2.0-4.0)	20.0 ml
Carvi fructus	(1:2.5-3.5)	10.0 ml
Cardui mariae fructus	(1:2.5-3.5)	10.0 ml
Melissae folium	(1:2.5-3.5)	10.0 ml
Menthae piperitae folium	(1:2.5-3.5)	5.0 ml
Chelidonii herba	(1:2.5-3.5)	10.0 ml
Liquiritiae radix	(1:2.5-3.5)	10.0 ml
Extraction solvent for all: ethanol 30% (V/V)		

- Vitamin(s)

Not applicable

- Mineral(s)

Not applicable

Information on period of medicinal use in the Community regarding the specified indication

See specific country-linked information. *Chelidonium majus* has been used since the middle ages. It was used for biliary and liver disorders. The fresh latex was used in the treatment of warts but also for other skin complaints such as corns, tinea infections, eczema and tumours of the skin. Therefore the fresh latex was dabbed on the warts. (*Barnes et al, 2007; Hager et al, 1999*)

In other literature it was mentioned that in 1896 a Russian doctor injected 1.50-5 g of an extract (concentration not specified) in combination with equal parts glycerine and distilled water in a tumour of the skin. The tumour was decreased partialy or disappeared completely. Further research showed that this painful method did not have an effect on the tumour but did only decreased secretion and hemorrhages. (*Leclerc, 1954*)

1.2. Information about products on the market in the Member States

Austria

See composition of Iberogast® under 1.1.

Belgium

Preparation

Aporil® contains tincture of *Chelidonium majus* 45 mg, tincture of Thuja species 45 mg, acetic acid 80 mg, lactic acid 20 mg, salicylic acid 135 mg in 9 ml solvent.

Indication

Topical treatment of warts.

Regulatory

According to the Royal Decree of 1997 Chelidonium is included in list 3: it can be used in medicines as well as in food supplements. There is no restriction with regard to maximal doses specified.

Bulgaria

Combination product with Chelidonii herba extractum fluidum (1:2,5-3,5): 10 ml/100 ml

Czech Republic

See composition of Iberogast® under 1.1.

Estonia

See composition of Iberogast® under 1.1.

Germany

See composition of Iberogast® under 1.1.

Slovenia

See composition of Iberogast® under 1.1.

Spain

Preparations

Combination of an extract of Chelidonium with a tincture of Thuja (1:10), salicylic acid, iodine and acetic acid.

Indication

Topical treatment of warts.

Regulatory

There are other combined preparations with 7 to 9 herbal substances for use as a herbal tea. The herbal substance is included in a list of plants restricted to be used as medicinal products and should not be used in food supplements.

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Combination prep Iberogast®
Belgium	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Combination prep. Aporil® External use
Bulgaria	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Combination product on the market
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Combination prep Iberogast®
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	No authorised or registered preparations Chelidonium not allowed in food supplements
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Combination prep. Iberogast®
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Combination prep. Iberogast®
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	No authorised or registered preparations
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Voluntary withdrawal of products for oral use
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not allowed in food supplements
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comminuted Chelidonium for tea infusion
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised or registered preparations
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised or registered preparations
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herb can be sold on prescription

Member State	Regulatory Status				Comments (not mandatory field)
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised or registered preparations
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Combination prep Iberogast®
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	No authorised or registered preparations for internal use Topical preparation only
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised or registered preparations
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorised or registered preparations

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

2. Non-Clinical Data

2.1. Pharmacology

2.1.1. Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

***In vitro* studies**

Antiviral effect

Amoros *et al*, 1977: Extracts of the whole plant of *Chelidonium majus* were tested for their antiviral properties. Alcoholic (96° ethanolic extract, 1 ml of the aqueous solution corresponds to 1 g of the fresh plant.) and alkaline (50 g of the dried and pulverised plant was eluted with ammonia solutions and extracted with chloroform) extracts were tested on the herpes simplex and the polio virus. The alkaline extract showed activity against herpes simplex virus but not against the polio virus, whereas the alcoholic extract didn't show any inhibitory effect. The authors note that this is only a preliminary search to antiviral properties of certain plants.

Sethi, 1981-1983-1985: Protoberberine and benzophenanthridine alkaloids were tested for inhibition of reverse transcriptase (RT) activity of RNA tumour viruses. Inhibition of reverse transcriptase activity was correlated with the structure and antileukemic activity of the protoberberine alkaloids,

	inhib of RT-activity (50% inhibition)		Antileukemic activity	
	µg/ml	µM	%	Dose (mg/kg)
coralyne acetosulfate	30	60	176	100
8-Et coralyne ethosulfate	35	72	219	100
8-Pr coralyne proposulfate	95	170	100	100
2,3-Me dioxy-10,11dimethoxy dibenzoquinolizinium acetosulphat	50	103	122	50
2,3-dimethoxy-10,11methylene dioxydibenzo quinolizinium acetosulphat	45	92	121	100
10-demethoxycoralyne acetosulphat	45	92	145	100
13-methylpalmatine iodide	80	158	116	100
palmatine chloride	60	124	107	12,5
norcoralyne chloride	30	70	/	/
stracoralyne acetosulfate	45	111	154	80
isocoralyne acetosulfate	60	115	180	80
fagaronine chloride	70	139	177	40
isomers of fagorine	6	16	265	100
isomers of fagorine	80	209	107	100
isomers of fagorine	12	32	147	100

Table 1: Comparison of inhibition of reverse transcriptase and antileukemic activities of protoberberine and benzophenanthridine alkaloids. (*Sethi, 1985*)

The phenolic and methoxy groups on the structure of the benzophenanthridine are important for anti-RT activity, so individual members of alkaloids show very different activity. The presence of the quaternary nitrogen in the molecule is necessary for activity. Compounds like chelidonine (IC_{50}=200 µg/ml) who do not have a quaternary nitrogen are very weakly active against RNA tumour viruses.

Kéry et al, 1987: The squeezed juice and a sodium chloride extract of the aerial parts of the plant was subjected to bioassays to test activity against adenoviruses and herpes simplex virus type I (HSV-I). Subfractions were taken from the aerial parts and the root and tested also for activity. After incubating the fractions with cells infected with adenovirus type 12, the subfraction with concentration of 35 µg/ml showed antiviral activity. This fraction showed also virucidal activity against HSV-1, achieving 100% loss of virus infectivity after 90 min of incubation (after 30 min, 75% and after 60 min, only 25% of the cells were infected with the virus). Although adenoviruses 5 and 12 were less

sensitive, they retained 50% infectivity after 120 min incubation (75% infections after 60 and 90 min for adenovirus 5 and 12.). This alkaloid fraction would belong to the benzophenanthridine fraction of *Chelidonium majus*.

Tan et al, 1991: Chelidonine on itself is only a weak inhibitor against human immunodeficiency virus type-I reverse transcriptase (IC_{50} around 200 µg/ml). Berberine chloride showed moderate inhibitory activity against the same enzyme (IC_{50} around 100 µg/ml).

Rogelj et al, 1998: Extract, with tris-HCl buffer pH 7, was taken from the green parts of a mature *Chelidonium majus* plant. The extract showed inhibitory activity, no concentrations are shown, against cysteine proteinases. Chelidocystatin was isolated from this extract and was the inhibitor of the cysteine proteinases cathepsin L ($Ki = 5.6 \times 10^{-11}$ M), papain ($Ki = 1.1 \times 10^{-10}$ M) and cathepsin ($Ki = 7.5 \times 10^{-9}$ M). The values are typical for phytocystatins since similar values were obtained for inhibition of the papain, cathepsin L and cathepsin H with phytocystatin from *Phaseolus vulgaris* seed.

Antimicrobial activity

Mitscher et al, 1978: Antimicrobial activity was found for pseudoalcoholates of sanguinarine and chelerythrine, who have greater intercellular penetration than the polar benzophenanthridine itself and are active (MIC= 6.25 µg/ml) against *Staphylococcus Aureus, Escherichia Coli, Salmonella gallinarum, Klebsiella pneumoniae, Mycobacterium smegmatis and Candida albicans.*

Lenfeld J et al, 1981: Fractions of *Chelidonium majus* containing chelerythrine and quaternary benzophenanthridine showed activity against some *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Candida albicans*. There was a significant antimicrobial effect upon gram-positive bacteria and upon *Candida* Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the quaternary benzophenanthridine fraction (QBF) and of chelerythrine are shown in the following table.

Organism	QBF		Chelerythrine	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
S. aureus	5	40	10	40
S. beta A	10	10	10	10
S. alfa	20	40	20	40
C. albicans	5	>160	5	>160

Table2: MIC and MBC of QBF and chelerythrine (*Lenfeld et al, 1981*)

Mahajan et al, 1982: *Alternaria*, *Aspergillus flavus*, *A. fumigatus*, *Candida albicans, Curvularia, Drechslera, Fusarium, Mucor, Penicillium, Rhizopus orizae and Scopulariopsis* growth was inhibited by Berberine in concentrations of 10-25 µg/ml

Fik et al, 1997: Drug resistant *staphylococci* and *enterococci* were found sensitive for a glycoprotein isolated from the juice of *Chelidonium majus* leaves and roots *in vitro*. The minimal bactericidal concentration, MBC, values of various methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), mupirocin-resistant MRSA, aminoglycoside-resistant *E. faecium* and aminoglycoside-resistant *E. faecium* were established, and were found 31-125, 31-250, 31-125, 125-500, 250-500 mg/l, respectively.

Matos et al, 1999: Antifungal activity *in vitro* was documented for *Chelidonium majus* extracts against *Fusarium* strains. A methanolic extract of the whole plant showed the highest antifungal activity. After five days of inoculation, the growth of the strain was reduced to less than 40% as compared to the control, but this was seen only in one strain with an alcoholic extract and no reduction was seen with a water extract. *Fusarium oxyxporum cubense* strain was most sensitive to the methanolic extract, *F. solani* was the most sensitive strain to all three extracts. Methanolic root extract achieved a better inhibition of growth in comparison with extracts from the aerial parts: growth of *F. oxysporum cubense*, *F. oxysporum melonis* and *F. solani* was reduced to less than 30% of that seen with control, although *F. culmorum* seemed rather insensitive. In further experiments, chelerythrine and sanguinarine were found to be active against *F. solani* and *F. culmorum*. *F. solani* is sensitive to berberine, but there was no activity found for chelidonine.

Ma et al, 2000: Chelidonine, dihydrochelerythrine and dihydrosanguinarine, isolated out of an extract of *Chelidonium majus* roots, have activity against *Cladosporium herbarum*. The lowest concentrations which showed activity against *Cladosporium herbarum*were 10. 6 and 4 µg/ml respectively (determined by TLC).

Zuo GY et al, 2008: extracts and compounds isolated from the aerial part of *Chelidonium majus* were tested for their antibacterial activity against strains of *methicillin-resistant Staphylococcus aureus* (MRSA). Bioassays led to the isolation of four benzophenanthridine-alkaloids (8-hydroxydihydro-sanguinarine (hhS), 8-hydroxydihydro-chelerythridine (hhC), dihydro-sanguinarine (hS) and dihydro-chelerythrine (hC)) who showed activity against MRSA strains, with $MIC_{50/90}$ values of 0.49/1.95; 0.98/7.81 µg/ml for hhS and hhC respectively. hS and hC showed moderate to no inhibitory effects at concentrations up to 3 mg/ml.

Anti-tumour effect

Krey and Hahn, 1969: Berberine has been shown to interact with nucleic acids by optical methods. They suggested that berberine binds to DNA, because calf-thymus DNA produced changes in absorbtion spectrum of berberine. This leads us to the fact that the planes of berberine lie parallel to those of purine and pyrimidine pairs in DNA (double, single DNA and ribosomal RNA).

Hladon et al, 1978: Cytotoxic activity of chelidonine, sanguinarine and berberine was tested in HeLa cell cultures, ED_{50} values were respectively 0.27; 0.54 and 3.5 to 30.0 µg/ml. ED_{50} values in normal rabbit kidney cell cultures were 1.35 µg/ml for chelidonine and 0.66 µg/ml for sanguinarine. Only weak activity for Erlich ascites carcinoma cell cultures was observed for chelidonine and sanguinarine.

Caolo and Stermitz, 1978: Anti-tumour activity of some quaternary benzophenantridine alkaloids was tested to determine the structure-activity relationship. It was found likely that the iminium site was involved in the biological activity of these compounds, a high iminium ion concentration (90-98%) in 50% ethanol is correlated with a good anti-tumour activity. Sanguinarine and Chelerythrine (resp. 3% and 10% iminium ion at pH7 in 50% EtOH) are inactive against P388 and L1210 mouse leukaemia.

Maiti et al, 1982: Anti-leukaemic activity of cytotoxic protoberberine alkaloids could be related to the structural conformation of the molecule and its DNA-binding properties. Free calf thymus DNA showed an absorption maximum of 325 nm, whereas bound DNA-sanguinarine showed an absorption of 342 nm. These results were evaluated against a control with ethidium (free showed an absorption max of 480 nm, whereas bound ethidium-DNA showed an absorption maximum of 520 nm. The melting points were also documented, they were 68°C and 91°C respectively. The biologic effects of sanguinarine can be attributed to the formation of a complex of sanguinarine with DNA.

Smekal et al, 1984; Smekal & Kubova, 1984; Faddejeva et al, 1984: DNA is intercalated by sanguinarine, the neutral form intercalates partially while cationic form intercalates totally into the DNA

double helix. There is a strong interaction with DNA, this indicates (like ethidium) that sanguinarine is involved into the DNA base pairing. Ethidium and sanguinarine are affecting the circular dichroism spectrum of DNA in the same manner.

Smekal et al, 1985: Chelerythrine binding differs from sanguinarine which suggests steric-hindrance effects of the substituents on ring D.

Ishii et al, 1985: Anti-tumour activity of chemically synthesized and natural non phenolic benzophenanthridine occurring alkaloids was evaluated for sarcoma 180 cell lines. Results showed the iminium site in the molecule was determinant for activity against these cells. Chelerythrine was found less active than sanguinarine, when tested *in vitro* against Kb cells and *in vivo* against L1210 and P288 leukaemia's. Chelerythrine, used in doses of 3 and 1 mg/kg/5days, gave resp. 26% and 81% tumor growth relative to the control. Sanguinarine, used in doses of 10 and 3 mg/kg/5days gave 1 and 87% tumor growth respectively (also when compared with the control).

Vavreckovà et al, 1996a and 1996b: *Chelidonium majus* antiproliferative effects were subjected into *in vitro* studies with rapidly multiplying human keratinocyte cell lines (HaCaT cells). A dry extract (0.68% alkaloids calculated as chelidonine) inhibited HaCaT cell growth with an IC_{50} value of 1.9 µmol/l. Sanguinarine, chelerythrine and chelidonine respectively gave IC_{50} values of 0.2; 3.2 and 3.3 µmol/l. The potency of sanguinarine was similar to that of the antipsoriatic agent anthralin (IC_{50} = 0.7 µmol/l), whereas berberine showed a low potency (IC_{50} = 30 µmol/l). Further investigation, by following lactate dehydrogenase release in the culture, showed more evidence for cytostatic activity than cytotoxic activity.

Song et al, 2002: A polysaccharide fraction from a water extract inhibited the proliferation of several tumour cell lines *in vitro*. A 100 µg/ml fraction showed over 50% cytotoxicity for the P815 and B16F10 cell lines.

Antispasmodic activity

Kardos et al, 1986: Protopine, cryptopine and allocryptopine enhance [^3H]-GABA binding to rat brain synaptic membrane receptors, this in comparison with diazepam binding. The following table gives the concentrations and enhancement (%) of the GABA binding. The effect of diazepam is more significant than the effect with the protopine alkaloids, indicating that the enhancement of GABA binding and binding to benzodiazepine receptor may be independent phenomena.

Conc (µmol/l)	% of control			
	Diazepam	Protopine	Cryptopine	Allocryptopine
0.01	1%	/	/	/
0.1	5%	0%	0%	0%
1	14%	7%	5%	10%
10	39%	31%	24%	30%
100	46%	/	/	/

Table3: Effect of diazepam and protopine alkaloids on ^{3}H-GABA binding (*Kardos et al, 1986*).

Boegge et al, 1996: An aqueous-methanolic extract of the flowering aerial parts of *Chelidonium majus* and the isolated constituents coptisine and (+)-caffeoylmalic acid, have been evaluated for its antispasmodic activity. *In vitro* tests involving isolated rat ileum was used. The extract contained 0.81% alkaloids, 2.00% flavonoids, 1.20% hydroxycinnamic acid derivates and 0.06% (+)-

caffeoylmalic acid. The extract showed a antispasmodic activity of 12.7% (SEM=4.0) in comparison to that of control group (Ach). Coptisine (at concentration of $1.0x10^{-5}$ g/ml organ bath) and (+)-caffeoylmalic acid (at concentration of $2.5x10^{-5}$ g/ml) were found to be the two constituents who contribute to the total antispasmodic activity of the extract, They exert a mean antispasmodic activity of 16.5% (SEM=3.0) and 6.9% (SEM=2.6) at these concentrations respectively. A lower concentration of coptisine ($0.5x10^{-5}$ g/ml organ bath) did not exhibit any statistically antispasmodic activity.

Häberlein et al, 1996: *In vitro* tests demonstrated effects of an extract of *Chelidonium majus* herb and certain constituent alkaloids at $GABA_A$ receptors. Radio receptor assays were evaluated with high concentrations of a dry ethanolic extract of the herb, who inhibited 50% of specific [^3H]-muscimol binding. At lower concentrations, specific binding of 115% indicated induction of positive co-operation. The alkaloid content (mg/100 mg dry extract) of the extract was determined allocryptopine 0.076, chelerythrine 0.009; protopine 0.465; sanguinarine 0.003 and stylopine 0.154. Further studies showed allocryptine, stylopine and protopine are responsible for the positive cooperative effect, mainly contributed by protopine. Concentration of chelerythrine and sanguinarine were considered to be too low to contribute.

Hiller et al, 1998: Two hydro alcoholic (ethanol 70% w/w) extracts of *Chelidonium majus* (at concentration of $5x10^{-4}$g/ml organ bath) relaxed, isolated guinea-pig ileums who were contracted using barium-chloride. The alkaloid content of both extracts was determined (by HPLC), containing chelidonine (0.38%), protopine (0.41%) and coptisine (0.32%) and extract two contained 0.59%; 0.48%; 0.26% respectively. Individual constituents were tested also and the mean percent of relaxation was 68.8% and 54.8% for chelidonine and protopine both at concentrations of $1x10^{-5}$g/ml organ bath. Coptisine up to a concentration of $3x10^{-5}$g/ml organ bath, showed no significant relaxation. Further experiments using these two active substances together and individual produced a concentration-dependent reduction of carbachol and electric-field-induced contractions. These results indicate that the antispasmodic effects of the herb comprise both musculotropic and neurotropic mechanisms.

Choleretic activity

Vahlensieck et al, 1995: Total ethanolic (70% EtOH) extract (total alkaloid content 1.6%; caffeic acid esters 1.9%), the phenolic and alkaloid fraction of *Chelidonium majus* herb were evaluated for their choleretic activity using isolated perfused rat livers. Choleresis was induced by the total extract (concentration of 10 mg/ml/minute), there was a significantly increased bile flow and after 40 min, the amount of bile was doubled as compared to the pre-treatment value. The two other fractions showed a slight increase of the bile flow, but it was not-significant. Even when used together they gave an increase of 20% over pre-treatment value, which was not significant.

Anti-inflammatory effect

Chung et al, 2004: effects related to anti-inflammatory activity have been evaluated for *Chelidonium majus* extract, specific plant parts not showed, the yield of the decocttion was approximately 9%, and for some alkaloids. Mouse peritoneal macrophages were used *in vitro*, nitric oxide production levels showed a significant increase when these cells were incubated with water extracts of the herb at different concentrations (0.01; 0.1; 1 mg/ml) together with recombinant murine interferon-gamma. This was compared to treatment with recombinant murine interferon-gamma only. The increased NO level indicates there is a cooperative induction of NO production. The increased NO levels were progressively inhibited by incubation with increasing levels of N-monomethyl-L-arginine and by addition of the antioxidant compound pyrrolidine dithiocarbamate (PDTC) (concentration: 100 µM). Furthermore, incubation of mouse peritoneal macrophages induced by *Chelidonium majus* extract plus

rIFN-gamma increased tumour-necrosis-factor-alpha (TNF-a) production in a concentration dependent manner. When nuclear factor kappa B inhibitor PDTC was added, there was a significant decrease in production of TNF-a, so *Chelidonium majus* extract increases TNF-a production via NF-kB activation.

Vavreckovà et al, 1996: The alkaloids sanguinarine and chelerythrine and a total extract of the herb showed inhibition of 5-lipoxygenase (5-LO) in isolated bovine polymorphonuclear leukocytes (PMNL) with IC_{50} values of 0.4; 0.8 and 1.9 µmol/l respectively. Sanguinarine and chelerythrine also inhibit 12-LO (from mouse epidermis; IC_{50} values 13 and 33 µmol/l respectively) whereas *Chelidonium majus* extract was inactive at a concentration of 170 µg extract/ml test solution. Chelidonine was tested also, but didn't show any activity against LO (IC_{50} = >100 µmol/l).

Vasopressin effect

Granger et al, 1992: Chelerythrine and sanguinarine exhibited affinity for rat liver vasopressin V_1-receptors. [^3H]-vasopressin were inhibited by those two alkaloids within the micromolar range (K_i, inhibition constant, $4x10^{-6}$M and $7x10^{-6}$M respectively). The interaction on the V_1-receptor is based on the position of the alkoxy group and the quaternary ammonium function.

Immunomodulatory activity

Song et al, 2002: Polysaccharide fractions of a water extract of *Chelidonium majus* were incubated for five days with spleen cells. Results showed an increase in the lytic activity of spleen lymphocytes to Yac-1 tumour cells from 0.9% to 30.0% and 34.2% for the fractions CM-Al and CM-Ala respectively. The optimal concentration for generation of activated T_c-cells was 5 µg/ml for both fractions. A second test, now with mouse peritoneal macrophages cultured with CM-Ala (10-100 µg/ml) gave also an increase in cytotoxicity, compared with the control test. The results were determined by the uptake of radio-labelled thymidine by the tumour cells.

Analgesic activity

Kim et al, 2001: analgesic activity was tested by testing receptor activity for GABA receptors. An aqueous extract of *Chelidonium majus* (no details specified) was used in patch-clamp experiments using fresh periaqueductal grey (PAG) neurons isolated from rats. Every 2 minutes the extract was applied at concentrations of over 0.3-10 mg/ml and elicited chloride ion current in a concentration-dependent manner. Bicuculline, a $GABA_A$ antagonist, reversibly inhibited this effect. Further tests showed the same results with lower concentrations of *Chelidonii herba* (0.03 and 0.1 mg/ml).

Shin, 2002: These tests have demonstrated that low concentrations (0.03 and 0.1 mg/ml) of a aqueous extract of *Chelidonii herba* (no details specified) suppress glycine-activated and increase glutamate-activated ion current in PAG (periaqueductal gray) rat neurons.

In vivo studies

Antiviral activity

Lozyuk, 1977: In influenza-virus-induced pneumonia mice, the total alkaloid extraction was injected. Total alkaloids given, were therapeutically effective when the quantity of virus injected in the mice was low.

Antimicrobial activity

Zhu and Ahrens, 1982 and Pitea and Marginanu, 1972: Berberine has been successfully used to control the intestinal secretions enhanced by *E. coli* enterotoxin. Berberine has positive influence because of the quaternary ammonium group.

Anti-inflammatory activity

Lenfeld et al, 1981: Anti-inflammatory activity was screened for sanguinarine, chelerythrine and a quaternary benzophenanthridine fraction in assays involving carrageenan-induced rat paw oedema. Sanguinarine, 5 mg/kg body weight, subcutaneous showed the highest anti-flogistic activity, chelerythrine (10 mg/kg body weight) was found less active, in compare to the control, what may be explained by the different oxygen electro-donating group.

Anti-tumour effect

Sokoloff, 1968: Chelidonine and protopine were tested for anti-tumour therapy in the treatment of sarcoma 180 and Erlich carcinoma. The total dose of 50 µg/kg body weight of the mouse chelidonine administered over 7 days showed an insignificant tumour inhibition (resp. 25% and 22% with mild cytotoxicity). Total dose of 350 µg/kg of protopine administered intraperitoneally in 7 days exerted only a mild tumour inhibition (resp. 15% and 26%).

Kim et al, 1969: An ethanol-water (1:1) dry extract of *Chelidonium majus* roots and rhizomes was inactive in animal models of leukaemia (L-1210 mice) and carcinosarcoma (Walker 256 rats). In contrary, the extract had cytotoxic activity in an assay utilising Eagle's 9KB carcinoma of the nasopharynx (ED_{50}<15 µg/ml). Coptisine and another, not named, alkaloid has also cytotoxic activity.

Kim et al, 1997: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 200 mg/kg body weight), a carcinogenic agent, was given to rats, together with a saturated sodium chloride solution or a 0.9% saline, for three weeks. A water methanol extract of *Chelidonium majus* (0.1% or 0.2% in the diet for 16 weeks) or no further treatment was combined, after 20 weeks the rats were killed. Pepsinogen-1-altered glands (PPAGs) in the pyloric mucosa of the stomach occurred in all groups of rats, but the mean number of PPAGs was significantly lower in animals treated with 0.1% *Chelidonium majus* herb extract, compared with MNNG and saturated sodium chloride alone, but the results didn't show a significant difference in rats with a 0.2% *Chelidonium majus* extract. There were also no significant differences between any groups in the number of animals with papilloma and squamous cell carcinoma lesions of the fore stomach.

Immunomodulatory activity

Song et al, 2003: the protein bound polysaccharide, CMAla, isolated from a water extract of *Chelidonium majus*, was given to mice (50 mg/kg) intraperitoneally 24 hours before sub lethal doses of irradiation. Results showed a significant increase in platelet numbers in mice treated with *Chelidonium majus* extract (evaluation: 5 days post radiation), also the white blood cells were significantly increased in CM-Ala treated mice, compared to the control (9 days post radiation). This indicates a haematopoietic recovery. In other experiments, mice were radiated with lethal doses of irradiation (9Gy), the survival rate for mice treated with CM-Ala (50 or 100 mg/kg) was 80% at 30 days post-irradiation, whereas all mice in the control group were dead after 15 days.

2.1.2. Assessor's overall conclusions on pharmacology

Viruses

The antiviral activity of total alkaloid extracts of *Chelidonium majus* shows activity against multiple viruses (herpes simplex virus, polio virus, several adenoviruses) *in vitro*. HIV-I virus was found to be less or more sensitive to berberine. There was only one study (mikrobiol 1977) that investigated activity against *influenza* virus, results showed activity in mice, when pneumonia was induced by a low quantity of *influenza* virus. Protoberberine was found active against reverse-transcriptase enzyme of RNA-tumour viruses, whereas its activity is dependent of the quaternary nitrogen in its structure. Chelidocystatin on its own has proved activity against cysteine proteinases. All these findings report a good indication for antiviral activity of *Chelidonium majus* extracts, but further investigations are necessary, especially *in vivo* trials.

Bacteria

Multi-drug resistant bacteria and MRSA strains were submitted in trials to test their sensitivity for *Chelidonium majus* extracts. Two benzophenanthridine alkaloids were found to be responsible for the activity, but no *in vivo* or clinical trials were conducted to date. Total extracts and pure substances (berberine, sanguinarine, chelerythrine and chelidonine) were tested for their antimicrobial activity against many strains (gram positive, gram negative and dermatophytes). Berberine, the quaternary nitrogen, showed *in vivo* activity against the enterotoxin production of *E. coli*.

Cancer

Most results of *in vitro* anti-tumour activity trials are pointing towards sanguinarine, chelidonine, chelerythrine and berberine for the anti-tumour effect of *Chelidonium majus* extracts. The iminium group in the structure of the molecules is hold responsible for the activity. Strongest anti-tumour agent of *Chelidonium majus* was found to be sanguinarine, who intercalates strongly with DNA, The activity is comparable with the anti-psoriatic agent anthralin. Chelerythrine, berberine and chelidonine are also active but are less potent. Nevertheless, *in vivo* trials didn't show any significant result for the anti-tumour activity, so much more trials are welcome for this indication. The results of the *in vitro* tests are very promising but they aren't proven in *in vivo* studies so there are additional tests needed.

Gastro-intestinal

Coptisine, chelidonine and (+)-caffeoylmalic acid, on there own and cooperative, are showing antispasmodic activity. Studies are indicating that there is a musculotropic and neurotropic effect and are indicating that there is also an effect at the GABA receptor. Choleretic activity was also evaluated but did not show any significant results with the total alkaloid extract of *Chelidonium majus* , this suggests that all the components of the total extract are necessary for activity, rather than the alkaloids only. Sanguinarine and chelerythrine extracts also showed a vasopressin receptor activity, so vasopressin couldn't bind to its receptor.

Inflammation

Chelidonium majus extract increases TNF-a production via NF-kB activation, so it plays a role in the anti-inflammatory process. 5 and 12-lipoxygenase are inhibited by sanguinarine and chelerythrine, but not by total extract or chelidonine. These enzymes are involved with leukotriene B_4 and 12-hydroxyeicosatetraenoic acid synthesis, so they have anti-inflammatory properties. These results were also shown in a *in vivo* study (*Lenfeld, 1981*).

Miscellaneous

A water extract of *Chelidonium majus* gave a significant increase in the cytotoxicity of C_T cells in a few *in vitro* studies. *In vivo* trials are showing also a positive immunomodulatory effect, but there are too few studies to conclude. Analgesic activity was evaluated with its activity on GABA receptors. Results showed an inhibition of GABA chloride currents which indicates an indirect mechanism for analgesic activity. The activity on glycine, an inhibitory neurotransmitter, and glutamate, an excitatory neurotransmitter, suggests also a nociceptive activity.

2.2. Pharmacokinetics

2.2.1. Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Only few studies have been executed on the pharmacokinetics of the *Chelidonium* alkaloids.

Sanguinarine and chelerythrine

In a study with adult rats they orally administered a single dose of sanguinarine (10 mg/kg body weight) in 1 ml water. Dihydrosanguinarine was identified as a sanguinarine metabolite in the plasma and liver and significantly higher levels of dihydrosanguinarine were found compared to levels of sanguinarine. Sanguinarine and dihydrosanguinarine were not detected in the urine (*Psotova et al, 2005*).

In a feeding experiment on pigs, where the daily dose was 5 mg/kg body weight, Sanguinarine was found in all body fluids and tissues except for muscles. Chelerythrine, whose proportion in the food was lower, was detected in faeces, liver, gingival and in the intestine and plasma (*Kosina et al, 2004*).

Coptisin

Coptisine was isolated from a traditional Chinese medicine and was administrated intravenous at a dose of 10 mg/kg in rats. The plasma-drug concentration time profile can be described as a two compartmental model. Coptisine was quickly eliminated with systemic clearance of 0.08 l/min/kg which is 147% of the hepatic blood flow (0.055 l/min/kg in rats), suggesting that the alkaloid was quickly cleared via hepatic clearance. The elimination half-life of coptisine was 288 min. The volume of distribution at terminal phase was 30.07 l/kg. This is greater than total body water at 0.67 l/kg, suggesting that coptisine may be widely distributed into extra vascular systems. The pharmacokinetics of coptisine after oral administration at a dose of 10 mg/kg can also be described as a two compartmental model. The terminal elimination half-life was 307.80 min. The oral bioavailability of coptisine was 7.80% and the time to reach the maximum plasma concentration was 14 min. The percent of coptisine excreted in the urine was 5.16% following intravenous administration and 0.26% after oral administration. The significantly lower excretion following oral than intravenous administration shows that coptisine may undergo the first pass effects. This low percentage strongly suggests either a strong first-pass effect or poor absorption (*Li HL et al, 2006*).

Berberine

The pharmacokinetic assay of berberine was executed on rabbits, using intravenous and intramuscular administration at a dose of 2 mg/kg. The plasma concentration of berberine as function of time can be described as a two compartmental model with a terminal elimination half-life of 5.28 h, a total plasma clearance of 5.64 l/h and a volume of distribution of 38.30 l. The amount of berberine excreted unchanged into urine was found to be only 4.93% of the dose given. Berberine was also excreted

biliary into the intestine and this may be one of the other major elimination pathways. The biliary excretion of berberine was found to be 0.5% of the dose given.

Berberine was also given by the intramuscular route. The bioavailability was evaluated by comparison with an i.v. bolus dose of 2 mg/kg. The absolute bioavailability of i.m. administration was 99.77%. After oral administration less than 0.1% of a dose is excreted in the urine unchanged in 24 hours. A reason for this finding may be intensive metabolism (elimination), but also poor absorption from the gastro-intestinal tract cannot be excluded (*Chen CM et al, 1995; Moffat et al, 2004*).

2.2.2. Assessor's overall conclusions on pharmacokinetics

Rats, rabbits and pigs were used as species to investigate the pharmacokinetics of *Chelidonium* alkaloids. The main alkaloids in *Chelidonium majus* may be intensively metabolized or poorly absorbed. The plasma concentration of the alkaloids as function of time can be described as a two compartmental model. The high volume of distribution suggests penetration into the extra vascular system. Up to now pharmacokinetic data on the components of *Chelidonium majus* are scarce. Further investigation on the pharmacokinetics of alkaloids and other components of *Chelidonium majus* is necessary.

2.3. Toxicology

2.3.1. Overview of available data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Single/repeated dose toxicity

In an animal experimental study the anti-tumour properties of *Chelidonium majus* L. were investigated. It was found that the fresh plant can cause acute toxicity due to the latex. Drying of the plant considerably reduces the toxicity. The use of therapeutic doses is safe due to the low quantity of alkaloids in the plant preparations. Large doses can irritate the gastro-intestinal tract. Excessive use for long periods should be avoided because of the risk of hepatotoxic effects, including severe hepatitis, severe cholestasis and fibrosis. A mechanism for *Chelidonium majus* induced hepatotoxicity has not been established. In literature deadly poisonings have been described with children after eating the plant (*Hänsel et al, 1992*).

The LD_{50} in mice was tested and determined as 1300 mg/kg, while in rats it was more than 2000 mg/kg (ESCOP monograph).

Genotoxicity

Sanguinarine was shown to induce DNA damage in mouse bone marrow cells. (*Ansari et al, 2006*)
In vitro DNA adducts formation with sanguinarine and chelerythrine in the presence of rat hepatic microsomes has been described *(Stiborova et al, 2002*).
Sanguinarine elicited weak positive responses in the *Salmonella* mutagenicity test after metabolic activation (*Frankos et al, 1990*).

Genotoxicity *in vivo* of *Macleaya cordata* (Willd.) R.Br. (Papaveraceae) was tested. The plant species contains sanguinarine. An isoquinoline mixture made from the plant was given to rats (5, 330 or 660 ppm isoquinoline alkaloids), mixed with the food. DNA adducts formation in liver was analyzed. No DNA damage was seen to rat lymphocytes or hepatocytes after 90 days administration (*Stiborova et al, 2008*).

Cytotoxicity

This study tested the cytotoxicity of sanguinarine and chelerythrine on human hepatoma cells. Concentrations of 0.01, 0.1, 1µM were found not to be cytotoxic.

It was also shown that these benzophenanthridine alkaloids do not interfere with the expression of the important P450 enzyme, CYP1A1 and that they do not affect the AhR (Aryl hydrocarbon receptor) transcriptional activity in human hepatoma cells. Chelidonine did not show a significant cytotoxicity or DNA damage (*Zdarilova et al, 2006; Dvorak, 2005; Kaminskyy et al, 2007*).

Cytotoxicity of chelidonine and protopine were investigated and cytotoxic activity was not observed for alcoholic and water extracts (*Saglam et al, 2003*).

Recently a hepatotoxic assessment of *Chelidonium* has been executed in primary hepatocyte cultures of different species. The aim was to elucidate the potential hepatotoxicity of *Chelidonium* in human, rat, canine and monkey hepatocyte cultures. For this purpose water-soluble extracts were used. The liquid extract showed a concentration-dependent toxicity in human hepatocytes and at concentrations of 7.5 mg/kg for animal hepatocytes (*Runge et al, 2009*).

The involvement of sanguinarine and chelerythrine was investigated in cell cycle regulation and cell death in various cell lines. Publications were mainly published from 1995 to 2006. These alkaloids seemed to be good candidates for chemotherapeutic regimens. They could also contribute to the development of successful immune therapies of some carcinomas due to their apoptotic potential. However the complete signalling cascade in which sanguinarne and chelerythrine treatment induces apoptotic cell death is not yet understood (*Malikova et al, 2006*).

Carcinogenicity

No data available

Reproductive and developmental toxicity

The alkaloid sanguinarine potently suppresses angiogenesis by inhibition of the VEGF signalling pathway in the angiogenic process (*Basini et al, 2007*).

Local Tolerance

Contact dermatitis has been reported in a woman who had used *Chelidonium majus* to treat warts. The woman experienced severe itching and erythema with papules at the application site. The reaction resolved within a few days without treatment (*Etxenagusia et al, 2000*).

2.3.2. Assessor's overall conclusions on toxicology

When the dried parts of *Chelidonium* are used in normal dose, the toxicity is limited. Severe and irreversible hepatotoxicity can occur after chronic administration of high doses. Further investigation is needed on the mechanisms of action. Four alkaloids, sanguinarine and chelerythrine, chelidonine and protopine were not found to be cytotoxic. The other components of *Chelidonium* need more investigation to make a conclusion of cytotoxicity of *Chelidonium.* A recent study also suggested that human hepatocytes seemed to be more sensitive to *Chelidonium* extracts than canine, rat and monkey hepatocytes. Further evaluation of possible toxicological effects on human hepatocytes is needed. It was found that sanguinarine and chelerythrine can induce DNA damage in rat and mouse cells. One case of contact dermatitis has been reported after topical application so it could be a hypersensitivity to one of the components. In summary, from the data available, the toxicity of *Chelidonium majus* can be assumed to be low and the plant can be considered as relatively safe to use. Nevertheless further

investigation is needed to exclude some toxic effects when the substance is used under normal administration.

3. Clinical Data

3.1. Clinical Pharmacology

3.1.1. Pharmacodynamics

3.1.1.1. Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

There are no intentional pharmacodynamic studies undertaken.

3.1.1.2. Assessor's overall conclusions on pharmacodynamics

No studies available.

3.1.2. Pharmacokinetics

3.1.2.1. Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

No data available on humans.

3.1.2.2. Assessor's overall conclusions on pharmacokinetics

Not applicable.

3.2. Clinical Efficacy[1]

Dose response studies.

3.2.1. No dose-finding studies.

3.2.2. Clinical studies (case studies and clinical trials)

Boulwere et al, 1985: The *Chelidonium majus* alkaloids sanguinarine and chelerythrine were found effective in the control of the production of bacterial sulphur compounds responsible for a halithosis. Subjects (five females, two males, average age of 34 years old) were asked to use a oral rinse (15 ml rinse for 15 sec each day at 9 AM). Saliva samples were taken at 8 minutes, 15 minutes and one hour after application. The subjects couldn't use any products like coffee, tea or couldn't have any morning mouth hygiene. Saliva samples were then worked on and incubated. The reduction of thiols available to react with reagents was evaluated for the different substances. Results are aligned into the following table.

[1] In case of traditional use the long-standing use and experience should be assessed.

Substance	Concentration	% of thiol reduction
Sanguinarine extract + ZnCl	0.030% / 0.20%	65.4%
ZnCl	0.22%	44.5%
Cetylpyridinium chloride + Domiphen Bromide	0.045% / 0.005%	43.9%
Essential oils	16.4mM	15.6%

Table 5: percent reduction of measurable volatile sulphur compounds from fermenting saliva by four commercial mouth rinses.

Thus, rinses capable of lowering populations of anaerobic bacteria, who produce volatile sulphur compounds, or those who are capable of covalently trapping sulphur, can be used to reduce bad breath compounds.

Southard et al, 1987: Benzophenanthridine alkaloids are used in the treatment of periodontal disease, they are integrated into toothpastes and mouth rinses The subjects (five females, eight males, with a average age of 29 years old) received a test irrigation fluid containing 22.5 µg/ml and 90 µg/ml or a oral rinse solution with a concentration of 0.03% or placebo. The subjects used the solutions twice a day for 14 days, without any other oral hygiene preparation. After day 4, 7 and 14, plaque and gingivitis score was evaluated in comparison with day 0. After this first period, the test persons did a cross over with the other test solution after a two week resting period that included optimal oral hygiene. Results are aligned in the following table, containing the kind of used preparation and the plaque and gingivitis score.

		Plaque score				Gingivitis score			
		Day 0	Day 4	Day 7	Day 14	Day 0	Day 4	Day 7	Day 14
oral rinse	0.03%	0	0.37	0.53	0.74	0.17	0.33	0.47	0.79
	PLACEBO	0	0.68	1.00	1.28	0.17	0.65	0.95	1.48
supragingival irrigation	22.5 µg/ml	0	0.37	0.56	0.83	0.4	0.34	0.42	0.67
	90 µg/ml	0	0.29	0.33	0.55	0.4	0.25	0.26	0.37

Table 4: Preparations in order to the concentration and the plaque and gingivitis score

Ritter et al, 1993: Tablets containing *Chelidonium majus* extract (containing 66.0 to 167.2 mg native dry extract, equivalent to 4 mg total alkaloids, calculated as chelidonine) or placebo pills were evaluated in a randomised, double-blind, placebo-controlled trial on 60 patients with functional epigastric complaints. The patients received two tablets, three times daily for six weeks. The reduction in symptom score, assessed using the Zerssen list, at the end of the trial was evaluated and was significantly greater in the *Chelidonium majus* group, compared with the placebo group (p=0.003). Physician's assessment of efficacy was that 18/30 patients in the treatment group were improved or symptom free, compared with 8/30 in the placebo group.

3.2.3. Clinical studies in special populations (e.g. elderly and children)

No data available.

3.2.4. Assessor's overall conclusions on clinical efficacy

Data from clinical studies are scarce. There is only one study with an extract of *Chelidonium majus*, the dose given to patients with epigastric pain being equivalent to 4 mg total alkaloids. Although the symptom score was positively influenced according to the Zerssen list as well as to the physicians' assessment, the number of patients is too low to support a well established use.

3.3. Clinical Safety/Pharmacovigilance

3.3.1. Patient exposure

In Germany there is a graduated plan (grade II) concerning Chelidonium-containing medicinal products for internal use which came into force on 09 April 2008.

The report is only available in German. Therefore only the key elements are mentioned.

1. All marketing authorisations for medicinal products are withdrawn which lead to a daily intake of more then 2.5 mg alkaloids from *Chelidonium majus* according to the posology of the SPC.
2. For medicinal products which lead to a daily intake of 2.5 µg to 2.5 mg alkaloids from *Chelidonium majus* according to the posology of the SPC the marketing authorization will be changed.

The following chapters of the package leaflet and the SPC are affected:

Contraindications

It is to quote that the intake of the medicinal product is contraindicated for persons which suffer from liver diseases or which had liver diseases in history or in cases of concomitant intake of other liver damaging medicinal products.

Special warnings and precautions for use

It is to quote that if signs of liver damage occur, the intake of the product should be stopped immediately and medical advice should be sought.

Undesirable effects

It is to quote that cases of liver damage (increase of liver enzymes and bilirubin up to drug-mediated hepatitis) as well as cases of liver failure occurred.

Pregnancy and lactation

It is to quote that the intake during pregnancy and lactation is contraindicated.

Duration of use

It is to quote that if the duration of use is longer than 4 weeks liver function test (transaminases) should be conducted.

For those medicinal products that are also authorized for the use in children, the maximum limit of alkaloids is to adjust according to the body weight (starting from 2.5 mg for a 70 kg adult).

The necessity for the graduated plan was justified with several cases of undesirable effects connected to liver diseases reported after the intake of Chelidonium-containing medicinal products. The maximum permissible value base on preclinical studies (*Notox project 320211 and 330222; Weleda 2002*). It is emphasized that for the dosage 2.5 µg-2.5 mg alkaloids/day no valid studies for the proof of efficacy exist.

3.3.2. Adverse events

Table 1 Summary of spontaneous reports ($n = 47$) of adverse drug reactions associated with single-ingredient *Chelidonium majus* preparations held in the Vigisearch database of the World Health Organization's Uppsala Monitoring Centre for the period up to end of June 2005.[a, 64]

System organ class. Adverse drug reaction name (number)	Total
Central nervous system. Paraesthesia (1)	1
Foetal. Biliary atresia (1)	1
General. Asthenia (3); death (1); fatigue (2); malaise (1); necrosis ischaemic (1)	8
Gastrointestinal. Abdominal pain (4); diarrhoea (2); faeces, discoloured (3); mouth dry (1); nausea (6); pancreatitis (1); tongue oedema (1); vomiting (3)	21
Liver-biliary. Bilirubinaemia (11); cholelithiasis (1); gamma-GT increased (6); hepatic enzymes increased (13); hepatic failure (1); hepatic function abnormal (1); hepatitis (19); hepatitis, cholestatic (8); hepatitis, viral (1); hepatocellular damage (4); jaundice (16); SGOT increased (7); SGPT increased (7)	95
Metabolic. Cholinesterase decreased (1); LDH increased (2); phosphatase alkaline, increased (5)	8
Psychiatric. Anorexia (1); insomnia (1); nervousness (2)	4
Respiratory. Dyspnoea (1); larynx oedema (1)	2
Skin. Pruritus (3); skin discoloration (1)	4
Urinary. Urine abnormal (3)	3
Total number of adverse drug reactions	**147**

Key: GT = glutamyl transferase; LDH = lactate dehydrogenase; SGOT = serum glutamic-oxaloacetic transaminase (= aspartate transaminase/aspartate aminotransferase); SGPT = serum glutamate pyruvate transaminase (= alanine transaminase/alanine aminotransferase)
[a]Caveat statement. These data were obtained from the Vigisearch database held by the WHO Collaborating Centre for International Drug Monitoring, Uppsala, Sweden. The information is not homogeneous at least with respect to origin or likelihood that the pharmaceutical product caused the adverse reaction. Any information included in this report does not represent the opinion of the World Health Organization

(*Barnes et al, 2007*)

Spontaneous reporting can be illustrated by published case reports.

Acute hepatitis

A woman (42y) was diagnosed with severe acute hepatitis 2 weeks after she started using a preparation with herbal ingredients (a.o. *Chelidonium majus herba* and *Curcuma longa rhizoma*) for dermatological conditions.

The following complaints were reported: high fever (40.5°C), epigastral pain, headache, hypersensitivity of the eyes, muscle pain and tiredness. The patient became icteric, her urine had a dark colour and faeces were discoloured. The patient declared that she had only taken 1 tablet of 500 mg paracetamol as co-medication.

Transaminases strongly increased: aspartate aminotransferase (ASAT): 838 U/l (0-40); alanine aminotransferase (ALAT): 1490 U/l (0-30); lactatedehydrogenase (LDH): 389 U/l (114-235); gamma glutamyltransferase (γ-GT) 286 U/l (0-65). Total bilirubine 200 µM/l (3-26). Viral infection with hepatitis A, B or C was excluded. Paracetamol intoxication was excluded by serum analysis.

After she discontinued the medication, her situation normalised within 2 months.

Causal relationship with Chelidonium was hypothesized but no causality scale or scoring was used (*Crijns et al, 2002*).

10 cases of acute hepatitis were observed over 2 years, probably induced by *Chelidonium majus*, frequently described to treat gastric and biliary disorders. The course of the hepatitis was mild to severe, with marked cholestasis observed in 5 patients. In all cases other possible causes of hepatitis were excluded (viral, autoimmune, hereditary, alcohol) by laboratory tests and imaging procedures.

Liver biopsies were consistent with drug-induced damage. After discontinuation of greater celandine, rapid recovery was observed in all patients and liver enzymes levels returned to normal in 2-6 months. Unintentional rechallenge lead to a second flare of hepatic inflammation in 1 patient. No causality scales were used (*Benninger et al, 1999*).

An 82 years old male patient was admitted to the geriatric ward of a local hospital. Cognitive impairment had become worse during the last months. There was a decline in Mini-Mental-State (MMS) from 24/30 to 14/30. When admitted to the hospital a MMS of 12/30 was found. There were no hepatic preconditions known. The liver had normal dimensions as was shown by sonography and by magnetic resonance imaging. The patient used a herbal tea preparation with self-collected Chelidonium as main component. Liver enzymes were enhanced in serum: GGT 197 U/l; GOT 687 U/l; GPT 904 U/l; LDH 297 U/l; bilirubine 2.32 mg/dl.

The diagnosis toxic hepatitis caused by Chelidonium was made. The patient declared that he prepared and used herbal medicine as told by friends and that he probably made his tea too concentrated. Five months after discontinuation of the herbal practice, liver enzymes were returned to normal. There were no signs of hepatic damage (*Bichler, 2009*).

Moro et al, 2009: investigated one case of a 65 year-old man with a sudden onset of asthenia, dyspepsia and jaundice. The first symptoms emerged 3 days before admission to the hospital and the patient noticed hyperchronic urine and scleral jaundice. Laboratory test showed a strong enhancement of transaminases and bilirubin levels: e.g. AST 3253 U/l (5-38); ALT 4765 (5-41). Apart from hepatitis C virus antibodies (HCV), the remaining for hepatic viruses were negative. The positive screening for HCV was most probably due to contact with patient's brother-in-law who died 5 years earlier of HCV-related hepatic cirrhosis.

The patient was currently taking lansoprazole (15 mg/d). Because of a persistent pyrrosis patient's wife recommended to take daily a cup of a *Chelidonium majus* decoction (boiling 4-5 spoons of dried leaves in 150 ml of water, straining and leaving it overnight) . This treatment went on for one month preceding the hospital admission. *Chelidonium majus* was identified by thin layer chromatography. The tea preparation as well as lansoprazol were discontinued after admission, infusions with normal saline were started, as well as treatment with glutathione (600 mg i.v. 2x/d) and ursodeoxycholic acid (300 mg p.o. 2x/d for 30 days). The patient was discharged from hospital after 10 days. He recovered completely, though a mild pyrosis persisted, and the liver parameters normalised within 2 months.

Additionally to this case, 16 cases of *Chelidonium majus* hepatitis from literature were analysed. Typical symptoms of cholestatic hepatitis (jaundice, itching and fatigue) usually arose after 2 or more months since the beginning of greater celandine oral consumption. The onset seems to be independent from dosage. This suggests that the adverse reaction may be of idiosyncratic nature. Typical manifestations are hepatomegaly, serum transaminases and bilirubin level over the normal range. Patients mostly tested negatively for antibodies against hepatitis viruses (sometimes slight positive hepatitis-C viruses reactions were detected). A possibility of a celandine-drug interaction must be considered, because a majority of patients took other (herbal or conventional) medicines. When the consumption of greater celandine was interrupted, symptoms regressed and liver parameters normalised until complete recovery within 2 to 3 months (rarely after more than 5 months), although recovery could also be seen after 2 weeks. Rechallenge occurred in 2 patients with liver impairment as results (*Moro et al, 2009; Stickel et al, 2001*).

A 58-year old woman was seen on consultation because of painless jaundice with dark urine and pale stools. The jaundice started one week before consultation. There was no medication used besides tablets of medicinal herbs for muscular pain since 6 weeks and – started after the jaundice –

supplements of vitamins. The patient took capsules with 50 mg of greater celandine, 50 mg of gentian and 100 mg of curcuma root.

The abdomen revealed a palpable and tender liver edge just beneath the costal arc. The gallbladder was also palpable. Liver enzymes were strongly increased: GOT (AST) U/l (1740 U/l; on day 14 lowered to 55 U/l); GPT (ALT) 1566 U/l; on day 14 lowered to 45 U/l). An idiosyncratic mechanism is put forward (*Hardeman et al, 2008*).

3.3.3. Serious adverse events and deaths

No data available.

3.3.4. Laboratory findings

See the graduated plan (Germany) under 3.3.1.

3.3.5. Safety in special populations and situations

See the graduated plan (Germany) under 3.3.1.

3.3.5.1. Intrinsic (including elderly and children) /extrinsic factors

See the graduated plan (Germany) under 3.3.1.

3.3.5.2. Drug interactions

See the graduated plan (Germany) under 3.3.1.

3.3.5.3. Use in pregnancy and lactation

As there exist some cytotoxicity, the herbal substance or preparations thereof are not recommendable during pregnancy. See also the German graduated plan.

3.3.5.4. Overdose

No systematic data reported.

3.3.5.5. Drug abuse

Not applicable.

3.3.5.6. Withdrawal and rebound

Not applicable.

3.3.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

Not reported.

3.3.6. Assessor's overall conclusions on clinical safety

The main concern goes to possible hepatic damage. In the Vigisearch database 95 cases of hepatic complications are indexed and hepatic toxicity has been repeatedly reported in literature. This concern leads to a graduated plan in Germany in 2008. This plan has been communicated to European

Medicines Agency. It essentially consists in limiting the daily intake of total alkaloids from Chelidonium to 2.5 mg. In the only clinical study reported patients took 4 mg. Apparently there were no major side effects, but the study was limited to 6 weeks. The German recommendations should be taken into consideration on a European level.

4. Overall conclusions

Chelidonium extracts have a well documented antiviral activity with perspective. *Herpes simplex*, polio as well as several adenoviruses are affected. There is even an *in vivo* anti-Influenza activity. Protoberberine was found active against reverse transcriptase of RNA-tumour viruses. Antimicrobial activity was mostly tested with separate compounds of Chelidonium. The same results were obtained for the anti-tumoral activity.

Choleretic activity seems to be linked to the totality of the components. Furthermore in different experimental models there are indications for anti-inflammatory and analgesic properties.

The alkaloids of Chelidonium seem to be intensively metabolised. A high volume of distribution should be taken into consideration.

Although the acute toxicology of the total extract is low, some alkaloids such as sanguinarine induced DNA damage in bone marrow cells. The same can be stated for the cytotoxicity.

There is only one clinical study with the total extract in patients with epigastric complaints, whereas there are warnings against possible hepatotoxicity of Chelidonium containing preparations. These warnings were translated into registration restrictions in Germany.

A risk-benefit analysis can be made for the herbal preparations containing Chelidonium. The herbal substance is described in the European Pharmacopoeia with a minimum of 0.6% of total alkaloids. The herbal substance as well as the alkaloids can be characterised without too much difficulty. Adulteration of the herbal substance is no point of concern.

Although voluntary intoxications with the herbal substance and preparations thereof are not reported, there is a concern with regard to possible hepatotoxicity. Indications for genotoxicity or foetotoxicity are known for single alkaloids of Chelidonium, not for the total extracts. Chelidonium preparations are used for epigastric discomfort and superficial warts. These conditions are not serious and other herbal as well as conventional medicines exist for internal use. Warts are mostly treated with preparations containing several plants which complicate the characterisation of mono preparations in the monograph. The fresh latex is traditionally used in folk medicine and cannot be considered in an industrial context. Patients with liver diseases should be considered as patients at risk and should not take Chelidonium containing preparations. Although the herbal substance has a long standing use in Europe, the risk-benefit balance can be considered as negative and the use of Chelidonium containing preparations should be restricted as type of patients and daily intake of alkaloids is concerned.

Annex

List of references