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Evaluation of Medicines for Human Use

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COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

ASSESSMENT REPORT ON *ARTEMISIA ABSINTHIUM* L., HERBA

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I. REGULATORY STATUS OVERVIEW[1]

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'

Member State	Regulatory Status				Comments[2]
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	combinations
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
Czech Republic	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	since 1996 and combinations
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	combinations
Estonia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	since 1999 and combinations
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	since 1976 and combinations
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	combinations
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Latvia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	since 1993 and combinations
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no response
Poland	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	since 1978
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
Romania	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	since 2004 and combinations
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	combinations

[1] This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.
[2] Not mandatory field

Member State	Regulatory Status				Comments[2]
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	combinations
Spain	☐ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	food supplement
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	no products

II. ASSESSMENT REPORT

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

(WELL-ESTABLISHED USE)

BASED ON ARTICLE 16D(1) AND ARTICLE 16F AND 16H OF DIRECTIVE 2001/83/EC AS AMENDED

(TRADITIONAL USE)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Artemisia absinthium* L.; basal leaves or slightly leafy, flowering tops, or mixture of these dried, whole or cut organs.
Herbal preparation(s)	a) comminuted herbal substance b) expressed juice from fresh Absinthii herba (1 : 0.5 - 0.9) c) tincture from Absinthii herba (1:5) ethanol 70% (v/v)
Pharmaceutical forms	Herbal preparation in solid or liquid dosage forms for oral use or as herbal tea for oral use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term
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Assessor	Dr. Jacqueline Koch Phone +49 228 207 5980 Fax: +49 228 207 5395 email: jkoch@bfarm.de

II.1 INTRODUCTION

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

- Herbal substance(s) [3]:

Absinthii herba [European Pharmacopoeia]

> Basal leaves or slightly leafy, flowering tops, or mixture of these dried, whole or cut organs of *Artemisia absinthium* L. Content: minimum 2 ml/kg of essential oil (dried drug); bitterness value: minimum 10,000

Synonyms: German- Magenkraut, Wermutkraut; Engl- Wormwood; French- Absinthe, Armoise amère; Polish- Ziele piolunu; Romanian- Iarbă de pelin.

Artemisia absinthium is a species of wormwood, native to temperate regions of Europe, Asia and northern Africa. It grows naturally on uncultivated, arid ground, on rocky slopes and wastelands; it can also be cultivated in dry soil.

The time of harvesting is important for the quality and the composition of the constituents. Metabolism processes change over the flowering period and ripenening of the fruit, e.g. during the flowering period the concentration of bitter constituents increases. [HÄNSEL & STICHER 2007].

Absinthii herba is used medically with a very long tradition, but it is also used as an ingredient in the liquor absinthe. In the beginning of the 19th century many countries banned the use of absinthe. Since 1988 the European Union permits a maximum thujone level of 5 mg/kg in alcoholic beverages with less than 25% volume of alcohol, 10 mg/kg in alcoholic beverages with more than 25% volume of alcohol, and 35 mg/kg in alcohol labelled as bitters. The use and sale of absinthe in the member states is permitted within this framework [88/388/EEC 1988].

Constituents: [HAGERROM 2006, WICHTL 2002, HÄNSEL & STICHER 2007]

Volatile oil:

Content: 0.2-1.5%. The composition depends on the plant provenance, the different chemotypes, and seasonal variations. The 4 main components described are: α-thujone, (Z)-epoxyocimene, trans-sabinylacetat and chrysanthenylacetat [CARNAT et al. 1992, HAGERROM 2006].

”pure”-chemotype:

α -Thujone is typical for plants grown in areas below 1,000 m a.s.l.. (Z)-epoxy-ocimene is the main component in plants grown in Europe at altitudes higher 1,000 m a.s.l.. In France, there are different chemotypes with trans-sabinyl-acetate and chrysanthenyl-acetate as main components, while plants from eastern Europe are mostly mixed types [CHIALVA et al. 1983].

Further volatile oil components are sequiterpenes like α-bisabolol, β-curcumen and spathulenol [WICHTL 2002].

3 According to the ‘Procedure for the preparation of Community monographs for traditional herbal medicinal products’ (EMEA/HMPC/182320/2005 Rev.2) and the ‘Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use (EMEA/HMPC/182352/2005 Rev.2)

"mixed"-chemotype:

(Z)-epoxy-ocimene+chrysanthenyl-acetate+thujone-chemotype: cis-chrysanthyl-acetate ~ 40%, cis-epoxyocimene ~ 30%, furthermore linalool, cis-chrysanthenol, cis-ocimene, trans-epoxy-ocimene [ARINO et al. 1999a] cis-chrysanthenol-chemotype (growing in Auvergne): cis-chrysanthenol ~ 70%, α-thujone ~ 8% (in november); cis-chrysanthenol ~ 20%, α-thujone ~ 50% (in August) [ARINO et al. 1999b, CARNAT et al. 1992]

(Z)-epoxy-ocimene+β-thujone-chemotype (growing in Croatia): β-thujone 14-43%, Z-epoxy-ocimene 6-38% [JUTEAU et al. 2003]

(Z)-epoxy-ocimene+chrysanthenyl-acetate-chemotype (growing in France): (Z)-epoxy-ocimene 25-65%, chrysanthenylacetat 15-50% (no α-thujone was detected in any sample) [JUTEAU 2003, ARINO et al. 1999c]

β-thujone-sabinyl-acetate-chemotype: β-thujone, sabinyl-acetate [CHIALVA et al. 1983]

Bitter constituents: 0.15–0.4%; the most bitter constituents belong to the structure of sesquiterpenlactones as absinthin (max. 0.28% in the drug), anabsinthin, artabsin (0.04 to 0.16% in the fresh drug) und matricin (0.007% in the drug) [HAGERROM 2006, HÄNSEL & STICHER 2007]

Other constituents: flavonoids (such as quercetin, rutin), caffeic acids, chlorogenic acid, syringic acid, salicylic acid, vanillic acid, carotenoids, coumarins, homo-diterpen peroxides, thiophene [HAGERROM 2006, HÄNSEL & STICHER 2007, TOSI et al. 1991, CANADANOVIC-BRUNET et al. 2005].

Extraction yields:

The extraction method modifies the content of thujone in the preparation as much as the variable amount of thujone in the starting material. TEGTMEIER & HARNISCHFEGER [1994] examined the thujone content of different preparations of *A. absinthium.*

Table 1: Influence of the extraction procedure on the thujone concentration in the extracts of *A. absinthium* [essential oil content: 0.5% (m/v); thujone content in the essential oil: 4.8% (m/v)]; thujone yield in % [thujone content in extract/actual thujone content] (mean ± SD; n=6) [TEGTMEIER & HARNISCHFEGER 1994]

method of extraction	extraction solvent	thujone yield
percolation (72 h; room temperature)	purified cold water	not detected
	ethanol 30% (v/v)	not detected
	ethanol 90% (v/v)	75.0%
digestion (30 min; 80°C)	ethanol 30% (v/v)	70.8%
Distillation	purified water	100.0%

GAMBELUNGHE & MELAI [2002] examined two ethanolic preparations. The first preparation (macerating *A. absinthium* for 30 days with ethanol 20%) contained 0.2 mg/l β-thujone, whereas the other sample (macerating *A. absinthium* for 6 months with ethanol 95%) contained 62 mg/l β-thujone. α-Thujone was not found in any sample.

NIESEL [1992] examined the extraction rates for different herbal substances referring to their contents of essential oil (tea preparation with boiling water). For peppermint leaves it was shown that 20-25% of the essential oil could be found in the preparation after 10 min. For fennel fruits anethole recovery rates of 25-35% were found after 10 min. Assuming similar physico-chemical characteristics for the essential oil of *A. absinthium,* a 35% transition rate into a tea preparation (boiling water) is estimated.

- Herbal preparation(s)[4]:

  i) comminuted herbal substance

  ii) expressed juice from fresh Absinthii herba (1:0.5-0.9)

  iii) tincture (1:5); extraction solvent: ethanol 70% (v/v)

- Combinations of herbal substance(s) and/or herbal preparation(s)[4]:

Absinthii herba is used in combinations with many other herbal substances / herbal preparations. The main combination substances such as Gentianae radix, Angelicae radix, Curcuma rhizoma, Millefolii herba, Taraxaci radix, Menthae piperitae herba, Levistici herba, Liquiritiae radix and Foeniculus fructus exhibit bitter and/or aromatic properties and are usually used for dyspeptic or choleretic complaints.

This monograph refers exclusively to Absinthii herba.

- Vitamin(s)[5]: not applicable
- Mineral(s)[5]: not applicable

II.1.2 Information on period of medicinal use in the Community regarding the specified indication

The following herbal substances and herbal preparations have been on the European market for a period of 30 years and were proposed for the monograph on traditional use.

i) comminuted herbal substance
ii) expressed juice (1:0.5-0.9)
iii) tincture (1:5) extraction solvent: ethanol 70% (v/v)

Posology and indications of the traditional herbal substance and preparations of Absinthii herba:

- comminuted herbal substance in tablets

  Indication: For the symptomatic treatment of dyspeptic complaints such as minor gastro-intestinal spasms, repletion and flatulence
  Posology: 3 times daily 4 coated tablets with 190 mg herb
  Single dose: corresponding to 760 mg herbal substance
  Daily dose: corresponding to 2.28 g herbal substance

- comminuted herbal substance for tea preparation

Poland →	Indication:	lack of appetite, dyspepsia
	Posology:	oral use, 1.0 g 2-3 times daily
Spain →	Indication:	appetizer (loss of appetite); dyspepsia
	Posology:	oral use, 2-3 cups/daily

[4] According to the 'Guideline on the clinical assessment of fixed combinations of herbal substances/herbal preparations' (EMEA/HMPC/166326/2005)

[5] Only applicable to traditional use

Germany →	Indication:	loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence, spasmodic functional disorders of the biliary tract
	Posology:	oral use, 1.5 g 2 times daily (as appetizer: 30 min before meals; all other indications 1 cup of tea after meals)

- expressed juice (1:0.5-0.9)

  Indication: loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence

  Posology: 2 x daily 5 ml liquid containing 100% expressed juice

- tincture (1:5); extraction solvent: ethanol 70% (v/v)

  Indication: loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence
  Posology: 3 x daily, single dose amount corresponding to 1 g herbal substance
  Single dose: corresponding to approx. 1 g herbal substance
  Daily dose: corresponding to approx. 3 g herbal substance

II.2 NON-CLINICAL DATA

II.2.1 Pharmacology

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

Herbal substance/Herbal preparations:

in vitro studies:

The free-radical scavenging activity using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and the reactive hydroxyl radical formed in the Fenton reaction was tested with different extracts (successive extraction with MeOH 70%, petroleum ether, chloroform, ethyl acetate, n-butanol and water) of *A. absinthium* (ESR spectroscopy). The total phenolic and flavonoid contents in the plant were 25.6 and 13.06 mg/g, respectively. The following order of antiradical activity was found: ethyl acetate > methanol > n-butanol > chloroform > petroleum ether > remaining water extracts. A concentration of 0.5 mg/ml of the ethyl acetate extract reduced all DPPH radical molecules, while for the methanol extract a concentration of 2 mg/ml led to the 100% antiradical effect. At this high concentration (2 mg/ml) the antiradical effects of n-butanol, chloroform and petroleum ether extract were 96.06%, 84.82% and 78.26%, respectively. The remaining water extract possessed an antiradical activity of 16.67% in the concentration of 2 mg/ml. For the antioxidant activity the above described order was proven, too. A concentration of 0.25 mg/ml of the ethyl acetate extract inhibited completely the formation of hydroxyl radicals. In this concentration, the methanol extract produced a scavenging effect of 74.65% while for the n-butanol (57.24%), chloroform (27.95%) and petroleum ether (16.64%) extracts only low scavenging effects were observed. The remaining water extract exhibited a high scavenging effect (95.68%) only in a high concentration (3.25 mg/ml) [CANADANOVIC-BRUNET et al. 2005].

Also Ramos et al. showed a DPPH reduction caused by a hydroalcoholic extract (*A. absinthium* from Cuba) with an IC_{50} of 121 µg/ml [RAMOS et al. 2003].

To evaluate human CNS cholinergic receptor binding activity, investigations with an ethanolic extract (80% EtOH) of leaves from *A. absinthium* were carried out. Human cerebral cortical cell membranes were used to prove the activity of the ethanolic extract to displace (N)-nicotine and (n)-scopolamine from nicotinergic and muscarinic receptors. The assay of the extract as dilution series resulted in a (n)-nicotine displacement curve resembling the dose-dependant sigmoidal displacement curves typical for carbamylcholine chloride and choline chloride (both known nicotinergic ligands) and indicating the presence of nicotine-like material in the extract. The IC_{50} value was calculated as 4.1 mg plant/ml while the choline content was measured with 1.3×10^{-4} M [WAKE et al. 2000].

For the evaluation of NGF-potentiating activities, methanol, ethyl acetate and aqueous extracts of *A. absinthium* from Paraguay were examined for their effects on the NGF-mediated neurite outgrowth from PC12D cells. At a concentration of 30 µg/ml all three extracts markedly enhanced the neurite outhgrowth induced by NGF from PC12D cells (50.53%, 41.53% and 51.43%, respectively) [LI & OHIZUMI 2004].

An aqueous crude extract of *A. absinthium* was used to analyze effects on the osmotic stability of human erythrocytes. The extract protected human erythrocytes against hypotonic shock. It was discussed that the flavonoids might be responsible for this effect which might lead to an exacerbation of the van der Waals contacts inside the lipid layer of the membrane [DE FREITAS et al. 2008].

In a growth inhibitory assay, aqueous and ethanolic extracts of *A. absinthium* were tested for their effect against *Naegleria fowleri*. Both extracts inhibited strongly the growth of *N. fowleri*. A sesquiterpene lactone fraction was prepared from the ethanolic extracts. For this fraction a LD_{50} value of 31.9 µg/ml was obtained in the test system. For artemisin (from *A. annua*) the IC_{50} was reported with 5 g/ml. Therefore it was postulated, that the sesquiterpene lactone fraction of *A. absinthium* may contain artemisin or a compound with similar activity [MENDIOLA et al. 1999].

Hernandez et al. prepared an aqueous extract as well as a sesquiterpene lactone fraction from *A. absinthium* and tested them in a growth inhibition test against *Plasmodium falciparum*. In the aqueous extract the maximum percentage of inhibition of growth (89.9%) was observed at the dilution 1:35. The LD_{50} value of the sesquiterpene lactone fraction was 31.4 µg/ml [HERNANDEZ et al. 1990].

in vivo studies:

Different semi-pure extracts from *A. absinthium* from Pakistan were tested for their antiulcer effects on acetylsalicylic acid induced ulcers in rats. The effects on volume of gastric juice, acid output, peptic activity and mucin activity were studied. The air-dried powdered plant material was extracted with ethanol (95% EtOH) and the extract was concentrated. The crude extract was defatted with hexane. The defatted material was then extracted successively with chloroform and carbon tetrachloride. The fraction finally obtained was dissolved in methanol and then the colouring matter was removed. The remaining extract was separated into five purified/semi-purified fractions by chromatography on silica gel.
The ulcerated rats were given the fractions orally at a dose of 5 mg/kg 3 h prior and 3 h after treatment with acetylsalicylic acid (200 mg/kg) for three days. On the fourth day the rats were operated (pylorus ligation) and gastric juice was collected for a period of 4 h. Thereafter the animals were killed and the stomach was removed. The average numbers of ulcers per stomach were recorded and the inhibition of ulcer formation calculated in percent. Acid output, peptic activity and mucin activity were also determined.
Phytochemical analysis of the fractions showed the absence of alkaloids and anthraquinones but indicated the presence of glycosidic sugars and saponins.
Significant antiulcer effects have been observed. Fractions I and II reduced the ulcer index by 65% and 44%, respectively. The other fractions decreased it by 33%, 11% and 27%. Also fractions I and II showed a decrease (40% and 33%, respectively). Fractions I, II and III also decreased significantly the volumes of gastric juice (~1/3). Furthermore, for fraction II a decrease in peptic activity was observed. After treatment

with acetylsalicylic acid a decrease in total hexoses in the gastric juice was recorded in the controls. In fractions I and II (together with acetylsalicylic acid) the amount of total hexoses within the carbohydrates corresponded to the controls but the amount of fucose increased. Also, the amount of total protein in the gastric juice increased after treatment with fractions I and II. Fraction I caused a significant change in the performance of rats in the swimming test (increased duration of swimming). During all studies, injurious or toxic effects were not observed and no lethal effects occurred with dosages up to 10 mg/kg. Subsequently no LD_{50} could be determined [SHAFI et al. 2004].

An i.v. injection of decoctions of Absinthii herba (equal to 5 g herbal substance) caused a threefold increase of bile secretion in dogs [KREITMAIR 1951].

Dried aerial parts of *A. absinthium* were extracted with methanol (80% MeOH) and tested for their hepatoprotective activity. The dry extract was administered in various experiments to mice or rats with a concentration of 500 mg/kg. Based on the yield of the dry extract (8%) this is equivalent to 6.25 g herbal substance/kg.
The aqueous-methanolic extract protected against both acetaminophen- and CCl_4-induced liver injuries when administered prophylactically to rats and against lethal doses of acetaminophen in mice. Because the plant extract led to a prolongation of phenobarbital sleeping time in mice, it was speculated that the plant extract might contain inhibitors of microsomal drug metabolizing enzymes which cause hepatoprotection. Furthermore it was assumed, that the compound sesartemin might be responsible for the observed effects. Calcium channel blocking activities were also discussed. In some experiments a curative effect against acetaminophen-caused liver damage was found. This effect was attributed to the content of flavonoids, ascorbic acid, carotenoids, tannins and lignans. The treatment with the plant extract did not reveal any symptoms of acute toxicity [GILANI & JANBAZ 1995].

A. absinthium leaves (fresh, stored frozen after collection) were extracted with organic solvents of different polarities and tested as repellents against host-seeking nymphs of *Ixodes ricinus*. The ethyl acetate extract had a repellent activity of 78.1%, while the hexane and methanol extracts had ~ 60% and 45% repellency, respectively. The main volatile detected in the ethyl acetate (myrtenyl acetate; 77.8%) was also the main component of the methanol extract (77.1%) [JAENSON et al. 2005].

An ethanolic extract (90% EtOH) from dried aerial parts of *A. absinthium* was fractionated into hexane-soluble and chloroform-soluble portions. The chloroform-soluble fraction was further separated into chloroform- and water-soluble fractions. All three extract fractions were dried and used to examine the oral antipyretic activity in rabbits (Himalayan strain). Pyresis was induced by subcutaneous yeast infections. After sixteen hours test substances were administered via a gastric tube (150 mg/kg). The mean temperature was determined 90, 180 and 270 min after application of the test substance. Aspirin was used as the reference antipyretic agent (150 mg/kg). An antipyretic activity was observed for all three extracts. The strongest effect was obtained with the hexane-soluble fraction (comparable to aspirin). No toxicity (single-dose toxicity) was observed in dosages up to 1600 mg/kg. The possible antipyretic constituent was identified as 24ζ-ethylcholesta-7,22-dien-3β-ol [KHATTAK et al. 1985, IKRAM et al. 1987].

For the screening of antimalarial effects leaves of *A. absinthium* were dried and extracted with ethanol (95% EtOH). The extract was dried, dissolved in deionised water, filtered and the filtrate was concentrated to dryness. Swiss albino mice were infected with 1x 10^7 parasitized (*Plasmodium berghei*) blood cells from a donor mouse. The ethanolic extract was given orally, subcutaneously or intra-peritoneally and the water soluble ethanolic extract was given orally. On day 4 the percentage suppression of parasitaemia was calculated in relation to the control. The highest suppression (96%) was observed with the ethanolic extract given orally in a concentration of 74 mg/kg; even 37 mg/kg led to a suppression of 80% [ZAFAR 1990].

Essential oil:

Freshly extracted essential oil from air-dried leaves of *A. absinthium* in a 1:1000 dilution showed antibacterial activity against *S. aureus*, a penicillin resistant strain of *S. aureus* (H57), *K. pneumoniae* and *P. aeroginosa*. No activity was observed against *S. thyphi*, *E. coli*, *C. albicans*, *C. utilis* or *A. niger* [KAUL et al.1976].

The essential oil of French *A. absinthium* showed antimicrobial activity against *C. albicans* and *S. cerevisiae* var. *chevaleri* (growth inhibitor concentration for 50% of the microorganisms: 0.1 and 0.05 mg/ml, respectively), while no activity could be found against *E. coli*, *S. aureus* and *E. hirae* [JUTEAU et al. 2003]. The essential oil of a carvone-rich chemotype of *A. absinthium* had no microbial activity against *S. aureus* [KARWOWSKA et al. 1997].
The anti-listerial activity of the essential oil from *A. absinthium* was studied and the minimal inhibitory concentration was given with 1:1280 [FIROUZI et al. 1998].
Dried plant samples of *A. absinthium* were extracted with $CHCl_3$ and tested for their antifungal (hyphal growth inhibition) and antibacterial (disk diffusion method) activity. The dose of 20 µl essential oil was found to be fungicide against the tested 34 agricultural pathogenic fungal species. The essential oil showed only a weak antibacterial activity against 13 of the 64 tested strains from plant, food and clinical origin (600, 900 and 1200 µg/disk) [KORDALI et al. 2005].

The freshly extracted essential oil from air dried leaves of *A. absinthium* was tested for its insecticidal activity. The essential oil was toxic to house flies in concentrations of 10% (mortality rate 3.3%), 15% (mortality rate 6.6%) and 20% (mortality rate 20%) [KAUL et al. 1978].

The essential oil of *A. absinthium* (hydrodestillation method) was found to be toxic to adults of *Sitophilus granarius* (Coleoptera). The concentration of 9 µl oil/l air caused a mortality rate of 86.7% after 48 h (53.3% and 73.3% after 12 and 24 h, respectively). Chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%) and caryophyllen oxide (4.3%) were the main constituents of the essential oil. The compounds 1,8-cineole (1.5% of the essential oil) and terpinen-4-ol (1.8% of the essential oil), were found to be more toxic against *S. granarius* adults, in comparison to the whole oil [KORDALI et al. 2006, KALEMBA et al. 1993]. In addition it was found that the essential oil was strongly toxic to *Rhizopertha dominca* (lesser grain borer) and mildly toxic to *Tribolium confusum* (darkling grain beetle) [KALEMBA et al. 1993].

Essential oils of *A. absinthium* were extracted by three methods (microwave assisted process, distillation in water and direct steam distillation) and tested for their relative toxicity as contact ascaricides to the two spotted spider mite, *Tetranychus urticae*. The LC_{50} from the oil obtained by direct steam distillation was significantly lower (0.04 mg/cm^2) than that of the microwave assisted process and distillation in water (both 0.13 mg/cm^2). Chromatographic analysis indicated that a sesquiterpene ($C_{15}H_{24}$) present in the direct steam distillation oil (absent in the two other oils) might enhance the toxicity [CHIASSON et al. 2001].

Thujone

The structure of a 3-thujone and its $\Delta^{3,4}$-enol was compared with (-)-Δ^9-THC and it was suggested that the 3-thujone or the $\Delta^{3,4}$ thujone-enol and THC (or their biologically active metabolites) share a common receptor in the CNS [DEL CASTILLO et al. 1975].

It was reported that in the hot-plate test (-)-3-isothujone was found to be codein-like and equipotent with (-)-Δ^9-THC, while (±)-3-isothujone was half as active and (+)-3-thujone was inactive (s.c., mice). Even though an antinociceptive action was observed, it could not be distinguished whether (-)-3-isothujone acts at the same site in the CNS as THC [RICE & WILSON 1976].

Following the suggestion that thujone binds to the cannabinoid receptor it was demonstrated that thujone exhibits only a weak affinity for cannabinoid receptors (CB_1 and CB_2) and fails to elicit typical cannabinoid receptor-mediated responses in rodents at doses as high as 30 mg/kg. The maximum attainable intake of thujone was estimated with 1 mg/ml (calculated for a 70 kg human, 200 ml alcoholic absinthe and a thujone concentration of 2.4 mM in the alcohol solution). Therefore a direct, low-affinity interaction of thujone and related compounds with cannabinoid receptors in the brain as the primary mechanism of action in absinthe intoxication was not considered likely [MESCHLER & HOWLETT 1999].

The mechanisms of α-thujone neurotoxicity in rats, mice and a *Drosophila* strain were investigated. The observations establish that α-thujone is a rapidly acting modulator of the GABA-gated chloride channel. The effect appears to be due to the parent compound, while metabolism leads to detoxification [HÖLD et al. 2000a].

Other compounds

Orally administered absinthin increased the amount of gastric juice and free HCL while this was not observed after gavage administration [KREITMAIR 1951].

A tetramethoxy-hydroxyflavone (p7F) isolated from Korean dried *A. absinthium* was investigated to determine whether it had an inhibitory effect on inflammatory mediators via suppression of NF-κB. The compound did not decrease cell viability of RAW 264.7 cells (macrophages) up to the highest tested concentration of 200 µg/ml. The p7F suppressed the expression of COX-2 and iNOS and the production of NO and PGE_2 in RAW 164.7 cells treated with LPS and it decreased efficiently the LPS-induced NF-κB activation [LEE et al. 2004].

II.2.1.2 Assessor's overall conclusions on pharmacology

It is long known that the bitter constituents stimulate the gustatory nerves in the mouth and increase the secretion of gastric juice and bile, thereby promoting appetite and digestion. Additionally, more recent studies show that taste receptors (bitter taste) could not only be found in the lingual epithelium but also in the gastrointestinal tract of animals [Rozengurt 2006].

These new findings support literature data which describe the use of Absinthii herba - not only in form of the herbal tea or hydroalcoholic preparations, but also in form of the powdered herbal drug - for the relief of mild dyspeptic/gastrointestinal disorders/complaints. The indication 'lack of appetite' should be restricted to fluid preparations only. For this indication, both the tradition of use (more than 30 years) and the plausibility are proven.

Other possible pharmacodynamic actions such as antimicrobial, anthelmintic, antipyretic, analgesic and hepatoprotective properties are also described.

II.2.2 Pharmacokinetics

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

Herbal substance/Herbal Preparations:

No data available.

Thujone:

After oral administration of a mixture of α- and β-thujone (ratio 9:2) at a dose level of about 650-800 mg/kg bw to male rabbits, two neutral urinary metabolites were identified as 3-β-hydroxy-α-thujane and 3-β-hydroxy-β-thujane. This indicated a stereo-specific reduction in spite of the different configurations of the methyl group [ISHIDA et al. 1989].
α-Thujone was rapidly metabolised by mouse liver microsomes forming 7-hydroxy-α-thujone as the major metabolite with five minor products (4-hydroxy-α-thujone, 4-hydroxy-β-thujone, two other hydroxy-thujones and 7,8-dehydro-α-thujone).

Incubation of α-thujone with rabbit (but not mouse) liver cytosol led to the reduction products, thujol and neothujol, in low yield [HÖLD et al. 2000a,b].

Site specificity and species differences in metabolism of the thujone diastereo-isomers were observed in mouse, rat and human liver microsomes and also in rats and mice *in vivo*. 2-hydroxylation was observed only in mice where the conjugated metabolite was a major urinary metabolite. 4-hydroxylation of α- and β-thujones is another major pathway and 4-hydroxy thujone is the major urinary metabolite in rats.

7-hydroxylation is another important pathway of metabolism but the conjugated product is a minor urinary metabolite except for β-thujone in the mouse. Site specificity in glucuronidation favours conjugation of the (2R)-hydroxy- and 4-hydroxythujone glucuronides rather than the other three hydroxy thujones. 7,8- and 4,10-dehydro metabolites have been identified *in vitro* and as urinary metabolites, respectively [HÖLD et al. 2001].

II.2.2.2 Assessor's overall conclusions on pharmacokinetics

Limited data are available on pharmacokinetics. For the herbal substance or the herbal preparation no data are available; therefore no conclusion can be drawn. For thujone and even absinthin more data exist, but these are not transferable to the herbal substance or herbal preparations.
Experimental data from animals indicate that the metabolism of thujone differs strongly in dependence of the animal species. The CYP system (Cytochrome P450) seems to be involved in the metabolic detoxification of thujone.

II.2.3 Toxicology

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

Herbal substance/Herbal preparations:

- single dose toxicity:
  The LD_{50} of an extract of *A. absinthium* (not specified) was given with 1 mg/kg in rats (i.p.) [HAGERROM 2006].

- repeat dose toxicity:
  In a 13-week repeated dose toxicity study Wistar Hannover (GALAS) rats were given water (ad libitum) containing 0, 0.125, 0.5 or 2% extract from *A. absinthium* (not defined). The corresponding amount of extract/day was calculated with 1.27 g/kg/day (males) and 2.06 g/kg/day (females) for the 2% preparation.

  All rats survived the end of the study, and no changes in body weight, haematological parameters and histopathological examinations were observed. In serum biochemical examinations, levels of total protein, albumin, blood urea nitrogen, Na und Cl were slightly but significantly increased in males of the 2% group. Because there were no other changes in other related parameters, these changes were considered to be of no toxicological significance. Relative liver weights were significantly increased in both sexes of the 2% group. However, since there were no increases in their absolute weights and no histopathological treatment related changes were observed in the liver, these changes were not interpreted as toxicological effects. Other effects were not seen [Muto et al. 2003].

  Chronic administration of an essence of Absinthii herba (not specified) caused epilepsy with consecutively stupor in dogs [Kreitmair 1951].

- reproductive and developmental studies:
  Antifertility studies were carried out in Wistar albino rats of proven fertility. Antifertility activity of an ethanolic dry extract (50% EtOH) of leaves of *A. absinthium* was assessed in terms of anti-ovulatory, anti-implantation or abortifacient effects in comparison with vehicle treated controls.

  No effects on ovulation (no influence of the di-oestrus or oestrus phase of cycle) were seen after treatment of female rats for 10 days. Pregnant female rats were treated with the dry extract from days 1 to 7 of pregnancy. On day 10 the numbers of implantation sites in each animal were recorded. Only 2 out of 6 rats became pregnant and the numbers of born pups per rat were reduced

in comparison to the control. Furthermore pregnant female rats were treated with the dry extract from days 11 to 13 of pregnancy. All the animals were examined for vaginal bleeding on days 12 to 16 and on day 20; they were killed and the numbers of live and dead foetuses were noted. *A. absinthium* (200 mg/kg) significantly reduced the sites of implantations (2 out of 6 rats became pregnant) and the numbers of born pups per rat were reduced in comparison to controls [Rao et al. 1987].

There are no data available on reproductive and developmental studies for aqueous preparations of *A. absinthium*.

- genotoxicity/carcinogenicity:
  No studies using herbal preparations of Absinthii herba were located.

<u>essential oil</u>

- single dose toxicity:
  The oral LD_{50} of the essential oil of *A. absinthium* was 0.96 g/kg in rats [Opdyke 1975]. The minimal dosage which caused spasms in cats was 0.03-0.04 ml for the diluted essential oil (1:20 with ethanol) [Kreitmair 1951].
- irritating properties:
  The undiluted essential oil was not irritating on the back of hairless mice and slightly irritating to intact or abraded rabbit skin for 24 h under occlusion [Opdyke 1975].
- sensitization:
  A 2% preparation in petrolatum produced no sensitization reaction in 25 volunteers (maximization test) [Opdyke 1975].
- photoxicity:
  No phototoxic effects were reported for undiluted essential oil on hairless mice and swine [Opdyke 1975].

<u>Thujone</u>

- single dose toxicity:
  The oral LD_{50} of a mixture from α- and β-thujone has been reported with 192 mg/kg in rats, 230 mg/kg in mice and 396 mg/kg in guinea pigs [Margaria 1963]. The LD_{50} (s.c.) value in mice was for α-thujone 134 mg/kg and for β-thujone 442 mg/kg [Rice et al. 1976]. The symptoms associated with acute intoxication are epileptiform convulsions with general vasodilation, hypotension, lower cardiac rhythm and increased respiratory amplitude. In rats, i.p. injections of thujone induced electro-cortical seizures associated with myoclonic activity. Both convulsant and lethal effects occurred at similar doses of 0.2 ml/kg bw [Pinto-Scognamiglio 1967, SCF 2002]. Höld et al. [2000a] reported for α-thujone a LD_{50} value of 45 mg/kg in mice (i.p.).

- repeated dose toxicity:
  Thujone was administered to rats by gavage at doses of 12.5, 25 or 50 mg/kg/day on five days per week for 13 weeks. There was an increased lethality of 60% in females and 37% in males at the top dose level. The NOEL for convulsions in the males was 12.5 mg/kg but no NOEL could be established in females in this study [Surber 1962].

  In a further study, thujone was administered to rats by gavage at doses of 0, 5, 10 or 20 mg/kg/day 6 times per week for 14 weeks. There were 3 deaths in females and 1 in males associated with convulsions at the top dose level. The NOEL for convulsions was reported to be 10 mg/kg in males and 5 mg/kg in females; no changes were reported in haematologic or histo-pathologic examinations [Margaria 1963].

α-Thujone and a mixture of α- and β-thujone have been included in the NTP testing programme. In the 14-day study, α-thujone was administered by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 1, 3, 10, 30 or 100 mg/kg. In mice, mortality was 4/5 males and 5/5 females in the top dose group; mortality was not increased in the lower dose groups. The increased mortality was associated with indications of neurotoxicity (hyperactivity, tremors, tonic seizures). Histological changes observed only at the top dose level included only mild renal tubular dilatation/focal degeneration, increased haematopoiesis in spleen, and bone marrow myeloid cell hyperplasia. No increased mortality occurred in male rats but there was increased mortality (3/5 animals) in females of the top dose group. As in mice, the increased death rate was associated with convulsions/seizures.

In the 14-day study on the mixture of α- and β-thujone (detailed composition not available), similar doses were administered by gavage to mice and rats of the same strains. In mice, at the top dose level, there was an increased mortality in males (5/5) and females (2/5), which was not associated with any notable gross or histopathological causation. In rats, there was death of 1/5 males in the highest dose group but gross and histological effects were minimal [SCF 2002].

- genotoxicity

  Negative results have been reported from *in vitro* mutagenicity tests in *Salmonella typhimurium* with α-thujone. The test was performed with the strains TA1535, TA100, TA97 and TA98 with and without metabolic activation. First toxicity signs were seen in dose units of 100. Negative results have also been reported from *in vitro* mutagenicity tests in *Salmonella typhimurium* with a mixture of α- and β-thujone. The test was performed with the strains TA1535, TA100, TA97 and TA98 with and without metabolic activation. First toxicity signs were observed in dose units of 333.

  In-vivo mouse micronucleus test was negative in males and positive in females with a mixture of α- and β-thujone. The test concentration range was 0-25 mg/kg (males) and 0-50 mg/kg (females), respectively [NTP 2003].

  Thujone was tested at 1.5 and 3% in DMSO for its effect on the mutagenicity of aflatoxin B1 in the *Salmonella typhimurium* strain TA100. The plates treated with thujone showed evidence of colony damage which indicates some mutagenic activity on the part of thujone (Kim et al. 1992).

- special studies on mechanisms of toxicity

  Several studies on the mechanism of neurotoxicity of α-thujone suggest a modulation of the GABA Type A receptor. It is a rapidly acting modulator of the GABA-gated chloride channel. The effects appear to be due to the parent compound and metabolism leads to detoxification [Meschler & Howlett 1999, Höld et al. 2000a].

- other biological effects

  Studies on primary cultures of chick embryo liver cells indicate that thujone is porphyrogenic, leading to accumulation of copro- and protoporphyrins. It induces 5-aminolaevulinic acid synthase in this test system [Bonkovsky et al. 1992].

II.2.3.2 Assessor's overall conclusions on toxicology

There are only limited preclinical safety data for Absinthii herba or preparations thereof.

Also for the essential oil the data are unsatisfactory. The limited toxicological data on thujone and the quality of the available studies, mainly published in the 1960s, were considered to be insufficient to set an maximum daily intake (MDI).
The results of the single dose toxicity studies show that the LD_{50}-data depend strongly on the way of application and the animal species.
Thujone has a neurotoxic potential and produces convulsions in animals by acting on the central nervous system. There are several anecdotal and case study reports on the acute effects of thujone-containing essential oils causing seizures in humans, which indicate that the animal data may be relevant to humans. However, for the essential oil or thujone, there are no reliable studies on the long-term effects of sub-convulsive doses on the nervous system and on the liver. Long-term toxicity studies, carcinogenicity studies and reproductive studies are missing for preparations of Absinthii herba, the essential oil and thujone, respectively. In the latter context, it has been suggested that porphyria may be a consequence of long-term ingestion of absinthe but this is conjectural [SCF 2002].
Thujone has been evaluated by the Council of Europe which allocated a temporary maximum daily intake (TMDI) of 10 µg/kg/d based on a NOEL for convulsions of 5 mg/kg in the female rat, dosed by gavage on 6 days per week for 14 weeks, to which a safety factor of 500 was applied [Council of Europe 2007]. The "temporary acceptable daily intake" is in this context a value for the acceptable daily intake proposed for guidance when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but are insufficient to conclude that the use of the substance is safe over a lifetime. A higher-than-normal safety factor is used when establishing a TMDI. When transferring this TMDI to herbal medicinal products, the daily dosage of thujone should not exceed 600 µg (= 0.6 mg) for a 60 kg human.
According to the Directive 88/388/EEC 1988 a maximum thujone level of 5 mg/kg in alcoholic beverages with not more than 25% volume of alcohol and of 35 mg/kg in alcohol labelled as bitters (40% volume of alcohol and more) are allowed. Taking into consideration a daily intake of 4-8 cl (40-80 ml) this amount corresponds to approximately 0.2-0.4 mg thujone per person (in 25% ethanol v/v) and 1.2-2.4 mg thujone per person (in bitters), respectively. This is without any restrictions for the duration of use.
The maximum daily intake (acceptable daily intake for food) is a measure of the amount of a specific substance that can be ingested (orally) over a lifetime without an appreciable health risk. For a short-term intake, the limits of thujone may therefore be less restrictive.
Dettling et al. [2004] had shown that ~15 mg thujone/person (60 kg) lead to changes in attention performances, while for the intake of ~1.5 mg thujone/person no such changes were described even though expected. The authors proposed for safety reasons a limit of 1.5 mg/person in a single dose, were effects could barely be seen.
At the time of this assessment a daily intake of 3.0 mg/person is considered acceptable for a maximum duration of use of 2 weeks. This is due to the fact, that this daily dose is supposed to be taken divided into three single doses. Therefore the single dose (1 mg thujone) is only 2/3 of the amount which was for safety concerns postulated as the lowest described limit of thujone action. The content of thujone must be shown for every batch.
The data from reproductive studies suggest that Absinthii herba might influence gravidity. Moreover it was proven that thujone stimulates the uterus [BIELENBERG 2002]. Therefore ethanolic preparations of Absinthii herba with high content of thujone should be contraindicated during pregnancy and lactation while aqueous preparations or preparations with a low content of thujone are not recommended during pregnancy and lactation.
Due to the lack of data on mutagenicity, carcinogenicity and reproductive and developmental toxicity, a list entry for Absinthii herba can not be recommended.

II.3 CLINICAL DATA

II.3.1 Clinical Pharmacology

II.3.1.1 Pharmacodynamics

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

Four healthy test persons (female, age 19-37 years) drank 100 ml of a test solution, which contained an ethanolic (EtOH 70%) preparation of *A. absinthium* (corresponds to 0.05 g Absinthii herba) within a time frame of 5 min. The amount of saliva was measured before and during the drinking. In the collected saliva the activity of amylase and the amount of hexosamine was measured. The Absinthii herba preparation caused in all 4 persons an increase in salivation of more than 100%, while the activity of amylase and the amount of hexosamine were not influenced or decreased. A non-spiced rice dish caused the same increase. A combination of a non-spiced rice-dish with the Absinthii herba preparation increased the amount of saliva in an additive manner. Furthermore the authors postulate, also in reference to other publications, that the action of bitters is more likely to be associated with the gastric juice secretion. They claim that bitters may act indirectly (due to sensations of the oral cavity) as activators of the secretion and inhibitors of the motor activity and directly by inducing hyperemia [Blumberger & Glatzel 1966].

In a clinical study, a dry ethanolic preparation of *A. absinthium* (not specified) was administered to 15 patients with hepatic disorders via a duodenal tube. First, the basal secretion was measured for 10 min before sample administration. In this way also the basis secretion of 22 healthy volunteers was measured for comparison. The following parameters were measured: volume of duodenal secretion, amount of bilirubin, cholesterol, lipase and α-amylase. All parameters were decreased in the patients with hepatopathy. After the preparation was administered, the duodenal secretion was measured for 100 min (fractionated in 10 x 10 min).
All parameters were significantly increased when the preparation was given. The stimulation of secretion of lipase (+163-647%), bilirubin (+55-170%) and cholesterol (+35-101%) was higher than the increase of α-amylase (+22-72%). The increased secretion of bilirubin and cholesterol, lipase and α-amylase was long lasting [Baumann 1975].
In a further clinical study, 2.5 mg of a dry ethanolic preparation of *A. absinthium* (not specified) and 10-20 mg of a thujone-free powder of *A. absinthium* were administered as aqueous solution to 14 (7/7) healthy test persons via a duodenal tube. The placebo group (8 test persons) received water. First the basal secretion was measured for 10 min. After administration of preparations/placebo the duodenal secretion was measured for 40 min (placebo), 100 min (preparation with thujone) and 120 min (preparation without thujone) in 10 min. intervals. The following parameters were measured: volume of duodenal secretion, amount of bilirubin, cholesterol, α-amylase and lipase. All parameters were significantly increased as compared to placebo. The thujone-free preparations had similar of even stronger effects than the thujone-containing preparation [BAUMANN et al. 1975].
In a double-blind, randomized clinical study an ethanolic preparation of *A. absinthium* (58.9% m/m) was administered to 20 healthy test persons (age between 18-35 years) via a duodenal tube. An ethanolic solution (58.9% m/m) was used as placebo. The basal secretion was measured twice for 10 min before 3 ml verum (100 g verum correspond to 0.65 g *A. absinthium*; and ~ 0.02 g Absinthii herba) or 3 ml placebo were administered. Five min after application the biliary secretion was measured for 60 min (fractionated in 6 x 10 min). Thereafter, the other preparation was given to the test persons following the same procedure. The parameters measured were: volume of biliary secretion, amount of protein, trypsin, chymotrypsine, α-amylase, and lipase.
All parameters were increased (10-20%) after verum application, but due to the variation the changes were not significant. The highest increase was 10-20 min after application [JAGUSCH 1988].
In a double-blind, randomized clinical study an ethanolic preparation of *A. absinthium* (58.9% m/m) was administered to 10 healthy test persons (age between 23-35 years) via duodenal tube. The procedure was according to Jagusch 1988. The following parameters were measured: volume and amount of bilirubin, total cholesterol, HDL-cholesterol, bile acids and alkaline substances.

The parameters volume, bilirubin, total cholesterol and HDL-cholesterol were increased after verum application but due to the variation the changes were not significant. The highest increase was 10-20 min after application. No increase was observed for the amount of bile acids and alkaline substances [KISTLER 1988].

II.3.1.1.2 Assessor's overall conclusions on pharmacodynamics

Ethanolic preparations from *A. absinthium* are able to stimulate gastric, intestinal and biliary secretion probably due to the content of bitter substances and essential oil. The essential oil acts antispasmodic in small amounts. In high dosages or after longer-lasting intake the essential oil acts as a convulsant poison.
Earlier hypotheses claimed that bitter tasting substances evoke secretory reflexes of the gastrointestinal tract via taste receptors in the lingual epithelium. The current notion is that additionally taste receptors are expressed in the gastrointestinal mucosa. It is postulated that activation of bitter taste receptors generate integrated responses as secretion, motility or absorption [STERNINI 2007].
In the studies of Jagusch 1988 and Kistler 1988 a dosage (0.02 g herbal substance) was used which is a hundred times below the dosage described for medicinal use. This might explain why the observed moderate effects were not significant.
The long-standing use of aqueous and ethanolic preparations of *Absinthii herba*, pharmacological studies and current findings of physiological properties justify the use of *Absinthii herba* for the treatment of loss of appetite and for the symptomatic treatment of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.

II.3.1.2 Pharmacokinetics

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

Within a drinking test two subjects (65 kg body weight) consumed 110 ml absinth with 3.85 mg thujone (content of absinth 35 mg/l) within 15 min. Blood samples were drawn 15, 30, 60, 90, and 120 min after drinking. The determination of the blood alcohol level was applied via head-space GC and the blood thujone content was determined via head-space solid-phase micro-extraction (HS-SPME) method.
Blood alcohol concentrations >1 g/l were determined, whereas thujone could not be detected in blood samples (detection limit 0.34 ng/ml). Conjugates of thujone were not determined. The two subjects showed typical signs of alcoholisation (e.g. staggering, chattiness) while hallucinogenic effects were not described by the two subjects [Kröner et al. 2005].

II.3.1.2.2 Assessor's overall conclusions on pharmacokinetics

According to the study by Kroener at al. (2005) in both subjects no free thujone could be detected in the blood samples. First measurements were taken 15 and 30 min after beginning of the drinking. It was not clear if other time frames could have shown other results. It remains unclear whether the measurement of unmetabolized thujone in blood is possible at all, because no data are known with regard to the first-pass effect in humans. Because of the lipophilic properties of thujone it is doubtful that noteworthy amounts of thujone can be detected in human blood samples.
Due to lack of comprehensive data no conclusions can be drawn.

II.3.2 Clinical Efficacy[6]

II.3.2.1 Dose response studies

II.3.2.2 Clinical studies (case studies and clinical trials)

In a multi-centre, randomized, double-blind trial 40 patients suffering from Crohn's disease receiving a stable dose of steroids at an equivalent of 40 mg or less of prednisone for at least 3 weeks were administered a product containing *A. absinthium* (3 x 500 mg/day) or a placebo for 10 weeks. Steroids, 5-aminosaliciyates (if dose remained constant for at least 4 weeks prior to entering the trial) and/or azathioprine (stable dose for at least 8 weeks) or methotrexate (stable for at least 6 weeks) were permitted as concomitant medications. The recruited patients were evaluated with the help of a Crohn's Disease Activity Index (CDAI) questionnaire, an Inflammatory Bowel Disease Questionnaire (IBDQ), the 21-item Hamilton Depression Scale (HAMD) and an 8-item Visual Analogue Scale (VA-Scale) in 2-week intervals during the first 10 study weeks, and then at week 12, 16 and 20, which were the medication free observation periods. The initial stable dose of steroids was maintained until week 2, after that a defined tapering schedule was started so that at the start of week 10 all the patients were free of steroids. At the end of week 10 the trial medication was also discontinued. The concomitant medications were maintained at the same dose levels till the end of the observation period that was the end of week 20.

The capsules contained powdered *A. absinthium* herb and powdered rose-petals, cardamom seeds and mastic resin while the placebo contained only powdered rose-petals, cardamom seeds and mastic resin. The powdered *A. absinthium* used for the product contained 0.2-0.38% absinthin and 0.25-1.52% essential oil, depending upon the batch.

The patients were 21-75 years old and suffered from Crohn's Disease 2.7-14.2 years (Crohn's Disease verified by coloscopy and histology). The median CDAI was 240-321 in the *A. absinthium* group and 238-317 in the placebo group, while median IBDQ was 110-152 in the *A. absinthium* group and 123-147 in the placebo group. Patients with serious pathological findings in ECG, liver, kidney and heart functions, or coexisting organic diseases such as history of cancer, asthma or other autoimmune disease requiring steroid treatments, were excluded from the trial.

Response to treatment was defined as a decrease in the CDAI score of at least 70 points from the qualifying score, or a decrease in 30% of CDAI score from the baseline score. For the HAMD total score, the primary outcome measure was the absolute decrease of the Hamilton total depression score between baseline and the following treatment weeks. Response was defined as a decrease in total score of >50% from baseline and remission as a score <10 points.

After week 2 in the placebo group 16 patients (80%) showed CD exacerbation due to reduction in steroid dose, whereas there were only two (10%) such patients in the group receiving *A. absinthium*. The exacerbation of CD symptoms necessitated the re-start of steroids in 11 patients in the placebo and 2 from the *A. absinthium* group.

At week 10 13 patients (65%) of the verum group were almost free of CD symptoms and there was no need to restart the steroid treatment in the follow-up weeks. Five patients from this group tolerated the reduction of the steroids. Their CDAI scores remained almost unchanged during the first 10 weeks, but they gradually improved in the following 10 weeks.

Nine patients of the placebo group tolerated the reduction of the steroids (unchanged CDAI score). After 6 weeks the number of patients who showed clinical improvement were significantly higher in the verum group as compared to the placebo group. This significant difference continued beyond week 10.

There was almost no change in the subjective feelings of illness in self-assessment (VA-scale) of the patients in the placebo group, whereas in the *A. absinthium* group the self-assessment evaluation of the patients indicated significant improvement. HAMD total scores, decreased by an average of 9.8 (SD 5.8) points for the verum group and by 3.4 (SD 6.6) points for the placebo group. At the end of the acute treatment phase (week 10) 70% of the patients of the *A. absinthium* group and none in the placebo group showed remission of depressive symptoms. The authors assumed that the efficacy might be due to the

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

anti-DNA virus properties, but also an immune system modulation caused by *A. absinthium* was considered. However, only one subgroup showed response to *A. absinthium*. It was striking that A. absinthium had also significant effects on the quality of life and mood [Omer et al. 2007].
Tahir et al. tested *A. absinthium* (powdered) in 20 patients against amoebiasis. All patients had symptoms and signs of amoebiasis i.e., abdominal pain and tenderness, loose motion mucous in stool often mixed with blood and tenesmus and Entamoeba histolytica was diagnosed in stool under the microscope. Patients suffering from other gastrointestinal, cardiovascular, respiratory, endocrinal, nervous system and sexually transmitted diseases were excluded from the study.
The powdered herbal substance was administered in the form of a capsule (500 mg). Three capsules every six hours were given to the patients for a period of fifteen days. The efficacy was assessed weekly in terms of improvement, in termination of the symptoms and signs of disease without the aid of any other drug for the management of this condition. The patients were between 14-65 years old (16 males, 4 females).
After treatment with 6 g *A. absinthium*/day for 15 days the following results were achieved:
All the patients had abdominal pain and loose motions at the beginning of the treatment. Regarding abdominal pain the relief in symptoms was recorded as complete in 13 patients and partial in 6 patients, while the relief in loose motions was noted in 14 (total) and 4 (partly) patients. Seventeen patients had abdominal tenderness before treatment and relief was noted in 11 (total) and 4 (partly) patients. Blood stained stool was present in 7 patients before treatment and relief was seen in 6 patients (total), while mucous stained stool was present in 10 patients before treatment and relief was seen in 8 (total) and 1 (partly) patients. Blood with mucous stained stool was present in 3 patients and in 2 patients a total relief was achieved. Tenesmus was seen in 13 patients at the beginning and a relief was recorded for 8 (total) and 2 (partly) patients). The average relief in all symptoms was noted in 84.66% of the cases, while 15.34% of the cases showed negligible or no relief. This reflects the fact that amoeba in stool disappeared in 70% of cases after 15 days treatment.
The authors report that the amoebicidal, anti-spasmodic, anti-inflammatory, analgesic and astringent properties of *A. absinthium* described in literature might be responsible for the effects observed [TAHIR et al. 1997].

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

None reported.

II.3.2.4 Traditional use

Artemisia absinthium has a long-standing traditional use for various indications.
Already the Egyptians used *A. absinthium* (or a closely related species) as an antiseptic, a stimulant and tonic, and as a remedy for fevers and menstrual pains (Ebers Papyrus). Hippocrates recommended Absinthii herba as a cure for jaundice. Pliny's "Historica Naturalis" describes the extract of Absinthii herba as having a long-standing benefit against gastro-intestinal worms and in Dioscorides' "De Materia Medica" it was also fully described. In the Middle Ages, the plant was used to exterminate tapeworm infestations while leaving the human host uninjured. Paracelsus considered it as a stomachic, anthelminthic herb which also acts as prophylaxis against sea-sickness. *A. absinthium* has been known to aid digestion, and as an effective treatment for upset stomach. Dioscorides, Galen and Hildegard von Bingen highlight the treatment of gastro-intestinal complaints with it [MADAUS 1976, ARNOLD 1989, HOSE 2002].
During the last decades *A. absinthium* is used as "amarum aromaticum" to promote the appetite in cases of gastritis, hypoacidity, and dyspepsia. The plant is also described as a choleretic [BHP 83, HAGERROM 2006, MARTINDALE 1989]. An ethnopharmacobotanical study (1977-2000) confirmed that *A. absinthium* is used in Central Italy for the treatment of lack of appetite [GUARRERA 2005]. A survey among Hakims in Pakistan showed that *A. absinthium* is used against liver diseases, hepatitis, blood purification, jaundice, diabetes, skin diseases, allergy, scabies, tetanus and as brain tonic [QURESHI et al. 2002]. In Russia,

Lithuania, Poland and America Absinthii herba was used in folk medicine against dyspeptic complaints [MADAUS 1976].
The dried comminuted herbal substance has been described in Pharmacopoeias and Pharmacognosy handbooks for decades, while the tincture was described in various German Pharmacopoeias [GERMAN PHARMACOPOEIA 1872, AB-DDR 1975] and handbooks [DAC 2007, HAFFNER & SCHULTZ 1979, HAGERROM 2006, MADAUS 1976, SCHULZ & HÄNSEL 2004; TEUSCHER 1989]. Authorized products with expressed juice of *A. absinthium* are currently on the German market.
The use for more than 30 years could be proven for

- comminuted herbal substance in tablets 3 times daily 760 mg herbal substance (2.28 g herbal substance daily) for the treatment of dyspeptic complaints such as minor gastrointestinal spasms, repletion and flatulence
- comminuted herbal substance for tea preparation 2-3 times daily 1 g (2-3 g herbal substance daily) for the treatment of loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion and flatulence and functional disorders of the biliary tract (as appetizer 30 min before meals, all other indications 1 cup of tea after meals)
- expressed juice (1:0.5-0.9) 2 times daily 5 ml for the treatment of loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion and flatulence
- tincture (1:5); extraction solvent ethanol 70% (v/v) 3 times daily, each single dosage equivalent to 1 g herbal substance to improve appetite and stimulate a digestion in cases of hypoacidity and chronic gastritis [SCHULZE & HÄNSEL 2004; SCHMID & SCHULTZ 1979].

II.3.2.5 Assessor's overall conclusions on clinical efficacy

Although *A. absinthium* has been used to treat loss of appetite, indigestion, biliary disorders, and other gastrointestinal problems, clinical data supporting these uses are lacking. Also for the described use as an antiparasitic and anthelmintic agent, there are no published studies evaluating the efficacy for these indications in humans.
The pharmacodynamic properties (clinical data) support the use of *A. absinthium* for the treatment of loss of appetite and for the symptomatic treatment of dyspepsia and mild spasmodic disorders of the gastrointestinal tract. This is also confirmed by results of another typical bitter drug (*Gentiana lutea*). *G. lutea* was tested as a dry extract in isolated rat stomach cells and in a multicentre uncontrolled study (205 patients). A concentration dependent rise in gastric acid production was observed in rat cells, while in patients a rapid and dramatic relief of symptoms (constipation, flatulence, appetite loss, vomiting, heartburn, abdominal pain, nausea) was achieved [GEBHARDT 1997, WEGENER 1998]. Such findings could explain why bitters, also when encapsulated, show therapeutic effects; suggesting that the reflex effect via lingual taste receptors is not the only mechanism of action.
Based on the pharmacological and clinical data and the long-standing use for more than 30 years the following indications are plausible. The wording of the indications was adjusted to other herbal substances/herbal preparations with similar effects after discussion in the MLWP:

a) Traditional herbal medicinal product used in temporary loss of appetite

b) Traditional herbal medicinal product used for mild dyspeptic/gastrointestinal disorders

According to the discussion concerning the safety of preparations of *A. absinthium* with respect to their content of thujone, following preparations and related posologies were agreed on:

a) comminuted herbal substance for tea preparation: 2-3 g herbal substance daily
expressed juice (1:0.5-0.9): 10 ml daily
to be taken 30 min before meals

b) comminuted herbal substance in tablets: 2.28 g herbal substance daily
comminuted herbal substance for tea preparation: 2-3 g herbal substance daily

expressed juice (1:0.5-0.9): 10 ml daily
tincture (1:5); extraction solvent: ethanol 70% (v/v): approx. 2-3 g herbal substance daily
to be taken after meals

The intake of thujone should not exceed 3.0 mg/day and the duration of intake is restricted to a maximum of 2 weeks. Chemotypes of *A. absinthium* with low content of thujone should be preferred.
The published study on the possible reduction in steroid dosages in Crohn's disease after *A. absinthium* intake is well conducted. However, well-established use is defined as well-documented use for a time frame of minimum 10 years, which is not fulfilled. Furthermore the study of Tahir et al. does not fulfill, besides other deficiencies, the requirements of a well-established use. Therefore a well-established use indication for Absinthii herba can not be supported.

II.3.3 Clinical Safety/Pharmacovigilance

II.3.3.1 Patient exposure

II.3.3.2 Adverse events

A 61 year-old woman was treated with a herbal medicinal product containing 5 plant extracts, including one preparation from *A. absinthium*. Some of the other plants of the combination product belong also to the Asteraceae (Compositae) familiy. After the intake, she complained of soreness and burning of the oral mucosa and tongue. Slight erythema could be seen in the oral cavity and on the tongue. Blood count and differential were normal. Patch test with the plant extracts of the herbal medicinal product showed positive reactions to the extract of *A. absinthium* as well as to all the other extracts [BAYERL & JUNG 1996].
Undesirable effects for aqueous preparations from the herbal substance are not reported. In the literature it is described, that contact with the flowers can provoke a scarlatina-like redness of the skin in very rare cases [HAUSEN & VIELUF 1997].

II.3.3.3 Serious adverse events and deaths

A 31 year-old man drank 10 ml of the essential oil incidentally. He was found in an agitated, incoherent and disoriented state. Later on tonic and clonic seizures with decorticate posturing were observed. On the second day the patient developed moderately intense, bilateral soreness of the leg muscles, followed by congestive heart failure. Among other changes he developed hyper-natriaemia, hypokalaemia and hypo-bicarbonataemia. By day 17 (after treatment which included diuretics and sodium restriction) biochemical abnormalities and blood chemistry had returned to normal [WEISBORD ET AL. 1997].

II.3.3.4 Laboratory findings

No data available.

II.3.3.5 Safety in special populations and situations

Dettling et al. [2004] investigated if the impact of thujone (absinthe) on attention performance and mood differs from the one experienced with beverages that contain only alcohol. 22 healthy subjects were tested using an attention performance test, which was developed for aptitude diagnostics in the area of performance and which is applied in the diagnostics of alcohol and drug-induced effects on visual orientation performance. Mood was assessed using two questionnaires that test different mood

dimensions: one (Masel Mood test) records the factors vitality, intra-psychic equilibrium, social extraversion and attentiveness, the other one (general activation –high activation state scale) records state anxiety and current subjective activation.
The calculated total amount of thujone consumed was 0.28 mg/kg and 0.028 mg/kg for men and 0.24 mg/kg and 0.024 mg/kg for women. The alcohol content was adjusted to 16 g/l in all beverages. The amount of liquid to be consumed depended on the weight of the subject. It was tried to attain a maximum blood alcohol concentration of 0.05% (= 0.5‰) for each subject. Before drinking every subject received a small standard meal; the beverage had to be drunk then within 10 min. All tests were performed before drinking (T0) and 30 (T1) and 90 min (T2) after drinking.
The results between T0-T1 and T0-T2 revealed no significant alterations in attention performance after the consumption of alcohol and low thujone concentration. When the subjects were under the influence of the high thujone concentration, the number of correct reactions in the peripheral field of attention decreased significantly and the reaction time in both the peripheral and central fields of attention increased significantly between T0-T1. Furthermore the number of "false alarm" reactions also increased. The changes in performance after 90 min (T2) revealed results that show a pattern similar to the results after 30 min but less pronounced (not significant). No significant differences in attention performance between the three treatments could be found either from T0-T1 or from T0-T2 (ANOVA t-test). It was assumed that the effects of the high thujone condition are quantitative but not qualitative. One possible explanation was the theory that the effects of alcohol on attention processing may be an inverted U-shaped function with thujone shifting the dose-effect to the left. The missing alteration in attention performance, at low thujone concentrations was explained by alcohol antagonizing the effect of thujone.
While within the treatment groups the mood state changed either from T0-T1 or from T0-T2, no significant differences (Friedman rank variance analyses) in the alteration of the tested mood dimensions could be found when comparing the treatment groups with each other. The most prominent difference was observed for the parameter "state anxiety". High thujone concentrations led to a decrease in state anxiety at T2. This effect was explained with the interaction of thujone with the GABA-receptor. The antagonistic effect of thujone on the GABA-receptor lead to an increase in fear sensations and also a stimulating and rousing effect, while ethanol acts as a GABA-enhancer (anxiolytic, sedative and amnesic).

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

No data available. Use in children and adolescents under 18 years of age is not recommended because data are not sufficient and medical advice should be sought.

II.3.3.5.2 Drug interactions

No data available.
The action of thujone is explained in the literature by its binding to the GABA receptor. The intake of Absinthii herba preparations might therefore influence the effect of medicinal products acting via the GABA receptor, even if such effects were not seen clinically.

II.3.3.5.3 Use in pregnancy and lactation

No data available. Most sources recommend contraindication in pregnancy and lactation due to the uterus stimulating effects of thujone.

Tests on reproductive toxicity have only been performed with a dry ethanolic extract of *A. absinthium* orally administered to pregnant rats. Results showed significantly reduced sites of implantations and a reduction in the numbers of born pups per rat.
Safety during pregnancy and lactation has not been established. Because of the amounts of thujone in the preparations covered by the assessment report, the proposed contraindication was changed into the sentence "The use should be avoided during pregnancy and lactation." after discussion in MLWP and HMPC.

II.3.3.5.4 Overdose

Limited data are available for Absinthii herba. After intake of a concentrated infusion of Absinthii herba a male developed dizziness, atony, tremor of the legs, lasting uresiaesthesia and burning in the glans penis [LEWIN 1929] and it is stated that excessive doses of Absinthii herba preparations may cause vomiting, severe diarrhea, retention of urine or dazed feelings [ROTH ET AL. 1994]. On the other hand it is stated that after excessive or long lasting intake of Absinthii herba preparations aversions against the intake may develop. Therefore, acute or chronic intoxications due to Absinthii herba preparations are not suspected [HÄNSEL & STICHER 2007].
Overdosage of alcoholic Absinthii herba preparations or the use of the essential oil may cause CNS disturbances which can lead to convulsions and ultimately to unconsciousness and death [GESSNER 1974; ROTH ET AL. 1994].
Cases with severe intoxications in humans have been reported after consumption of essential oil rich in thujone [CENTINI & LAURINI 1987, MILETT ET AL. 1981]. Convulsions resembling epilepsy have been reported after the ingestion of isolated thujone [COBB 1922].
There are no cases of overdose reported concerning herbal tea preparations.

II.3.3.5.5 Drug abuse

No data available.

II.3.3.5.6 Withdrawal and rebound

No data available.

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

No data available.
Attention performance [DETTLING ET AL. 2004] was changed under the influence of high thujone concentrations. For safety reasons affected patients should not drive or operate machinery after intake of Absinthii herba preparations.

II.3.3.6 Assessor's overall conclusions on clinical safety

Limited data are available. From the study of Dettling et al. [2004] it can be assumed that at least a concentration of 0.24-0.28 mg thujone/kg might lead to changes in attention performances and mood conditions (even if not significant). For a 60 kg adult this corresponds to a single dose of 14.4-16.8 mg thujone per person. For the concentration of 1.44-1.68 mg per person an effect was postulated but not proven.

The use in children and adolescents is not recommended. The use of Absinthii herba during pregnancy or lactation should be avoided. Absinthii herba should not be used in cases of obstruction of the bile duct, cholangitis, or liver disease. Medical advice is needed in cases of gall stones or other biliary disorders. The intake of Absinthii herba preparations might influence the effect of medicinal products acting via the GABA receptor. After intake of preparations from Absinthii herba, patients should not drive or operate machinery for safety reasons. Duration of use should be limited to a maximum of 2 weeks.

II.4 ASSESSOR'S OVERALL CONCLUSIONS

Absinthii herba is well known and derived traditional herbal medicinal products have been used for centuries in European countries. Sufficient data are available to develop a Community monograph on the traditional use of *Artemisia absinthium* L., herba - provided the indications are suitable for self-medication. The proposed indications are:

a) Traditional herbal medicinal product use in temporary loss of appetite

b) Traditional herbal medicinal product used for mild dyspeptic/gastrointestinal disorders

The pharmacological studies *in vitro* and *in vivo* showed stimulating effects on the gastric, intestinal and biliary secretion.
The intake of thujone should not exceed 3.0 mg/day and the duration of use should be limited to 2 weeks. Chemotypes of *A. absinthium* with low content of thujone should be preferred.
Use of Absinthii herba should be avoided during pregnancy and lactation and Absinthii herba should not be taken in children and adolescent under 18 years of age and in patients with obstruction of the bile duct, cholangitis or liver disease. The intake of Absinthii herba preparations might influence the effect of medicinal products acting via the GABA receptor. For safety reasons, patients should not drive or operate machinery after intake of preparations from Absinthii herba.
Because the minimum required data on mutagenicity (Ames test) are not available for herbal preparations of Absinthii herba, an inclusion to the Community list of traditional herbal substances and preparations is not recommended.

III. ANNEXES

III.1 COMMUNITY HERBAL MONOGRAPH ON *ARTEMISIA ABSINTHIUM* L., HERBA

III.2 LITERATURE REFERENCES