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Committee on Herbal Medicinal Products (HMPC)

Assessment report on Artemisia absinthium L., herba

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Artemisia absinthium* L., herba
Herbal preparation(s)	Comminuted herbal substance Powdered herbal substance Expressed juice from the fresh herb (1: 0.5-0.9) Tincture (1:5), extraction solvent ethanol 70% V/V
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea or tablets for oral use. Herbal preparation in solid or liquid dosage forms for oral use.
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1. Introduction

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

- Herbal substance(s)

Absinthii herba (European Pharmacopoeia Monograph ref.: 1380).

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).

Constituents: (Blaschek *et al*., 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; Blaschek *et al*., 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).

"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 (Ariño *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) (Ariño *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; Ariño *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) (Blaschek *et al*., 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 (Blaschek *et al*., 2006; Hänsel & Sticher, 2007; Tosi *et al*., 1991; Canadanovic-Brunet *et al*., 2005).

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 hours; room temperature)	Purified cold water	Not detected
	Ethanol 30% (V/V)	Not detected
	Ethanol 90% (V/V)	75.0%
Digestion (30 minutes; 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 minutes. For fennel fruits anethole recovery rates of 25-35% were found after 10 minutes. 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)

i) comminuted herbal substance

ii) powdered herbal substance

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

iv) tincture (1:5); extraction solvent: ethanol 70% (V/V)

- 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.

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 Foeniculi fructus exhibit bitter and/or aromatic properties and are usually used for dyspeptic or choleretic complaints.

This monograph refers exclusively to Absinthii herba.

1.2. Search and assessment methodology

Available literature on *Artemisia absinthium* (Absinthii herba) at the "Spanish Agency of Medicinal Products", European Pharmacopeia and the incoming, on the "call for scientific data for use in HMPC assessment work on *Artemisia absinthium* L., herba", was used for a literature search. For most current publications a literature search in the DIMDI-database XMEDALL, Derwent Drug File (DD83), AMED (CB85), IPA (IA70), Biosis Previews (BA70), Medline (ME00), Embase (EM00) was performed on October-November 2016 for the scientific information update. Articles were filtered by using the following terms: *Artemisia absinthium*, Absinthii herba, wormwood, Language in English, German, French or Spanish and concerning humans and pharmacological *in vitro* and *in vivo* studies.

Only articles found to be relevant for assessment are included in the list of references.

Search engines used: Google, Google Scholar

Scientific databases: Medline, PubMed, EMBASE, BioMed Central

Other resources: Library of the Faculty of Pharmacy of Ljubljana

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

The following herbal substances and herbal preparations have been on the European market for a period of 30 years.

a) comminuted herbal substance

b) powdered herbal substance

c) expressed juice (1:0.5-0.9)

d) 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 for tea preparation**

Poland Indication: lack of appetite, dyspepsia.

Posology: oral use, 1.0 g 2-3 times daily.

Spain Indication: appetiser (loss of appetite); dyspepsia.

Posology: oral use, 2-3 cups/daily.

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 appetiser: 30 minutes before meals; all other indications 1 cup of tea after meals).

- **Powdered herbal substance**

Indication: 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.

- **Expressed juice from fresh herb (1:0.5-0.9)**

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

Posology: 2 times 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 times daily, single dose amount corresponding to 1 g herbal substance.

Single dose: corresponding to approximately 1 g herbal substance.

Daily dose: corresponding to approximately 3 g herbal substance.

Information on medicinal products marketed in the EU/EEA

Table 2: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
Powder herbal substance	Symptomatic treatment of dyspeptic complaints such as minor gastro-intestinal spasms, repletion and flatulence	Coated tablets 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	Germany (withdrawn from the market in 2008) Since 1976 WEU

Comminuted herbal substance	Lack of appetite, dyspepsia Loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence, spasmodic	Herbal tea Poland: 1.0 g 2-3 times daily Spain: 2-3 cups daily Germany: 1.5 g 2 times daily (as appetiser: 30 minutes before meals; all other indications 1 cup of tea after meals)	Poland Spain Germany: 1986, Standard Marketing Authorisation
Expressed juice from fresh herb (1:0.5-0.9)	Loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence	2 times daily 5 ml expressed juice (100% expressed juice)	Germany Since 1976, TUR according to national legislation
Tincture (1:5); extraction solvent ethanol 70% (V/V)	Loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence	1 g herbal substance 3 times daily Single dose: corresponding to approximately 1 g herbal substance Daily dose: corresponding to approximately 3 g herbal substance	Latvia, since 1993, WEU

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

Not applicable

2.1.2. Information on products on the market outside the EU/EEA

Not applicable

2.2. Information on documented medicinal use and historical data from literature

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 1983; Blaschek *et al*., 2006; Martindale, 1989), digestive and hepatoprotective remedy (Ghédira & Goetz, 2016). 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, and also as a remedy for Inflammatory Bowel Disease (Algieri *et al*., 2015; Ng *et al*., 2013; 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, 1969; Blaschek *et al*., 2006; Madaus, 1976; Schulz & Hänsel, 2004; Teuscher, 1989). Authorised 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 appetiser 30 minutes 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; Haffner & Schultz, 1969).

Table 3: Overview of historical data

Herbal preparation	**Documented use / traditional use**	**Pharmaceutical form** ***Posology*** **Duration of use**	**Reference**
Artemisia absinthium	Gastrointestinal complaints	-	Madaus, 1976 Arnold, 1989

Herbal preparation	Documented use / traditional use	Pharmaceutical form *Posology* Duration of use	Reference
			Hose, 2002
Artemisia absinthium	To promote appetite in case of gastritis, hypoacidity Dyspepsia Choleretic For liver diseases, hepatitis, blood purification, jaundice, diabetes, skin diseases, allergy, scabies, tetanus, as brain tonic	-	Madaus, 1976 BHP, 1983 Martindale, 1989 Guarrera, 2005 Blaschek *et al*., 2006
Dried comminuted herbal substance		Herbal tea	German Ph., 1872 AB-DDR, 1975 Madaus, 1976 Teuscher, 1989 DAC, 2007 Haffner & Schultz, 1969 Blaschek *et al*., 2006
Tincture	To improve appetite To stimulate the digestion in cases of hypoacidity and chronic gastritis	Liquid preparation: Tincture (1:5), extraction solvent Ethanol 70% (V/V) 3 times daily, each single dose equivalent to 1g herbal substance	Haffner & Schultz, 1969 Schultz & Hänsel, 2004

2.3. Overall conclusions on medicinal use

Table 4: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Powder herbal substance	Symptomatic treatment of dyspeptic complaints such as minor gastro-	Coated tablets Posology: 3 times daily 4 coated tablets with 190 mg	At least since 1976 in Germany

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	intestinal spasms, repletion and flatulence	herb Single dose: corresponding to 760 mg herbal substance Daily dose: corresponding to 2.28 g herbal substance	
Comminuted herbal substance	Lack of appetite, dyspepsia Loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence, spasmodic	Herbal tea Poland: 1.0 g 2-3 times daily Spain: 2-3 cups daily Germany: 1.5 g 2 times daily (as appetiser: 30 minutes before meals; all other indications 1 cup of tea after meals)	Since 1986 in Germany
Expressed juice from fresh herb (1:0.5-0.9)	Loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence	2 times daily 5 ml liquid extract containing 100% expressed juice	Since 1976 in Germany
Tincture (1:5); extraction solvent ethanol 70% (V/V)	Loss of appetite, dyspeptic complaints such as minor gastrointestinal spasms, repletion, flatulence	1 g herbal substance 3 times daily Single dose: corresponding to approximately 1 g herbal substance Daily dose: corresponding to approximately 3 g herbal substance	References since 1979 (Haffner & Schultz, 1969) In the market, at least since 1993 in Latvia

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Herbal substance/Herbal preparations

***In vivo* studies**

Antiulcer effects

Different semi-pure extracts from *A. absinthium* from Pakistan were tested for their antiulcer effects on acetylsalicylic acid induced ulcers in rats (Shafi *et al*., 2004). 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. Phytochemical analysis indicated the presence of saponins and glycosidic sugars in the extract.

The ulcerated rats were given the fractions orally at a dose of 5 mg/kg 3 hours prior and 3 hours 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 hours. 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. Propylene Glycol 5mg/kg i.p. was used for the control group.

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).

Effects on gastric juice and bile secretion

An i.v. injection of decoctions of Absinthii herba (equal to 5 g herbal substance) caused a threefold increase of bile secretion in dogs, while orally administered absinthin increased the amount of gastric juice and free HCl while this was not observed after gavage administration (Kreitmair, 1951).

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 mucosa of animals (Rozengurt, 2006).

Earlier hypotheses claimed that bitter tasting substances evoke secretory reflexes of the gastrointestinal tract via taste receptors in the lingual epithelium. It is postulated that activation of bitter taste receptors generate integrated responses as secretion, motility or absorption (Sternini, 2007).

3.1.2. Secondary pharmacodynamics

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 times 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 analyse 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*., 1991).

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**

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 was used to estimate the protective effect of plant extract against lethal dose of acetaminophen (1 g/kg) in mice. Animals were divided into 2 groups; the first group received 500 mg/kg per os, followed by oral administration of acetaminophen (1 g/kg) after 1 hour. The second group served as a control and received the same treatment except that normal saline (0.9% NaCl) was administered instead of plant extract. The mortality was observed for 48 hours post-administration of acetaminophen. While acetaminophen induced 100% lethality in mice, animals pretreated with *A. absinthium* extract showed a 80% protection against lethal effect of acetaminophen (only 2 out of 10 animals died) (Gilani & Janbaz, 1995).

Pre-treatment of rats with the extract (500 mg/kg, orally twice daily for two days) prevented ($p<0.01$) the acetaminophen (640 mg/kg) as well as CCl_4 (1.5 ml/kg)-induced rise in serum transaminases GOT and GPT. Post-treatment with the extract (500 mg/kg, 6 hours) restricted the hepatic damage induced only by acetaminophen ($p<0.01$). The observed prolongation of the sleeping time induced by pentobarbital, together with the increased strychnine-induced lethality in mice suggested an inhibitory effect on microsomal drug metabolizing enzymes (MDME) which could explain the hepatoprotective effect of *A. absinthium*. 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 minutes 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 107 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 hours (53.3% and 73.3% after 12 and 24 hours, 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).

Both essential oil and plant extract collected from the Balkan peninsula showed antibacterial and antioxidant activity (Stanković *et al*., 2016a, 2016b).

Isolated compounds

Thujone

The structure of a 3-thujone and its Δ3,4-enol was compared with (-)-Δ9-THC and it was suggested that the 3-thujone or the Δ3,4 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 (-)-Δ9-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).

Data on the mechanism of action of thujone are summarised in the Public Statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010).

Other compounds

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 PGE2 in RAW 164.7 cells treated with LPS and it decreased efficiently the LPS-induced NF-κB activation (Lee *et al*., 2004).

Table 5: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Posology**	Experimental model	Reference	Main non-clinical conclusions
Ethanolic extract (different fractions)	5mg extract/kg bw, twice daily, 3 days Oral route	*In vivo* (rats)	Shafi *et al*., 2004	Antiulcer effect No toxic/lethal effects
Aqueous-methanolic extract	Pretreatment with 500 mg extract/kg bw, twice daily, 2 days Oral route Post-treatment with 500 mg extract/kg bw, 6h 500 mg extract/kg bw	*In vivo* (mice)	Gilani & Janbaz, 1995	Prevention of the rise in serum transaminases induced by acetaminophen and CCl_4 Reduction of hepatic damage by acetaminophen Prolongation in pentobarbital-induced sleep and increase in strychnine-induced lethality Possible inhibition of MDME
Decoction Isolated absinthin	Equiv to 5g herbal substance Intravenous Oral route	*In vivo* (dogs)	Kreitmair, 1951	Threefold increase of bile secretion Increase of gastric juice and free HCl amount

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

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.

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

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

Herbal substance/Herbal preparations

No data available.

Isolated compounds

Thujone

Metabolism of thujone has been investigated in mouse, rat and human liver preparations *in vitro* and in mice, rats and (partially) rabbits *in vivo*. Hydroxylations at various positions, followed to a different extent by glucuronidation, and reductions as minor reactions are principal metabolic pathways, although *in vitro* and *in vivo* metabolic profiles do not necessarily agree with each other (Ishida *et al*., 1989; Höld *et al*., 2000a; 2001).

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).

Some studies indicated that among human recombinant P450 enzymes studied, CYP3A4 and CYP2D6 were the most active enzymes, producing 7-hydroxy-α-thujone, 4-hydroxy-thujone (in this order of abundance) and some minor metabolites. CYP1A2, CYP2C9, CYP2C19, and CYP2E1 were less active, catalyzing only about 1% conversion in one hour of incubation (Höld *et al*., 2001; Jiang *et al*., 2006). The latter study (Abass *et al*., 2011) with a more comprehensive set of recombinant enzymes indicate that the principal CYP enzyme metabolizing α-thujone is CYP2A6, followed by CYP3A4 and, to a small extent, CYP2B6. The major metabolites produced were 7- and 4-hydroxyl compounds. Extrapolation of microsomal metabolic clearances suggested that α-thujone is a liver blood flow–dependent substance.

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*, 2000; 2001). 7,8- and 4,10-dehydro metabolites have been identified *in vitro* and as urinary metabolites respectively (Höld *et al*., 2001).

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.

Pharmacokinetic studies of thujone, are summarised in the Public Statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010).

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

Toxicological studies of thujone, are summarised in the Public Statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010).

3.3.1. Single dose toxicity

Herbal substance/Herbal preparations

The LD_{50} of an extract of *A. absinthium* (not further specified) was given with 1 mg/kg in rats (i.p.*)* (Blaschek *et al*., 2006).

Essential oil

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).

Isolated compounds

Thujone

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 a LD_{50} value of 45 mg/kg in mice for α-thujone (i.p.).

3.3.2. Repeat dose toxicity

Herbal substance/Herbal preparations

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 per day was calculated with 1.27 g/kg per day (males) and 2.06 g/kg per 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).

Isolated compounds

Thujone

Thujone was administered to rats by gavage at doses of 12.5, 25 or 50 mg/kg per 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 per 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).

Some results of the long-term toxicity studies from the NTP (NTP 2010, TR No. 570) show the effect of an isomeric mixture of thujone, administered by gavage to B6C3F1 mice at doses of 0, 3, 6, 12, and 25 mg/kg body weight per day and to Fischer 344 rats at doses of 0, 12.5, 25, and 50 mg/kg body weight per day for 2 years. In both species, the increased mortality observed in the top dose group, and in the rat also in the middle dose group. Clonic and tonic seizures were observed in the middle and top dose groups in rats and in the top dose group in mice. A small increase in clonic seizures was observed also in the low dose group in rats. In the rat, NOEL value was 12.5 mg/kg for mortality and tonic seizures (no NOEL for clonic seizures). In the mouse, the NOEL was 12 mg/kg body weight for seizures and mortality.

Repeat-dose toxicity studies of thujone are also summarised in the Public Statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010).

3.3.3. Genotoxicity

Isolated compounds

Thujone

In connection with the NTP study (NTP 2010), genotoxic potential of racemic thujone and α-thujone were investigated according to the NTP protocols. The Ames test results of both compounds were negative in the presence or absence of the activating enzyme system. *In vivo*, daily exposure by gavage to racemic thujone (6.25, 12.5, 25, 50, or 75 mg α, β-thujone/kg body weight) for 3 months did not result in an increase in micronucleated erythrocytes in the peripheral blood of male B6C3F1 mice. However, female mice had a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-month study. Racemic thujone did not induce bone marrow toxicity.

Further evaluation of the genotoxicity of Absinthii herba, related to thujone, are summarised in the Public Statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010).

3.3.4. Carcinogenicity

Herbal substance/Herbal preparations

No studies with herbal preparations of Absinthii herba and carcinogenesis have been published.

Isolated compounds

Thujone

According to the NTP report (NTP 2010) on 2-year gavage studies with rats (dose levels 12.5, 25, and 50 mg/kg) and mice (dose levels 3, 6, 12, 25 mg/kg), there was some evidence of carcinogenic activity of α, β-thujone in male F344/N rats based on increased incidences of preputial gland neoplasms at the dose level of 25 mg/kg (all rats at 50 mg/kg died before the end of the study); increased incidences of benign pheochromocytoma of the adrenal medulla may have been related to administration of α, β-thujone in male F344/N rats administered 12.5 or 25 mg/kg. There was no evidence of carcinogenic activity of α, β-thujone in female F344/N rats administered 12.5 or 25 mg/kg. There was no evidence of carcinogenic activity of α, β-thujone in male or female B6C3F1 mice administered 3, 6, or 12 mg/kg.

In the same 2-year study, administration of α, β-thujone resulted in increased incidences of seizures in F344/N rats and B6C3F1 mice in a dose-dependent manner and increased incidences of nonneoplastic lesions in the brain and spleen of male and female F344/N rats, the kidney of male F344/N rats and the pituitary gland of female F344/N rats usually at the two highest dose levels (25 and 50 mg/kg).

3.3.5. Reproductive and developmental toxicity

Herbal substance/Herbal preparations

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*.

Isolated compounds

Thujone

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).

Reproductive toxicity studies have not been performed.

3.3.6. Local tolerance

Essential oil

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 hours under occlusion (Opdyke, 1975).

Sensitization: A 2% preparation in petrolatum produced no sensitization reaction in 25 volunteers (maximization test) (Opdyke 1975).

3.3.7. Other special studies

Essential oil

Photoxicity: No phototoxic effects were reported for undiluted essential oil on hairless mice and swine (Opdyke, 1975).

Isolated compounds

Thujone

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).

3.3.8. Conclusions

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

The essential oil of Absinthii herba contains constituents like thujone, which have toxic effects affecting the central nervous system at high doses. Based on existing data it can be concluded that because of

the toxic properties of the essential oil, one should not exceed recommendations concerning the posology of Absinthii herba.

For further information on these recommendations, see section 5.6 and the Public statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010 Rev.1). The "therapeutic margin" of thujone where effects may start at those borderline effects and end in seizures is not known and its determination would need further studies. However, on the basis of the limit doses of 3.5 and 6.6 mg per day, it is recommended that the amount of thujone in a preparation needs to be specified and that exposures in the range between 3 and 7 mg per day do not pose special concerns. For higher concentrations a case-by-case benefit/risk assessment would be necessary. The amount of dietary intake of 1 mg in average may not cause special concerns. However, for the upper limit of the additional intake from medicinal products, the highest safe amount was reduced by the possible intake by food, to give 6 mg as a limit of daily exposure (EMA/HMPC/732886/2010).

Tests on reproductive toxicity have been performed with a dry ethanolic extract of Absinthii herba administered orally to pregnant rats. Results showed reduced sites of implantations and a reduced rate of born pups. Thujone is known for its uterus stimulating activity.

Due to the lack of data on mutagenicity, carcinogenicity and reproductive and developmental toxicity, a list entry for Absinthii herba cannot be recommended.

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on Absinthii herba to support the proposed indications are very limited. The reported pharmacological effects are not considered contradictory to the traditional uses. In particular, results with some Absinthii herba preparations indicated an antiulcer effect which could be in favour of use for dyspeptic or gastrointestinal disorders. Toxicity studied showed no acute toxicity at the assayed doses.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of Absinthii herba is scarce.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed with Absinthii herba.

Absinthii herba contains thujone, which have toxic effects in high doses. Toxicological dose limits have been set based on the available toxicological data and other studies. The toxic effect appears to be of central nervous origin with convulsions as the main symptom. For further information, see the Public statement on the use of herbal medicinal products containing thujone (EMA/HMPC/732886/2010 Rev.1).

The "therapeutic margin" of thujone where effects may start at those borderline effects and end in seizures is not known and its determination would need further studies. However, on the basis of the limit doses of 3.5 and 6.6 mg per day, it is recommended that the amount of thujone in a preparation needs to be specified and that exposures in the range between 3 and 7 mg per day do not pose special concerns. For higher concentrations a case-by-case benefit/risk assessment would be necessary. The amount of dietary intake of 1 mg in average may not cause special concerns. However, for the upper limit of the additional intake from medicinal products, the highest safe amount was reduced by the possible intake by food, to give 6 mg as a limit of daily exposure (EMA/HMPC/732886/2010).

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Herbal substance/Herbal preparations

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 minutes. 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 hyperaemia (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 minutes 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 minutes (fractionated in 10 times 10 minutes). 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 minutes. After administration of preparations/placebo the duodenal secretion was measured for 40 minutes (placebo), 100 minutes (preparation with thujone) and 120 minutes (preparation without thujone) in 10 minutes 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, randomised 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 minutes before 3 ml verum (100 g verum correspond to 0.65 g *A. absinthium*; and ~ 0.02 g Absinthii herba) or 3 ml placebo was administered. Five minutes after application the biliary secretion was measured for 60 minutes (fractionated in 6 times 10 minutes). 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 minutes after application (Jagusch, 1988).

In a double-blind, randomised 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 minutes after application. No increase was observed for the amount of bile acids and alkaline substances (Kistler, 1988).

Assessor's comment:

Another typical bitter drug *Gentiana 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.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Herbal substance/Herbal preparations

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 minutes. Blood samples were drawn 15, 30, 60, 90, and 120 minutes 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).

4.2. Clinical efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

In a multi-centre, randomised, 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 times 500 mg per 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 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* 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* per day for 15 days the following results were reported:

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).

A randomised, double-blind multicentre study including 20 patients diagnosed with Chron's disease was performed (Krebs *et al*., 2010). Patients received 3x750mg per day of dried powdered *A. absinthium*. After 6 weeks of treatment, 80% patients on wormwood group and 20% on placebo group achieved clinical remission (defined as Chron's disease activity index below 170 or a reduction in this by 70 points).

Table 6: Clinical studies on humans in Inflammatory Bowel Disease (IBD).

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Omer et al, 2007	Randomised double-blind multicentre study versus placebo 10 weeks	3 × 500 mg per day	40	Inflammatory Bowel Disease (IBD) types C,D	After 8 weeks of treatment with wormwood, there was almost complete remission of symptoms in 65% of the patients, whereas no beneficial effect was observed in those receiving the placebo	Response was defined as a decrease in total score of >50% from baseline and remission as a score of <10 points. Statistical methods: Student's t-test and w2-test. Significance was at p-value 0.01 w2-test was used to compare patients in both groups Median CDAI, IBDQ and HAMD scores were compared at pre-defined study visits	Remission in 65% patients on wormwood Remission on placebo: 0%
Krebs et al., 2010	Randomised, double-blind multicentre study	3 × 750 mg powder / day (in addition to standard therapy) 6 weeks	20	Inflammatory Bowel Disease (IBD) types C,D	Wormwood administration promoted the clinical improvement of the symptoms in all the patients. The beneficial effect was associated with a significant decrease in TNFα serum levels in comparison with those obtained in the placebo group, where no amelioration in the disease was observed	Baseline continuous data were compared using the 2-sample t-test and baseline categorical data were compared using the w 2-test or Fisher Significance was at p-value 0.05	Improvement in wormwood group: CDAI-Score < 150

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

None reported

4.4. Overall conclusions on clinical pharmacology and efficacy

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 has been reported to act antispasmodic in small amounts. In high dosages or after longer-lasting intake the essential oil acts as a convulsant poison.

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 study by Krebs *et al*., 2010, showed a 80% remission of symptoms in patients with Crohn's disease after 6 weeks of treatment with *A. absinthium*.

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 long-standing use and experience of aqueous and ethanolic preparations of Absinthii herba, are in favour of the plausibility of efficacy of the herbal preparation(s) containing Absinthii herba for the traditional use for temporary loss of appetite and for mild dyspeptic/gastrointestinal disorders. This is also confirmed by results of another typical bitter drug *Gentiana lutea*.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

No adverse effects have been reported with *A. absinthium* preparations.

5.2. Patient exposure

No data available.

Aside from market presence and data from studies, there are no concrete data concerning patient exposure.

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 (Council Directive 88/388/EEC).

5.3. Adverse events, serious adverse events and deaths

A 61 year-old woman was treated with an 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) family. 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).

A case report after cutaneous use of a cosmetic containing *Artemisia absinthium* indicated redness and sensitivity of the face, causing a chemical burn of the first degree (El Makrini & Hassam, 2016).

A case report involving a 10-month-old male infant who received a home-prepared extract of *A. absinthium* and suffered severe diarrhoea was published. Nonetheless, no data on the preparation is included (posology, composition) and so no clear relationship between wormwood consumption and the adverse event can be stated (Kocaoglu & Ozel, 2014).

Cases with severe intoxications in humans have been reported after consumption of essential oil rich in thujone (Centini *et al*., 1987, Milett *et al*., 1981). Convulsions resembling epilepsy have been reported after the ingestion of isolated thujone (Cobb, 1922). 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). Although it is difficult to determine exposing doses in these cases, SCF (2002) concluded that humans are at least as sensitive to thujone neurotoxicity as experimental animals.

In a clinical study by Dettling *et al*., 2004, 25 volunteers were exposed to absinthe containing high (100 mg/l) and low (10 mg/l) concentrations of thujone. Approximate thujone amounts consumed were 0, 1.5 mg and 15 mg. The simultaneous administration of alcohol containing a high concentration of thujone had a negative effect on attention performance and some mood dimensions at the earliest examination time (30 minutes). Alcohol alone or with a low concentration of thujone did not result in similar effects. The authors interpreted the observations at a high thujone dose as the antagonistic effect of thujone on the GABAA receptor.

In a pilot absinthe drinking study by Kröner *et al*., 2005, two subjects consumed 110 ml absinthe with 3.85 mg thujone (content of absinthe 35 mg/l) within 15 minutes, 15 and 30 minutes and then every 30 minutes until up to 2 hours after drinking blood samples were drawn. Blood alcohol concentrations >1 g/l were observed whereas no thujone could be detected in blood samples (detection limit 0.34 ng/ml). Conjugates of thujone were not determined. The two subjects showed typical signs of alcohol effects (e.g. staggering, chattiness) while hallucinogenic effects were not described (EMA/HMPC/732886/2010).

Serious adverse events

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).

5.4. Laboratory findings

One case report involving a 10-month-old male infant who received a home-prepared extract of *A. absinthium* indicated a persistent metabolic acidosis (Kocaoglu & Ozel, 2014).

5.5. 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 minutes. All tests were performed before drinking (T0) and 30 (T1) and 90 minutes (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 minutes (T2) revealed results that show a pattern similar to the results after 30 minutes 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 leads to an increase in fear sensations and also a stimulating and rousing effect, while ethanol acts as a GABA-enhancer (anxiolytic, sedative and amnesic).

5.5.1. Use in children and adolescents

No data available.

The use in children and adolescents under 18 years of age is not recommended because data are not sufficient and medical advice should be sought.

5.5.2. Contraindications

The use of Absinthii herba preparations is contraindicated in case of obstruction of the bile duct, cholangitis or liver disease.

5.5.3. Special warnings and precautions for use

Patients with gallstones and any other biliary disorders should consult a doctor before using Absinthii herba preparations. Please consider the contraindications.

If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

None reported.

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. However, such effects have not been reported.

Studies on human liver preparations and enzymes *in vitro* indicate that CYP2A6, and CYP3A4 and CYP2B6 to a lesser extent, are principal thujone-metabolizing enzymes at least *in vitro* (Höld *et al*., 2001; Jiang *et al*., 2006; Abass *et al*., 2010). Clearance calculations point to a possibility of a prominent first-pass metabolism. Induction and inhibition interactions with drugs after oral administration in humans are probably not likely because of multiple metabolizing enzymes and a fairly rapid metabolism. However, metabolic and pharmacokinetic characteristics remain inadequately defined and need further studies (EMA/HMPC/732886/2010).

5.5.5. Fertility, 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. There are no preclinical or clinical studies which would permit reliable scientific assessment of potential consequences regarding exposure of sensitive groups (i.e. pregnant women, lactation, etc.). Thus the use of thujone-containing herbal medicinal products in these groups is not recommended during pregnancy and lactation.

5.5.6. 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 diarrhoea, 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.

5.5.7. 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.

5.5.8. Safety in other special situations

Not applicable

5.6. 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. After intake of preparations from Absinthii herba, patients should not drive or operate machinery for safety reasons.

The use in children and adolescents is not recommended. The use of Absinthii herba during pregnancy or lactation is not recommended. 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.

If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

6. Overall conclusions (benefit-risk assessment)

Absinthii herba is well known and derived traditional herbal medicinal products have been used for centuries in European countries.

Based on the available data, the HMPC concluded that European Union herbal monograph on *Artemisia absinthium*, herba, can only be established for traditional use.

For the well-established medicinal use, the following requirement set out in Article 10a of Directive 2001/83/EC for a herbal preparation/product is not fulfilled: the requirement regarding coherence of scientific assessment in the WEU criteria is not fulfilled. Although pharmacological *in vivo* studies showed stimulating effects on the gastric, intestinal and biliary secretion, there are no clinical data regarding the efficacy of the preparations.

In conclusion, on the basis of the available data, the HMPC concluded that due to lack of scientific data, a well-established use cannot be established for a European Union herbal monograph on *Artemisia absinthium*, herba.

Based on the data on its long-standing use in the European Union and on the available bibliographic references, traditional use for more than 30 years and acceptable safety are documented for *Artemisia absinthium*, herba: comminuted herbal substance; powdered herbal substance; expressed juice from fresh Absinthii herba (1:0.5-0.9); tincture (1:5), extraction solvent ethanol 70% (V/V).

The above-cited Absinthii herba preparations can be accepted as traditional herbal medicinal products in the following indications:

a) Traditional herbal medicinal product for temporary loss of appetite
Therapeutic area for browse search: Loss of appetite

b) Traditional herbal medicinal product for mild dyspeptic/gastrointestinal disorders
Therapeutic area for browse search: Gastrointestinal disorders

If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

The presence of thujone in wormwood preparations mentioned in the monograph is restricted to a daily exposure of 6.0 mg/person (Public Statement on the use of herbal medicinal products containing thujone EMA/HMPC/732886/2010).

The use of Absinthii herba is not recommended during pregnancy and lactation and in children and adolescent under 18 years of age. Absinthii herba is contraindicated in patients with obstruction of the bile duct, cholangitis or liver disease. For safety reasons, affected patients should not drive or operate machinery after intake of preparations from Absinthii herba, as it might affect performance in driving and operating machinery.

A European Union list entry is not supported due to lack of data on genotoxicity.

Annex

List of references