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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Humulus lupulus* L., flos

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

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Humulus lupulus*. L., flos
Herbal preparation(s)	a) Comminuted herbal substance b) Powdered herbal substance c) Liquid extract (DER 1:1), extraction solvent ethanol 45% V/V d) Liquid extract (DER 1:10), extraction solvent sweet wine e) Tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent ethanol 60 % V/V f) Dry extract (DER 4-5:1), extraction solvent methanol 50% V/V
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use. Herbal preparations 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

Humulus lupulus L., flos is the dried, generally whole female inflorescences of *Humulus lupulus* L. (European Pharmacopoeia).

Constituents

Bitter principles consisting mainly of prenylated phloroglucinol derivatives known as alfa-acids or humulones (2-12% of dried strobile), principally humulone (35-70%), and beta-acids or lupulones (1-10% of dried strobile), principally lupulone (30-55%) (Verzele, 1986, De Keukeleire *et al.*, 1999, Wohlfart *et al.*, 1993, Hölz, 1992, Hänsel and Schultz, 1986).

Essential oil (0.5-1.5%) consisting mainly of myrcene (monoterpene) and beta-caryophyllene, humulene and farnesene (sesquiterpenes) (Verzele, 1986, Eri *et al.*, 2000). Although only traces of the sedative constituent, 2-methyl-3-buten-2-ol , are found in freshly harvested hop strobile (Hänsel *et al.,* 1982, Wohlfart *et al.*, 1983) the amount is higher in stored material increasing to a maximum of about 0.15% of the dry weight (up to 20% of the volatile constituents) after 2 years due to degradation of humulones and lupulones (Wohlfart *et al.*, 1993, Hölz, 1992, Hänsel *et al.*, 1982, Wohlfart *et al.*, 1983).

Flavonoids (0.5-1.5%) including quercetin and kaempferol glycosides (McMurrough, 1981, De Cooman *et al.*, 1998), and at least 22 prenylated or geranylated flavonoids, notably the chalcones xanthohumol (up to 1% of the dried strobile and 80-90% of total flavonoids), desmethylxanthohumol and dehydrocycloxanthohumol, and the flavanones isoxanthohumol, 8-prenylnaringenin (25-60 mg/kg) and 6-prenylnaringenin (Rong *et al.*,2000, Stevens *et al.*, 1997, 1999a, 1999b, Milligan *et al.*, 1999).

Other constituents include proanthocyanidins (2-4%), phenolic acids, proteins (15%), polysaccharides (40-50%) and minerals (Verzele, 1986, De Kekeleire *et al.*, 1999, Hölz, 1992).

- Herbal preparations

a) Comminuted herbal substance

b) Powdered herbal substance

c) Liquid extract (DER 1:1), extraction solvent ethanol 45% V/V

d) Liquid extract (DER 1:10), extraction solvent sweet wine

e) Tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent ethanol 60% V/V

 f) Dry extract (DER 4-5:1), extraction solvent methanol 50% 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.

 Not applicable

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

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Belgium	☒	☐ TRAD	☒	☒Other Specify: Food Suppl.	Comb. only. Food Suppl.
Bulgaria	☒MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Croatia	☒MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☒MA	☒TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only.
Denmark	☒ dry extract	☒	☐ Other TRAD	☐ Other Specify:	Comb. MA and Trad
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
France	☐ MA	☒ Powder	☐ Other TRAD	☐ Other Specify:	
Germany	☒	☒ Dry Extr	☐ Other TRAD	☐ Other Specify:	Comb. MA and Trad
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☒MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☒MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Italy	☐ MA	☒TRAD	☐ Other TRAD	☒ Other Specify: Food Suppl.	Comb. only
Latvia	☒	☒TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☒Food Suppl.	No products
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☒Food Suppl.	No products
Poland	☒MA	☒Herbal tea	☐ Other TRAD	☐ Other Specify:	Comb.: MA and Trad
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Serbia					No products
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☒	☒TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

Member State	Regulatory Status				Comments
Sweden	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Comb. only

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

From this overview it can be concluded that herbal preparations fulfil the criteria of traditional use in Denmark, France, Germany and Poland. Besides these countries, in several other countries only combinations of hop preparations with other sedative plant preparations, usually valerian, and sometimes other plants, up to 5 ingredients, have been marketed or registered. These countries include Belgium, Bulgaria, Croatia, Czech Republic, Ireland, Italy, Latvia, Slovenia and Sweden. In Malta and Norway, hop preparations are only available as food supplements. Most hop preparations used in the combination products are dry extracts prepared with water, methanol 30%-45% or ethanol 70%. Some preparations consist of powdered herbal substances or liquid extracts prepared with 70 %-90% ethanol.

1.3. Search and assessment methodology

In preparing this report a number of data sources have been taken into account *viz* the ESCOP monographs published respectively in 1997 and 2003, the bibliographic references made available by ESCOP at the end of 1997, the monograph of the European Pharmacopoeia in 2005 and the results of literature searches in electronic databases including BioMed Central Journals (since 2007), Evidence based Medicine Reviews (Cochrane), Medline, SciFinder (since 2007), and PubMed. The key words used were hops and *humulus lupulus*.

Results; Cochrane: 169, PubMed: 494, BioMed Central Journals: 15, SciFinder: 16 and Medline: 2480. Restriction to medicinal use was applied to the titles and after selection the abstracts were screened manually and all publications deemed relevant were assessed and included in the assessment report.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Herbal substance

Although hops have been used in beer primarily for their bitter taste and preservative action for over 1000 years, their medicinal or tonic properties were apparently also valued from very early times. It was observed that hop pickers tired easily, apparently as a result of the accidental transfer of some hop resin from their hands to their mouths, and the hop plant gained a reputation as a sedative and hypnotic but also as a topical antibactericidal agent. Pillows filled with hops have been used for sleeplessness and nervous conditions (Hänsel *et al.*, 1976, Schiller *et al.*, 2006). Koch and Heim (1953) following up on the folk legend "that women who normally live a distance from hop gardens regularly begin to menstruate 2 days after arriving to pick hops" reported that hops contain the equivalent of 200-300 microgram oestradiol/gr. Since 1973 it has been claimed that brewing sludge baths containing ca. 30% hops could be used for the treatment of a variety of gynaecological disorders (Fenselau and Talahay, 1973).

The dried, generally whole female inflorescences of hops are described in the European Pharmacopoeia (2005).They are also described in an ESCOP monograph (2003) as having a therapeutic indication

similar to valerian root *viz.* tenseness, restlessness and sleep disorders. Hops is included in a monograph in DAB 10 (1991), PF 10 (1989) and the British Herbal Pharmacopoeia (1983). The German Commission E monograph described hop strobiles and hop extracts as sedative in 1984 (BAnz nr 228, dated 05.12.1984).

Herbal preparations

Humulus lupulus strobiles have a long traditional medicinal use in Europe, in the form of herbal tea, infusions, powdered herbal substance or alcoholic extracts for the relief of insomnia, excitability and specifically for restlessness associated with nervous tension, headache and/or indigestion. Hops is well documented in a number of handbooks (British Herbal Pharmacopoeia, 1983, Hagers Handbuch der Pharmazeutische Praxis, Hänsel *et al.* Ed. 1993, ESCOP, 2003).The German Commission E approved use of hops for mood disturbances such as restlessness and anxiety as well as sleep disturbances (Blumenthal *et al.*, 2000). Hops are mostly used in combination with other sedative herbs such as valerian, passion flower and lemon balm for the treatment of sleep disturbances (Blumenthal *et al.*, 2000, Schulz *et al.*, 2001). Hop extracts have also been reported to be active as antioxidants, cancer chemopreventives, anti-inflammatory agents, antimicrobials (antibacterials and antifungals) and cytotoxics (Chadwick *et al.*, 2006).

The dried inflorescences are used in comminuted form as such or in tea mixtures or they are prepared as infusions. Lipophilic extracts are used for the preparation of bath oils whereas hydroalcoholic liquid extracts are prepared for internal use as sedatives, mostly in combinations with other sedative plant extracts. In several cases, the hydroalcoholic liquid extracts are prepared with sweet wine.

2.2. Information on traditional/current indications and specified substances/preparations

Data on the following preparations are reported in standard sources:

German Commission E: mood disturbances such as restlessness and anxiety as well as sleep disturbances: single dosage of 0.5 g of cut or powdered strobile or dry extract powder for infusions, decoctions or other preparations (BAnz. Nr 228 of 05.12.1984). Infusion or decoction: 0.5 g hop strobiles (1 to 2 teaspoons) in 150 ml hot water 2 to 3 times daily and before bedtime (Teedrogen, Wichtl, 1989, Phytotherapie Manual, Fintelmann *et al.* 1993, Blumenthal *et al.*, 2000).

British Herbal Pharmacopoeia (1983): restlessness associated with nervous tension; headache and/indigestion: dried strobile: dose 0.5-1g or by infusion; 1-2 g as a hypnotic. Liquid extract (1:1 in 45% alcohol): 0.5-2.0 ml. Tincture (1-5 in 60% alcohol):1-2 ml (Hagers Handbuch, Hänsel *et al.*, 1993, Herbal Medicines, Newal *et al.*, 1996).

ESCOP monograph (2003): tenseness , restlessness and sleep disorders; Internal use: adults and children over 12 years of age: 0.5 g of the drug as infusion, 2-4 times daily; 0.5-2 ml of liquid extract (1:1, 45% ethanol) or 1-2 ml of tincture (1:5 , 60 % ethanol) up to 3 times daily; other equivalent preparations. External use: infants and young children: up to 500 g of dry hop strobiles (previously stored for 1-2 years) in a hop pillow. Duration of administration: no restriction.

The following information about products currently on the market was obtained from the Member States:

Denmark

No	Preparation	Period of medicinal use	Dosage Form	Posology	Indication
1	Powdered herbal substance	Before 1993	Tablet (60mg of powdered herbal substance/tablet)	no information	HMP in uneasiness and difficulties in falling asleep
2	Herbal tea (WEU)	1993-2007	Herbal tea (23g/100g)	no information	HMP in uneasiness and difficulties in falling asleep

France

No	Preparation	Period of medicinal use	Dosage Form	Posology	Indication
1	Powder (TRAD)	From 1980	Hard capsule (195 mg of herbal substance/tablet)	Adults: 2 caps/twice daily Adolescents: 1 caps/twice daily	Traditionally used in the symptomatic treatment of neurotonic conditions in adults and adolescents notably in cases of mild disorders of sleep

Germany

No	Preparation	Period of medicinal use	Dosage Form	Posology	Indication
1	Dry extract (4-5:1), methanol 50%/V/V (TRAD)	From 1976	Coated tablet (125mg of dry extract, 4-5:1, extraction solvent methanol 50%/V/V)	Adults and adolescents over 12 years a. 2-3 times 1 coated tablet b. 1hr before bedtime 1-2 coated tablet	a. to improve general condition in mental stress b. to aid sleep
2	Liquid extract (1:10), sweet wine	From 1978	Oral liquid	2-3 times; Single dosage equivalent to 1.9 g herbal substance	To support mental relaxation
3	Liquid extract (1:12.4-12.6), ethanol 16%, m/m	From 1965	Oral quid	2-3 times; daily dosage equivalent to 0.8 g herbal substance	To support mental relaxation
4	Liquid extract (1:94-95), sweet wine	From 1978	Oral liquid	2-3 times ; daily dosage equivalent to 0.4-0.6 g herbal substance	To support mental relaxation

Poland

No	Preparation	Period of medicinal use	Dosage Form	Posology	Indication
1	Herbal tea (TRAD)	Before 1980	Herbal tea	a. 0.5 in 200 ml of boiling water as a herbal infusion 2-4 times daily b. 0.5g-1.0g in boiling water as a herbal infusion 30min before bedtime	a. Mild symptoms of nervous tension b. Difficulty of failing asleep

The requirements of medicinal use in the indications to relieve symptoms of mental stress and to aid sleep for at least 30 years and an appropriate posology according to Directive 2004/24/EC are fulfilled for the following preparations:

a.   Comminuted herbal substance

b.   Powdered herbal substance

c.   Liquid extract (DER 1:1), extraction solvent ethanol 45% V/V (only for mental stress)

d.   Liquid extract (DER 1:10), extraction solvent sweet wine (only for mental stress)

e.   Tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent ethanol 60% V/V (only for mental stress)

f.   Dry extract (DER 4-5:1), extraction solvent methanol 50% V/V

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

For the relevant indications the following oral dosages have been recorded:

Herbal tea:

0.5 g of cut or powdered hop strobile (1 teaspoon= ca. 0.4 g) in ca. 150 ml of boiling water as a herbal infusion 2 to 3 times daily or before bedtime (BAnz nr 228, 05.12.1984, Fintelmann _et al._, 1993 and Blumenthal, 2000).

0.5-1 g as infusion and 1-2 g infusion as hypnotic (BHP, 1983, Hänsel _et al._, 1993, Newal _et al._, 1996)

Adults and children over 12 years: 0.5 g of the drug as an infusion, 2-4 times daily (ESCOP, 2003)

Denmark: herbal tea: 23 g/100 g, no further information.

Poland: 0.5 g in 200 ml of boiling water as herbal infusion, 2-4 times daily (nervous tension),

0.5-1.0 g in 200 ml of boiling water as a herbal infusion, 30 min before bedtime (to aid sleep).

Powdered herbal substance:

0.5-1.0 g or 1–2 g as hypnotic as single dose (BHP, 1983, Wichtl, 1989, Hänsel _et al._, 1993, Newal _et al._, 1996)

3 g of powdered drug after meals 2 times daily (Fintelmann, 1993)

Denmark: 60 mg of powdered herbal substance /tablet. No further information.

France: 195 mg of powdered substance /hard capsule: Adults: twice daily 2 capsules; Adolescents: 1 capsule twice daily.

Liquid extract (DER 1:1), extraction solvent ethanol 45% V/V

Dose: 0.5-2 ml (BHP, 1983, Hänsel _et al._, 1993, Newal _et al._, 1996).

Dose: 0.5-2 ml, up to 3 times daily (ESCOP, 2003)

Fluid extract 1:1 (g/ml), no extraction solvent: 0.5 ml (Blumenthal, 2000)

Tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent ethanol 60% V/V

Dose: 1-2 ml (BHP, 1983, Hänsel _et al._, 1993, Newal _et al._, 1996)

Dose: 1-2 ml up to 3 times daily (ESCOP, 2003)

Tincture 1:5 (g/ml), no extraction solvent: 2.5 ml (Blumenthal, 2000)

Marketing authorisation by Germany (more than 30 years)

Liquid extract (1:10), prepared with sweet wine

Dose: Single dosage corresponding to ca. 1.9 g herbal substance 2-3 times daily (mental stress).

Liquid extract (1:94-95), prepared with sweet wine

Dose: daily dosage corresponding to 0.4-0.6 g herbal substance in divided dosages of 2-3 (mental stress)

Liquid extract (1:12.4-13.6), extraction solvent ethanol 16% m/m

Dose: daily dosage corresponding to about 0.8 g herbal substance in divided dosages of 2-3 times (mental stress)

Dry extract (DER 4-5:1), extraction solvent methanol 50% V/V

Dose: Adults and adolescents over 12 years: 125 mg of dry extract/coated tablet, 2- 3 times 1 coated tablet (mental stress) and 1 hour before bedtime 1-2 coated tablets (to aid sleep).

Native dry extract 6-8:1 w/w, no extraction solvent, 0.06-0.08 g (60-80 mg) (Blumenthal ,2000).

Although the liquid extracts (1-12.4-12.6 and 1:94-95) prepared with ethanol 16% and sweet wine, respectively, have been marketed in German medicinal products for more than 30 years, their daily dosages, corresponding to less than 1 g herbal substance daily, are not plausible with the posology of the other traditional herbal medicinal products . Therefore, these preparations should not be included in the monograph on hops. The same conclusion can be drawn for the native dry extract (DER 6-8:1) because the extraction solvent as well as the daily posology are not known.

A restriction in the duration of use has not been documented.

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

3.1.1.1. Sedative effects

3.1.1.1.1. *In vitro* studies

Over the past decade considerable pharmacological research has been carried out on hop strobiles and its constituents particularly with respect to oestrogenic activity. However, the publications on *in vitro* studies relating to the sedative effects of hops are scarce. On the contrary, some spasmolytic effects have been reported for hops.

An alcoholic extract of hop strobile (1 g of dried drug in 10 ml of 70% ethanol) produced a strong spasmolytic effect on isolated smooth muscle from guinea pig intestine with ED_{50} values equivalent to 37×10^{-6} g of hop strobile per ml for acetylcholine-induced contractions compared to 60×10^{-9} g/ml with atropine, and 39×10^{-6} of hop strobile per ml for barium chloride-induced contractions compared to 57×10^{-7} g/ml with papaverine. The extract also inhibited contractions of rat uterus with an ED_{50}

equivalent to 31x10^{-6} g of hop strobile per ml (Caujolle *et al.*, 1969). An effect on calcium flux has been detected.

A methanolic extract from hop strobile showed strong inhibitory activity on calcium fluxes, inhibiting depolarization-induced $^{45}Ca^{2+}$ uptake in clonal rat pituitary cells by 94.7% at 20 µg/ml (p<0.001). The activity was attributed to prenylated flavonoids, although individual compounds from hop strobile have not so far been tested in this way (ESCOP, 2003).

Meissner and Häberlein (2006) investigated the influence of xanthohumol (X) on the binding of muscimol-Alexa-Fluor 532 (Mu-Alexa), a fluorescently labeled GABA$_A$ receptor agonist by fluorescence correlation spectroscopy. An incubation of hippocampal neurons with 75 nM of X increased the specific Mu-Alexa binding with ca. 17%, which was selectively found in GABA$_A$ receptor Mu-Alexa complexes with hindered lateral motility [D_{bound2} = (0,11 ± 0,03) µm$^{2/}$s$^)$] as described with midazolam, a benzodiazepine agonist. It was further shown that the modulatory activity of X on the GABA$_A$ receptor was not mediated via an interaction with benzodiazepine receptors. The authors concluded that X may play an important role in the sedative effects of hop preparations.

3.1.1.1.2. *In vivo* studies

Old reports have indicated that preparations of hops have sedative-like activity in frogs, pigeons, gold fish and golden carp. According to Wohlfart (1982, 1993), however, these animal tests show many methodological shortcomings.

Only a few animal experiments have been carried out in recent times to study the supposed sedating effects of hop extracts. Following gavage of different extracts from hops or lupulone, Hänsel and Wagener (1967) observed no indication of sedation in mice or rats. They used three hop extracts, two produced with ethanol and another with methylisobutylketone. Both ethanolic extracts were dried and administered in oil. Locomotor activity was unaffected up to doses of 500 mg/kg b.w. and no antagonistic effect against metamphetamine-induced stimulation was observed. Hexobarbital-induced sleeping time remained unchanged. In the Rotarod test up to 200 mg/kg b.w. caused no impairment of coordination, and no muscle relaxation was observed.

On the contrary, Bravo *et al.* (1974) described a reduction in locomotor behaviour of mice following the intraperitoneal administration of three different extracts of hops (aqueous, ethereous and alcoholic). The ethereous extract was the most active, since it completely inhibited mice motor activity, 20 minutes after injection. However, whilst this effect was only seen in very high doses, this study clearly showed that the sedative effect of hops is strongly dependent on the type of solvent used for the extraction procedure.

Lee *et al.* (1993a) observed a dose-dependent suppression of spontaneous locomotor activity in mice after the intraperitoneal administration of 100 mg/kg (p<0.05), 250 mg/kg and 500 mg/kg (p<0.001) of hop extract (96% ethanolic dry extract). The same authors further investigated the CNS effects of hop extract, using other behavioural tests such as potentiation of pentobarbital-induced sleep, hypothermic analysis and anticonvulsant tests (Lee *et al.*, 1993b). Pentobarbital-induced sleeping time increased dose-dependently; not significant at 100 mg/kg, by 1.9-fold at 250 mg/kg (p<0.05) and 2.6-fold at 500 mg/kg (p<0.01). In the hot plate test, latency time for licking the forepaws increased with doses of 100 and 250 mg/kg (p<0.01). Rotarod performance decreased by 59% and 65%, respectively, at 250 mg/kg and 500 mg/kg (p < 0.001 after 120 minutes). The time to onset of convulsion and survival time after administration of pentylenetetrazole were significantly lengthened by 500 mg/kg (p<0.001), but not by 250 mg/kg. A significant and time dependent fall in rectal temperature was observed after a dose of 500 mg/kg (p<0.001 after 120 minutes). Thus hop strobile extract showed sedative and hypnotic properties at lower doses (100-250 mg/kg), and at a higher dose of 500 mg/kg it also produced anticonvulsive and hypothermic effects.

Assessors Comment: *The relevance of these findings remains unclear, since all experiments were carried out after intraperitoneal injection and no experiments were done following oral application, so that the bioavailability of the preparation used remains questionable.*

The central effects of a hop CO_2 extract and a fraction containing the α-acids were studied in animal experiments following oral application of the hop extract dissolved in Tween 80 (10%) or the α-acid fraction dissolved in peanut oil. Acute effects on locomotor activity and pentobarbital sleeping time were studied, as well as behavioural parameters in the elevated maze and the forced swimming test. The authors observed a significant increase of the pentobarbital-induced sleeping time in rats without affecting the latency to the loss of the righting reflex. This effect was dose-dependent starting from a minimal dose of 10 mg/kg. Neither the extract nor its α-acid fraction affected the locomotor activity in the open-field test or exerted an anxiolytic effect in rats submitted to the elevated plus- maze test. Interestingly, both hop CO_2 extract and the α-acids containing fraction significantly reduced the period of immobility in the forced swimming test, when administered three times (24 h, 5 h and 1 h), before the test indicating an antidepressant activity. It was concluded that hop CO_2 extract and a hops fraction containing α-acids exert a pentobarbital-enhancing property without influencing the motor behaviour of rats and both had antidepressant activity. These results show that the α-acids present in hop CO_2 extract may explain the use of hops in sleep disturbances and that the α-acids could be a new class of compounds for the development of natural antidepressant agents (Zanoli *et al.*, 2005).

Schiller *et al.* (2006) confirmed the sedating effects of the liphophilic extract reported by Zanoli *et al.* (2005). They investigated several ethanolic extracts (40% V/V and 90% m/m) and CO_2 extracts from diverse hop varieties as well as α-acids and β-acids fractions and pure oil. All hop extracts increased ketamine-induced sleeping time in mice. The increase in duration of ketamine narcosis proved to be a specific central effect and not caused by a pharmacokinetic interaction, as could be confirmed by a comparable increase in ether-induced sleeping time. In contrast to the findings of Zanoli *et al.* they also observed a reduction of locomotor activity. The low doses Zanoli *et al.* applied (20 mg/kg/b.w. as maximum) may be the reason for this discrepancy. Like Zanoli *et al.* no anxiolytic effects of hop preparations were found. A decrease in body temperature induced by all hop extracts as an additional parameter was observed, which confirms the sedating activity of the hop preparations. The results of Schiller *et al.* are similar to those of Lee *et al.* (1993), who found a marked sedation *viz.* a reduction of locomotor activity, increase of pentobarbital-induced sleeping time and antagonistic effects against pentylenetetrazol-induced convulsions following intraperitoneal injection of a hop extract. On the contrary, the results of Hänsel and Wagener (1967) are in clear contrast to the findings of Schiller *et al.* (2006). The latter authors explain this discrepancy by the use of different raw materials, different conditions of storage by which the content of α- and β-acids might be reduced significantly and different extraction solvents used.

Finally, Schiller *et al.* showed that not only the α-acids, but also the β-acids and the hop oil, although to a lesser extent, exert distinct sedating effects and contribute to the activity of the plants. They also do not rule out the presence of further sedating components in hops.

A degradation product of the bitter acids, humulones and lupulones, the five carbon olefinic alcohol, 2'-methyl-3-buten-2-ol, given intraperitoneally to mice at high dosage, 800 mg/kg showed central nervous depressant activity (Hänsel *et al.*, 1980; Wohlfart *et al.*, 1983a). Although present only in small amounts in fresh hops, higher levels of this compound may be generated *in vivo* by metabolism of the bitter acids, reaching its maximum concentration after 2 years of storage at room temperature (Hänsel *et al.*, 1982 ; Wohlfart *et al.*, 1982, Wohlfart, 1983b, Hänsel and Schultz, 1986). The sedative effect of this alcohol is comparable, in the same dosage range, to that of the structurally related drug methylpentynol (Wohlfart *et al.*, 1983a).

Thus, if this compound can fully explain the sedative activity attributed to hops, then it must be formed *in vivo* from hop constituents, such as bitter acids, that would then be considered as "pro-drugs" analogous to the case of the oestrogenic activity (Chadwick *et al.*, 2006).

The hypothesis, however that this alcohol can be formed *in vivo* by metabolisation of α-acids has not been demonstrated to date (Zanoli *et al.*, 2005). Similarly, the proposal that the sedative effect of hops was due to its content of myrcene, which has shown to have analgesic activity in mice (Hänsel and Wohlfart, 1980) has not been established.

Grundmann *et .al.*, (2006) showed that the hypothermic effects of hops could be antagonized with the competitive melatonin receptor antagonist, luzindole. Based upon a study in which it was found that a combination of valerian and hops interacts with serotoninergic 5-HT$_{4e}$, 5-HT$_6$, 5-HT$_7$ and melatoninergic ML$_1$ and ML$_2$ receptors (Abourashed *et al.*, 2004, Butterweck *et al.*, 2007, Brattström, 2007), these authors evaluated the hypothermic activity of hop extract in mice. In a dosage of 250 mg/kg hops extract significantly decreased body temperature in male BL6/C57 mice (ΔT-1,1°C) 2 h after oral administration. The effects of the plant extract were comparable to melatonin (50 mg/kg, ΔT-0.8°C, 2 h after i.p. injection). The hypothermic effects of both melatonin and hop extract could be antagonised with the competitive melatonin receptor antagonist, luzindole.

The authors concluded that this data suggests that the hypothermic effects of hop extract are mediated through activation of melatonin receptors. Since it is known that melatonin has both hypnotic and hypothermic effects at physiological levels and that the hypnotic effect may be mediated via the hypothermic action of melatonin (Zemlan *et al.*, 2005), a similar effect may be suggested for hop extract. The authors also concluded that neither the α- and β-acids, nor the essential oil were responsible for these effects (Personal communication by Butterweck).

In 2012, Franco *et al.* investigated the sedative effects of hop dry extracts on the activity/rest rhythm on common quails on the basis that these animals have a similar diurnal sleep/wake rhythm to humans. The doses administered, close to the content of non-alcoholic beer, were 1, 2, and 11 mg dry extract of hops as one capsule per day, at 18.00 h for one week. A control group received capsules with methylcellulose only and a basal group received no treatment. The chronobiological analysis of the animal's activity captured and logged by the software DAS24 was performed using the Ritme computer program. With the dose of 2 mg there was a statistically significant ($p=$smaller than 0.05) reduction of the arithmetic mean nocturnal activity with respect to the basal control and other doses groups i.e. 1 mg and 11 mg. The authors concluded that the concentration of 2 mg hop extract, similar to the concentration in non-alcoholic beer was more effective in reducing nocturnal activity than other doses of hop extracts, as well as in preserving the circadian activity/rest rhythm. Consequently, on basis of this investigation, they recommended administration of non- alcoholic beer (assuming that normal beer contains about 0.3% hops) as an aid to nocturnal sleep.

3.1.1.2. Oestrogenic effects

3.1.1.2.1. *In vitro* studies

3.1.1.2.1.1. Oestrogenic activity of hop strobiles

Circumstantial evidence over many years, including menstrual disturbances reported to be common among female hop pickers, linked hop strobiles with potential oestrogenic activity (Verzele, 1986; De Keukeleire *et al.*, 1999). In Germany, hop baths were used to treat gynaecologic disorders and hop extracts have been reported to reduce hot flushes in menopausal women (Goetz, 1990). However, early studies to confirm this activity experimentally were inconclusive or contradictory due to methodology of inadequate sensitivity (De Keukeleire *et al.*, 1997, 1999).

In a recent screening of plant drugs for oestrogenic activity, a 50% ethanolic extract (2 g of hop strobile to 10 ml) exhibited binding to oestrogen receptors in intact, oestrogen-dependent [ER(+)], human breast cancer MCF-7 cells with a potency equivalent to 0,5 µg of oestradiol per 2 g of dried strobile (for comparison, the potencies of 2 g of thyme or red clover were equivalent to 0.5 or 3 µg of oestradiol, respectively).

The extract also showed significant ability to stimulate cell proliferation in ER (+)T47D, but not in ER(-) MDA 468, breast cancer cells (Zava *et al.*, 1998). In contrast, in a different series of experiments, a similarly prepared extract of hop strobile at concentrations of 0.01-1.0% V/V was found to significantly inhibit serum-stimulated growth of ER(+)T47D breast cancer cells (p<0.001) (Dixon-Shanies and Shaikh, 1999).

Ovarian cells isolated from immature female rats, which 48 hours previously had been injected (primed) with pregnant mare's serum gonadotrophin, were incubated with follicle-stimulating hormone to induce oestradiol secretion. Addition to the culture medium of purified water-soluble fractions F_1 or F_2 from defatted hop strobile extract reduced the amounts of oestrogen E_2 released from the ovarian cells (p<0.01) with a probably related decrease in cAMP release (p<0.05) (ESCOP,2003).

In 2005, Overk *et al.* compared the oestrogenicities of the extracts of hops and red clover (*Trifolium pratense*) and those of their individual constituents, including prenylated flavanones and isoflavonoids, using a variety of *in vitro* oestrogenic assays. The hop extract consisted of a chloroform partition of a methanolic extract from a previously SF-CO_2-extracted Nugget hops cultivar and the red clover extract was an ethanolic extract containing 30% isoflavonoids prepared for a phase II clinical trial.

The IC_{50} values for the oestrogen receptor α and β binding assays (according to Obourn *et al.* (1993) and Liu *et al.* (2001), were 15 and 27 µg/ml, respectively for hops and 18.0 and 2.0 µg/ml, respectively, for the red clover extract.

Both of the extracts also demonstrated significant activities of transiently transfected ERE-luciferase, quantitative RT-PCR of an oestrogen-inducible gene, and AP-enzyme induction assays (EC_{50} values of 1.1 for hop extracts and 1.9 µg/ml for red clover extracts).

3.1.1.2.1.2 *Oestrogenic activity of 8-prenylnaringenin (8-PN)*

8-PN, a flavanone occurring in hop strobiles at levels of 25-60 mg/kg (Rong *et al.*, 2000), has been shown to be a potent phyto-oestrogen with activity greater than that of other established plant oestrogens (Milligan *et al.*, 1999).

Oestrogenic activity of a much lower order (less than one-hundredth of that of 8-PN) has also been detected in three other hop flavanones, 6-prenylnaringenin (6-PN), 8-geranylnaringenin (8-GN) and 6,8-diprenylnaringenin (6,8-PN), while xanthohumul (X) and isoxanthohumol (IX) were found to be inactive (Milligan *et al.*, 2000). EC_{50} values for 17β-oestradiol, 8-PN, 6-PN, coumestrol, genistein and daidzein were 0.3, 40, > 4000, 70, 1200 and 2200 nM, respectively in a screen using oestrogen-inducible yeast (*Saccharomyces cerevisiae*) expressing the human oestrogen receptor, and 0.8, 4, 500, 30, 200 and 1500 nM respectively, for stimulation of alkaline phosphatase activity in a human endometrial cell line (Ishikawa Var I). The relative binding affinities of 17β-oestradiol, 8-PN, coumestrol and genistein with rat uterine cytosol containing soluble oestrogen receptor were 1, 0023, 0008 and 0.003 respectively (Milligan *et al.*, 1999).

The high oestrogenic activity of 8-PN was confirmed by Zierau *et al.* (2002) using two different bioassays *viz.* a yeast based oestrogen receptor assay, containing a stably transfected oestrogen receptor α (ERα) construct and an expression plasmid carrying oestrogen-responsive sequence

controlling the reporter gene lac-Z encoding the enzyme β-galactosidase and a transactivation assay using MVLN cells, a bioluminescense MCF-7 derived cell-line.

The same research group showed, using a yeast-based androgen receptor assay, strong anti-androgen activities for 8-PN and 6-(1,1-dimethylallyl)naringenin (6-DMA-N), while the parent compound naringenin was inactive. In an androgen receptor activity-assay based on the analysis of putative specific antigen (RSA) concentrations in the supernatants of treated PC3(AP)2 cells only 6-DMA-N showed antiandrogenic activity at concentrations of 10^{-5}. 8-PN and naringenin had no detectable antiandrogenic effects. 6-DMA-N is a structurally related compound, isolated from the African tree *Monotes engleri*
(Zierau *et al.*, 2003).

The oestrogenic activity of 8-PN was further confirmed in competitive binding assays using purified human recombinant oestrogen receptors α and β (ER_α and ER_β). 8-PN competed strongly with 17β-oestradiol for binding to both receptors with a relative binding affinity of about 0.1, compared to 1.0 for 17β-oestradiol and 0.001 for 8-GN (Milligan *et al.*, 2000).

In another study, involving displacement of [^3H]-17β-oestradiol, 8-PN showed competitive binding affinity for the oestrogen receptor in bovine uterine cytosol with an IC_{50} of 140 nM, compared to 1,0 nM for 17β-oestradiol and 320 nM for genistein. 8-PN also dose-dependently stimulated the proliferation of cultured, oestrogen-dependent, human breast cancer MCF-7 cells with an EC_{50} of 1,9 nM, compared to 0,0032 nM for 17β-oestradiol and 47 nM for genistein, suggesting that it was an oestrogen receptor agonist (Kitaoka *et al.*, 1998).

The same authors synthetized racemic 8-PN, separated and assayed both enantiomers, and reported that there was no significant difference in ER binding potency between the 2R and the 2S forms (Kitaoka *et al.*, 1998).

In an *in vitro* receptor binding assay, using recombinant human ER_α and ER_β from cytosolic SF_9-cell extracts, Schaefer *et al.*, (2003), showed that 8-PN exhibited > 2-fold higher affinity for ER_α than ER_β. Using a mammalian cell-based transactivation assay consisting of U_2-osteosarcoma cells transient transfected with either ER_α or ER_β and a luciferase reporter gene construct these authors demonstrated that 8-PN is the strongest plant-derived ER_α agonist identified so far, being 10-fold more potent than coumestrol and 100-fold stronger than genistein, but only 70 times weaker than 17β-oestradiol. The transactivational analysis revealed also a >3,6-fold higher oestrogenic activity of 8-PN at ER_α than ER_β, a strong contrast to primarily ERβ-activating coumestrol and genistein. At the same time they found that the *in vivo* oestrogenic activity of 8-PN in reproductive tissue was about 20,000-fold weaker compared to 17β-oestradiol. As such, 8-PN mimics the effects of the endogenous hormone and could be a natural SERM (selective oestrogen receptor modulator) with promising potential for the treatment of various oestrogen deficiency-related conditions (Schaefer *et al.*, 2003).

In 2004, Bovee *et al.* developed a rapid yeast oestrogen bioassay stably expressing oestrogen receptors α and β and green fluorescent proteins and tested various phyto-oestrogens. They confirmed the earlier results of Schaefer *et al.* that 8-PN is relatively more potent with ER_α and less potent than coumestrol and genistein with ER_β.

In 2005, Overk *et al.* compared the oestrogenicities of the extracts of hops and red clover and those of their individual constituents *viz.* 8-PN, 6-PN, IX and X from hops and daidzein, formononetin, biochanin A and genistein from red clover. The results of the extracts have been discussed under point 3.1.1.2.1.1. Here we give the results of the individual constituents. The competitive ER_α and ER_β binding assays using tritiated 17β-oestradiol are based on the method of Obourn with minor modifications (Liu *et al.*, 2001). As already mentioned the red clover extract preferentially bound to the ER_β receptor nine times greater than to ER_α. The hop extract had nearly a 2-fold preference for the ER_α

as compared with ER_β. For the isolated compounds, the relative binding affinities for ER_α were as follows: genistein ≈ 8-PN > daidzein > biochanin A > formononetin > IX, while the relative binding affinities for ER_β were genistein > 8-PN ≈ daidzein > biochanin A > formononetin ≈ IX. Genistein and 8-PN had similar affinities for ER_α with IC_{50} values of 0.51 and 0.3 μM respectively, whereas genistein was more selective for ER_β as indicated by the IC_{50} value of 0.020 and 1.7 μM, respectively. Both of the extracts, genistein and 8-PN activated the oestrogen response element (ERE) in Ishikawa cells, while the extracts, biochanin A, genistein, and 8-PN significantly induced ERE-luciferase expression in MCF-7 cells. Hop and red clover extracts as well as 8-PN up-regulated progesterone receptor (PR) m-RNA in the Ishikawa cell line. In the MCF-7 cell-line, PR m-RNA was significantly up-regulated by the extracts, biochanin A, genistein, 8-PN and IX. The two extracts had EC_{50} values of 1.1 and 1.9 μg/ml respectively in the alkaline phosphatase induction assay. The EC_{50} values of the individual compounds are consistent with values reported by Milligan *et al.* (1999) *viz.* 8 PN >> genistein ≈ daidzein > XI > biochanin A. None of the compounds had anti-oestrogenic activity.

3.1.1.2.2. *In vivo* studies

3.1.1.2.2.1. Antigonadotrophic effects of hop strobiles

Purified water-soluble fractions from defatted hop strobile extract were administered subcutaneously twice daily for 3 days to immature female rats primed with 25 IU of pregnant mare's serum gonadotrophin (PMSG). None of the fractions induced a change in uterine weights. However, fractions F_1 (20 mg/rat) and F_2 (50 mg/rat) significantly suppressed PMSG-induced gain in ovarian weights by about 25% (p<0.05) compared to controls. Under the same conditions, two further fractions (4 mg/rat) purified from F_1 suppressed gain in ovarian weights by 42% and 33% (p<0.01) compared to controls (ESCOP, 2003). In further experiments on PMSG-primed immature rats, by comparison with saline-treated control animals, subcutaneously administered fractions F_1 and F_2 reduced the number of ovulations (p<0.05) ; suppressed levels of serum luteinizing hormone (p<0.001) ; suppressed thymidine kinase activity in uterine tissue (p<0.01) ; reduced 17β-oestradiol E_2 secretion in cultures of ovarian cells from the rats (p<0.001) ; and reduced progesterone production in cultures of luteal cells from the rats (p<0.05 to p<0.001) (ESCOP, 2003).

To date no other *in vivo* studies on the oestrogenic effects of hop strobiles have been published.

3.1.1.2.2.2. Oestrogenic effects of 8-prenylnaringenin (8-PN)

In vivo studies with 8-PN are scarce. Only one report highlighted favourable effects of hops on hot flushes in humans (Goetz, 1990), but preclinical studies aimed at evaluating the efficacy of 8-PN for alleviating menopausal symptoms and discomforts are still ongoing. Most investigators to date have focused on animal models. It should be noted that the relative potency of oestrogenic compounds *in vivo* largely depends on a number of factors, including the route of administration and the nature of the response monitored. Moreover, estimates of oestrogenic activity are markedly affected depending on the nature of the *in vivo* bioassay used.

Milligan *et al.* (2002) tested 8-PN in two *in vivo* assays, *viz.* an assay based on the rapid response of the uterine vasculature to oestrogenic stimulation and an assay based on the uterotrophic response and mitotic responses of the uterine and vaginal epithelium. In both assays ovariectomized female Swiss albino mice, about 2-3 months of age, were used. In the first assay, a quantitative index of the vascular permeability was obtained from the leakage of radiolabelled albumin from the circulation. Several test substances were subcutaneously administered to the ovariectomized mice and after 4 hours a significant increase in the vascular permeability was obtained for oestriol and 17β-oestradiol, but both coumestrol and 8-PN were considerably less potent (< 1% relative to 17β-oestradiol). The

dose-response relationship for 8-PN was similar to that of coumestrol, and a large stimulatory effect was produced by 100 mmoles 8-PN. The amount of genistein required to produce the same effect was at least ten-fold greater. The administration of daidzein produced no detectable uterine vascular response at the doses used. The responses of 8-PN and 17β-oestradiol were blocked completely by prior treatment of the animals with anti-oestrogen
(ICI 182, 780).

In the second assay the oestrogenic potency of the test substances was tested after continuous administration in the drinking water by monitoring the uterotrophic response and cell mitosis in the vaginal and uterine epithelia. Both 8-PN (100 μg/ml) and 17β-oestradiol (100 ng/ml) produced significant increase in vaginal mitosis after 72 h compared with the negative control ($p<0,05$ and $p<0,005$, respectively). However, although 17β-oestradiol also produced significant increases in uterine mass and in epithelial mitosis, there were no significant differences in the mice exposed to any of the 8-PN treatments.

They concluded that in addition to its *in vitro* activity 8-PN shows oestrogenic effects in the two *in vivo* test systems. It was noted that 100 μg 8-PN/ml in the drinking water (equivalent to an intake of about 15 mg per kg per day) was able to induce the characteristic oestrogenic mitotic response in the vaginal epithelium of ovariectomized mice. The apparent lack of effect of 8-PN on uterine epithelial mitosis may reflect with the limited amounts given or the temporal differences in mitotic responses induced by oestrogens in the uterine luminal, uterine glandular and vaginal luminal epithelia of ovariectomized mice (Finn and Publicover, 1981).

Coldham and Sauer (2001) showed that a dietary supplement for breast enhancement containing hop extracts was only weakly active in mouse uterotrophic assays following administration in feed or after subcutaneous injection of the extract at doses of 8-PN up to 250 times higher than that recommended for women. They concluded that the dietary supplement is unlikely to produce oestrogenic effects *in vivo* at the level of the uterus.

In other experiments ovariectomized rats, as a model for oestrogen deficiency induced osteoporosis, were treated subcutaneously with racemic 8-PN (OVX + 8-PN) at 30 mg/kg/day or with 17β-oestradiol (OVX + OE) at 0.01 mg/kg/day, or with vehicle only (OVX). Another group of rats was sham-operated, i.e. subjected to ovariectomy surgery without removing the ovaries, and treated with the vehicle only (sham). After 2 weeks of treatment, 24-hour urine samples were collected and body weight gain, uterine weight and bone mineral density were determined. The uterine weights of sham and OVX + 8-PN rats were found to be 165% higher ($p<0.001$), and of OVX + OE rats 235% higher ($p<0.001$), than those of OVX rats. Urinary excretion of hydroxyproline (a conventional marker of bone resorption) was 1.62 μg/g/day from OVX rats compared to 1.18 and 1.16 μg/g/day for sham and OVX + OE rats respectively Body weight gains of sham, OVX + OE and OVX + 8-PN were significantly lower ($p<0,05$) than those of ($p<0.01$), and 1.01 μg/g/day from OVX + 8-PN rats ($p<0.001$). The levels of urinary hydroxypyridinium cross-links (assayed as pyridinoline and deoxypyridoline), which are recognized to be directly related to bone matrix degradation, were significantly lower in sham rats ($p<0.01$), and in OVX + OE and OVX + 8-PN rats ($p<0.001$), than in OVX rats. Bone mineral densities of 139.1, 141.9 and 141.9 mg/cm^2 in sham, OVX + OE and OVX + 8-PN rats, respectively, were significantly higher ($p<0.001$) than that in OVX rats (132.1 mg/cm^2). It was concluded that 8-PN functions as an oestrogen receptor agonist in reproductive tissues and that the dosage used had completely prevented ovariectomy-induced bone loss (Miyamoto *et al.*, 1998).

Recently, the influence of 8-PN on oestrogen-related gene expression in liver and uterus tissues was investigated in rats (Diel *et al.*, 2004). At a 100-fold dose with respect to 17β-oestradiol, 8-PN induced a qualitatively similar, but less pronounced expression profile. On the other hand, 8-PN was found to be more potent than 17β-oestradiol in inducing expression of IGFBP-1 (Insulin growth factor binding

protein-1). This factor has been correlated to an improvement of the vascular endothelial function and blood pressure homeostasis (Laughlin *et al.*, 2004), while higher concentrations of IGFBP-1 are also associated to a decreased risk of prostate cancer (Ngo *et al.*, 2003).

Schaefer *et al.* (2003) determined the oestrogenic potency *in vivo* and *in vitro* of 8-PN using the classic uterine growth assay and vagina growth assay in juvenile rats. Their findings confirm the conclusions of Diel *et al.* (2004) that 8-PN is a pure oestrogen agonist *in vitro*, exhibiting an oestrogenic activity profile comparable to oestron. The *in vivo* oestrogenic activity of 8-PN in representative tissue, however, is 20,000-fold weaker compared to 17β-oestradiol. They also suggested that 2S(-)-8-PN has moderately higher ER affinity and oestrogenic activity, *in vitro* and *in vivo* than 2R(+)-8-PN.

In 2005, Hümpel *et al.* studied 8-PN in adult ovariectomized rats, an established animal model to mimic hormone dependent osteoporosis in menopausal women. The study demonstrated that 8-PN can completely protect from ovariectomy induced bone-loss while exhibiting minimal (dose independent) trophic effects on uterus and endometrium. It is estimated that at equivalent bone protective doses of 17β-oestradiol and 8-PN, the phyto-oestrogen has a 10-fold lower stimulatory effect on uterus and endometrium. The tissue specific effect of 8-PN was confirmed in a transgenic receptor mouse model (ERE-LUC mice). Here they also found pronounced oestrogenic activity in prostate.

In a recent *in vivo* study using the medaka sex reversal/vtg gene expression assay no oestrogenic effects of all the naringenin-type flavonoids including also 8-PN were observed. Natural sex steroid hormones and isoflavonoids such as genistein, on the contrary, can functionally reverse the phenotype sex of fish (Zierau *et al.*, 2005).

3.1.1.3. Conclusions on sedative and oestrogenic effects

Sedative effects

Although no *in vitro* studies, directly related to the sedative activity of hop strobiles have been published, several studies in animals have been carried out to investigate the neuropharmacological properties of *Humulus lupulus*, traditionally used in the treatment of different CNS disorders such as insomnia, excitability and restlessness.

One of the studies showed that hop strobile extracts exert sedative and hypnotic properties in mice at lower doses (100-250 mg/kg) and produce other activities such as anticonvulsive and hypothermic effects at higher doses of 500 mg/kg.

Hop extracts were able to prolong the pentobarbital sleeping time without affecting the latency to the loss of the righting reflex in rats, confirming the first study concerning the hypnotic properties and the traditional observation of sleepiness in hops-pickers. In the same study it was shown that the α-acids were responsible for the pentobarbital sleep-enhancing properties as well as for the antidepressant activity found for the hop strobile extract.

In a study, carried out with several ethanolic and CO_2 extracts of hops, administered by gavage to mice, it was clearly shown that all extracts reduced the spontaneous locomotor activity, increased the ketamine-induced sleeping time and reduced body temperature, confirming a central sedating effect. No indications of anxiolytic activity were found for any of the test preparations. The sedating activity could be attributed to three categories of constituents of lipophilic hop extracts, however, Whilst the α-bitter acids proved to be the most active constituents, the β-acids and the hop oil clearly contributed to the sedating effect of lipophilic hop extracts as well.

The authors also suggested that the contradictory results obtained by different research teams in the determination of different pharmacological effects was due to the use of different raw materials,

different storage conditions leading to various amounts of active components and the use of different extraction solvents for the production of the preparations.

Furthermore, candidate molecules for the sedative effects of hop strobiles such as myrcene and 2'-methyl-3-buten-2-ol have been suggested, but their role as sedative agents has not been substantiated.

It has been shown that one of the chalcones, *viz.* xanthohumol, influences the $GABA_A$ receptors and their lateral mobility at hippocampal neurons in a similar way as the benzodiazepine agonist midazolam without interfering with the benzodiazepine receptors. Thus, xanthohumol may play an important role in the sedative effect of hop preparations, but this should be confirmed by *in vivo* studies.

More recently the *in vitro* study in which it was suggested that a combination of hops and valerian interacts with melatoninergic ML_1 receptors was confirmed by an *in vivo* investigation on mice. It was shown that hop extracts (250 mg/kg) had comparable hypothermic effects as melatonin (50 mg/kg), which could be blocked by the competitive melatonin receptor antagonist luzindole. Since it is known that the hypnotic effect of melatonin may be mediated via its hypothermic action, a similar effect for hops may be postulated.

The authors also concluded that this effect was not due to the acids or the essential oil of hops, as both were absent in the hop extract used in this study.

Oestrogenic effects

In contrast to the studies on the sedative effects, the phyto-oestrogen, responsible for the oestrogenic activity of hop strobiles has been isolated and identified as the prenylated flavanone, 8-prenylnaringenin or hopein (8-PN). The oestrogenic activity proved to be considerably greater than that of established phyto-oestrogens such as coumestrol (present in red clover) and genistein and daidzein (present in soy). The oestrogenicity has been examined in great detail by a number of independent research groups worldwide. The other prenylated flavonoids were extremely weakly oestrogenic or devoid of any oestrogenicity. 8-PN exerts its activity through oestrogen receptor-mediated mechanisms. It binds very strongly to both oestrogen receptor isoforms (ER_α and ER_β), but in contrast to most known phyto-oestrogens, 8-PN is selective for the oestrogen receptor-α and as such it mimics the effects of the endogenous 17β-oestradiol. Both have similar profiles, however, the activity of 8-PN is 5- to 100-fold weaker depending on the test system and the particular reaction conditions.

8-PN is orally active in ovariectomized mice as a significant increase in vascular permeability was observed within 4 hours after subcutaneous injection of both 17β-oestradiol and a 100-fold dose of 8-PN. Administration of 17β-oestradiol (100 ng/ml) or 8-PN (100 µg/ml) to the drinking water showed, after 71 h, a considerable increase in vaginal mitosis. Although 17β-oestradiol caused also a substantial increase in uterus weight and in epithelial mitosis, significant differences with 8-PN were not observed.

In other experiments, ovariectomized rats were injected with 8-PN over a period of 14 days. A favourable effect on bone metabolism was observed. Removal of the ovaria normally results in a drastic increase in levels of bone resorption markers in urine, a decrease in mineral bone density and a reduction in uterus weight. Treatment with 17β-oestradiol (0,01 mg/kg/day) or 8-PN (30 mg/kg/day) gave quantitatively comparable effects on bone and uterus showing that 8-PN functions as an oestrogenic agonist.

The influence of 8-PN on oestrogen-related gene expression in liver and uterus tissues was investigated in rats. At a 100-fold dose with respect to 17β-oestradiol, 8-PN induced a qualitatively similar, but less pronounced expression profile. On the other hand a stronger up-regulation of the

expression of IGFBP-1 was observed. This growth factor has been correlated to an improvement of the vascular endothelial function and blood pressure homeostasis, while higher concentrations of IGFBP-1 are also associated with a decreased risk of prostate cancer.

In conclusion, 8-PN, present in hop strobiles at levels of 25-60 mg/kg, should be considered to be the active oestrogenic compound of hops. It should be noted that desmethyl-xanthohumol serves as a pro-oestrogen, since it can be metabolised into a mixture of 8-PN and 6-PN.

3.1.2. Secondary Pharmacodynamics

3.1.2.1. Other pharmacological activities of hop strobiles and/or its constituents

In a very comprehensive review article, De Keukeleire and Heyerick (2005) discussed the intriguing biological activities of hops and its constituents.

3.1.2.1.1. Hop essential oil and hop acids

Antimicrobial activity

Some antimicrobial activity of hop essential oil has been reported and hops are included in various cosmetic preparations. The lupulones (β-acids) have a longstanding reputation as antibacterials (against gram-positive bacteria), which had led to some applications of hop products rich in β-acids, particularly in the sugar industry. It appears that the three prenyl groups present in lupulone interfere with the building up and the functioning of the bacterial cell walls leading eventually to leakage of the cell contents.

Resistance of gram-negative bacteria to the resin acids is attributed to the presence of a phospholipid-containing outer membrane, as lupulones and humulones are inactivated by serum phospholipids. Structure-activity relationship studies have indicated the requirement of a hydrophobic molecule and a six-membered central ring for such activity.

The acids are thought to possess little activity towards fungi or yeasts. However, antifungal activity has been documented for the bitter acids towards Trichophyton, Candida, Fusarium and Mucor species (De Keuleleire and Heyerick, 2005).

Antidiabetic activity

Isohumulones are the main bittering principles in beer. Recently, interesting antidiabetic properties of isohumulones have become apparent. Via a reporter system, it was found that agonistic effects were exerted on the activity of the nuclear receptors PPARalpha ('Peroxisome Proliferator-Activated Receptor-alpha') and PPARgamma. The PPAR's are important regulators of the glucose and fat metabolisms and agonists are applied to treat non-insulin-dependent diabetes (diabetes type II) and hyperlipidemia. Administration of isohumulones to a mouse model for diabetes type II (KK-A^y) led to a decrease of plasma triglycerides and free fatty acids in a dose-dependent manner. Moreover, the glucose levels were significantly diminished in KK-A^y-mice. The results indicate that intake of isohumulones may favourably influence conditions of hyperglycemia and hypertriglyceridemia. Treatment of 20 subjects suffering from mild diabetes with isohumulones (twice a day for 12 weeks), in dosages that were equivalent to only a few glasses of strongly hopped beers, showed a decline in glucose levels and other parameters, indicating that oral intake of isohumulones ameliorates insulin sensitivity in patients with diabetes type II(De Keukeleire and Heyerick, 2005).

Activity on osteoporosis

Humulone, the major constituent of the mixture of α-acids, has been shown to inhibit bone resorption using an *in vitro* "pit formation assay" (formation of pits on dentine slices incubated with mouse bone cells). Xanthohumol and humulone have been identified as inhibitors of bone resorption at concentrations at or above 10^{-6} and 10^{-11}, respectively ($p<0.01$).

Humulone showed high inhibitory activity with an IC_{50} of 5.9×10^{-9} M .These findings indicate that hops may be active against osteoporosis (ESCOP, 2003).

It should be noted, however, that xanthohumol and especially humulone, have no oestrogenic activities, which may indicate that the inhibition of bone resorption of hops is not associated with oestrogenicity (De Keukeleire *et al.*, 1999).

Anti-inflammatory activity

Humulone proved to be a potent inhibitor of the expression of cyclooxygenase-2 (COX-2) via interaction with NFκB, which translates into pronounced anti-inflammatory activity (ESCOP, 2003). *In vivo* anti-inflammatory effects have also been seen for humulone, which appears to be the active anti-inflammatory agent of hop strobiles.

A dry methanolic extract of hop strobile, applied topically at 2 mg/ear, inhibited 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced ear oedema in mice by 90% ($p<0.01$) six hours after TPA treatment. Humulone, isolated from hop strobile by bioassay-guided fractionation and identified as an anti-inflammatory constituent, inhibited the oedema with an ID_{50} of 0.2 mg/ear (ID = inhibitory dose).

Topically applied humulone also inhibited arachidonic acid-induced inflammatory ear oedema in mice with an ID_{50} of 2.2 mg/ear ($p<0.01$ against controls) compared to 0.4 mg/ear ($p<0.01$) for indometacin (ESCOP, 2003).).

Antiproliferative activity

Humulone has been found to inhibit angiogenesis (formation of new blood vessels, which is essential for tumour growth), as well as proliferation (uncontrolled growth) of endothelial cells (Shimamura *et al.*, 2001). The latter effect has been confirmed by an *in vivo* test in which tumour promotion could be inhibited.

Humulone applied topically at 1 mg/mouse to the backs of mice markedly inhibited the tumour-promoting effect of TPA on 7,12-dimethylbenz[a]-anthracene-initiated skin tumour formation. In the control group, 100% of mice developed tumours (first tumour appeared in week 6), compared to only 7% in the humulone-treated group (first appearance in week 16). Humulone treatment resulted in a 99% reduction in the average number of tumours per mouse at week 18 ($p<0.01$) (ESCOP, 2003).

3.1.2.1.2. Hop prenylflavonoids

Cancer chemopreventive activity

8-Prenylnaringenin (8-PN) is effective in the aggregation of MCF-7/6 breast cancer cells, which indicates that the compound may inhibit metastasis (Rong *et al.*, 2001). Moreover, it was shown that 8-PN inhibits angiogenesis *in vitro* and *in vivo* (Pepper *et al.*, 2004). Various tests using different cancer cell lines showed no toxicity of 8-PN at levels below 50 μg, while antiproliferative activities were also observed (Tokalov *et al.*, 2004).

Xanthohumol (X) and to a lesser extent dehydrocycloxanthohumol (DHX) and isoxanthohumol (IX) show a broad spectrum of inhibitory mechanisms at the initiation, promotion and progression stage of carcinogenesis (Gerhauser *et al.*, 2002).

On examining proliferation of human breast cancer cells (MCF-7 and MDAMB 231), colon cancer cells (HT-29), prostate cancer cells (LNCaP and PC-3) and cancer cells of the ovaries (A-2780), a dose-dependent inhibition of the growth of all cancer cells was noted on addition of low concentrations of X (low micromolar range) (Gerhauser et al., 2002). The activity is correlated with inhibition of DNA polymerase-alpha, the only eukaryotic enzyme capable of initiating DNA synthesis de novo. In the presence of X, many cancer cells undergo apoptosis and programmed cell death. An additional antiproliferative mechanism was found in HL-60 leukemia cells (Gerhauser et al., 2002). Terminal cell differentiation is induced whereby cancer cells start to behave normally again. In these tests, X was 10-fold more active than genistein present in soy.

Inhibition of carcinogenesis ex vivo was also demonstrated for X using the so-called mouse mammary gland organ cultures. In this model system, breast tissue is removed from mice and small cancer lesions are caused by addition of a potent carcinogen. Extremely low concentrations of X (low nanomolar range) completely inhibited formation of these lesions (Gerhauser et al., 2002). Interestingly, resveratrol, a stilbenoid-type polyphenol held responsible for a number of health-beneficial effects of red wine, was 210-fold less active in this assay.

X showed an inhibitory activity on specific cytochrome P450 enzymes that mediate the conversion of procarcinogens to carcinogens (Miranda et al., 2000a; Henderson et al., 2000). Also, it was observed that X induces the activity of the enzyme quinone reductase in vitro, which is indicative of detoxification of a variety of carcinogens (Dietz et al., 2005). The compound was able to scavenge reactive oxygen species and to interfere with formation of radicals. On trapping of hydroxyl and peroxy radicals, X was 9- and 3-fold, respectively, more active than a well-known reference, Trolox (Gerhauser et al., 2001b), while inhibition of oxidation of low-density lipoproteins (LDL) was more effective than that caused by alpha-tocopherol (Miranda et al., 2000c).

Dorn et al. (2010) have shown that X at a concentration of 25 μM induced apoptosis in two HCC cell lines (HepG2 andHuh7). Furthermore, X repressed proliferation and migration, as well as TNF-kappaB activity and interleukin-8 expression in both cell lines at even lower concentrations. In contrast, X concentrations up to 100 μM did not affect viability of primary human hepatocytes in vitro.

Interestingly, studies have shown that X has anti-inflammatory properties by inhibition of the activities of cyclooxygenases (COX-1 and COX-2) that account for the production of prostaglandins (Gerhauser et al., 2002).

Effects on the synthesis of triglycerides

Xanthohumol interferes considerably in the synthesis of triglycerides. The activity of diacylglycerol transferase, a liver enzyme which is involved in the formation of glycerides, was substantially inhibited (ESCOP, 2003). This interaction is associated with positive effects on hypertriglyceridemia including a decreased risk for associated diseases such as atherosclerosis and diabetes. It was found, using HepG2 cells as model system, that X decreased apolipoprotein B secretion in a dose-dependent manner under both basal and lipid-rich conditions (Casaschi et al., 2004). Furthermore, X inhibited the synthesis of triglycerides in the microsomal membrane and the transfer of newly synthesized triglycerides to the microsomal lumen indicating that triglycerides availability is a determining factor in the regulation of apolipoprotein B secretion. The inhibition of triglycerides synthesis is caused by a reduction in diacylglycerol transferase activity suggesting that X is a potent inhibitor of apolipoprotein B secretion.

Antiplatelet activity

Xanthohumol was shown to possess antiplatelet activity which may initially inhibit the P13-kinase/Akt , p38 MAPK, and PLCy2-PKC cascades, followed by inhibition of the thromboxane A2 formation, thereby leading to inhibition of Ca-ions, and finally to inhibition of platelet aggregation (Lee et al., 2012).

Antiviral activity

Xanthohumol enhances the antiviral effect of interferon alpha-2b against bovine viral diarrhoea virus, a surrogate of hepatitis C virus (Zhang *et al.*, 2010).

3.1.2.1.3. Conclusions on other pharmacological activities

Besides having the most potent phyto-oestrogen currently known with possible health beneficial properties (8-PN), hops also contains xanthohumol, the quantitatively most important prenylchalcone (up to 1,3% m/m), which displays potential therapeutic properties (Gerhauser and Frank, 2005)

This compound shows a wide spectrum of inhibition mechanisms at all stages of carcinogenesis. Low concentrations of X dose-dependently inhibit the growth of cancer cells among them breast cancer cells (MCF-7 and MDAMB-231) and cancer cells of the ovaries (A-2780).

Consequently, if hop-based phytotherapeuticals were to be developed as potential alternatives to hormone replacement therapy for menopausal women, not only the high oestrogenic potential of 8-PN, but also the cancer chemopreventive activity of xanthohumol should be taken into account. Standardisation of both active components in hop-derived extracts would need to be considered. The studies described in this assessment report may be considered as a rationale for further studies and trials to establish efficacy.

3.1.3. Interactions

Limited evidence from one animal study suggests that hops may potentiate the effects of sedative drugs (Lee *et al.*, 1993b).

Interactions with other phyto-oestrogens such as alfalfa, black cohosh, bloodroot, burdock, kudzu, licorice, red clover, soy and others, are theoretically possible. It should be noted, however, that like 17β-oestradiol, 8-PN is an agonist, whereas phyto-oestrogens containing isoflavonoids as active oestrogenics are known to be mixed agonists/antagonists. Soy isoflavonoids act as agonists when no endogenous oestrogens are available, but become antagonists when high endogenous levels of oestrogens are available.

Laboratory research shows that oestrogen-like substances in hops may have effects on oestrogen-sensitive parts of the body. It is not clear what interactions may occur when hop extracts are used together with other hormonal therapies such as birth control pills, hormone replacement therapy, tamoxifen, or aromatase inhibitors such as letrozole, because no studies have been conducted. Hops may interfere with the way the human body processes certain drugs using the liver's 'cytochrome P450' enzyme system, so that the levels of certain drugs may be decreased in the blood, but they have not been confirmed by studies. Clinical cases of drug interactions with hops have not been reported to date.

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

3.2.1. Herbal substance

So far no *in vivo* pharmacokinetic investigations were performed on hop extracts, not only due to the complexity of the phytochemical composition but also due to the many metabolisations of the different compounds of hops, which take place after intake in humans.

Several research teams have published their findings on the metabolism of 8-prenylnaringenin (8-PN), xanthohumol (X) and isoxanthohumol (IX). A first indication on the ADME characteristics of these three compounds can be deduced from CaCo-2 experiments performed by Bracke *et al.* (Bracke *et al.*, 2006. personal communication). They determined "the apparent permeability coefficient" (P_{app}) as an important parameter for the absorption efficacy through the gut epithelium. In this *in vitro* system, P_{app} > 1x10^6 cm/s values are correlated with a good absorption, whereas values p_{app} < 1x10^7 cm/s are correlated with weak absorption (< 1%). For values in between, there is no real relationship between P_{app} and absorption, but the absorption is considered between > 1% and < 100% (Grès *et al.*, 1998). 8-PN and IX showed P_{app} values of respectively 1.3x10^{-6} cm/s and 3.3x10^{-6} cm/s, showing that these compounds could have a good bioavailability, whereas the P_{app} of X (6.7x10^{-7} cm/s) showed that it might be less bioavailable. Beside the information on the absorption efficacy, the CaCo-2 system also indicated that phase-II metabolites (glucuronidation and sulphation) are formed. A similar study has been published by Nikolic *et al.* (2006). The apparent permeability coefficients were 5.2 ± 0.7 x 10^{-5} and 4.9 ± 0.5 x 10^5 cm/s respectively, indicating a good absorption via passive diffusion. According to these authors, the P_{app} values are similar to those of drugs such as propanolol and testosterone, which are often used as high permeability standards.

The metabolism of prenylated flavonoids is not well documented. The biotransformation of flavonoids takes place at several sites in the body, but mainly in the liver and the gut lumen, so that the gut flora might play a significant role as well. As cytochrome P450-enzymes (CYP) are abundantly present in the liver, they also are important elements in the metabolization process of flavonoids.

Several studies showed that flavonoids are mostly excreted as glucuronides in humans and animals. Glucuronidation is the most important reaction route for the phase II detoxification process for most of the xenobiotics. The functionalization is catalysed by the membrane-bound UDP-glucuronosyltransferase, mainly catalysed in the endoplasmatic reticulum of the liver. In an *in vitro* study with rat-derived and human liver microsomes, the glucuronidation of X was studied. Two important glucuronidates were characterized as respectively C-4'- and C-4-monoglucuronides of X. It is also important to mention that the conjugated flavonoids have a significant biological activity (e.g. antioxidant effects).

As for 8-PN, an *in vitro* study with human liver microsomes has revealed several metabolites. LS-MS analysis showed that the most important breakdown products are the result of oxidation (**Fig. 1**). In this process, a hydroxyl group can appear on the prenyl chain, but also a modification of the flavanone skeleton has been observed. The most frequent metabolites were oxidation products of the prenyl group, in which the terminal methyl function was hydrolysed. This is in contrast to xanthohumol, which did not show a similar metabolism (Nikolic *et al.*, 2004; Zierau *et al.*, 2004).
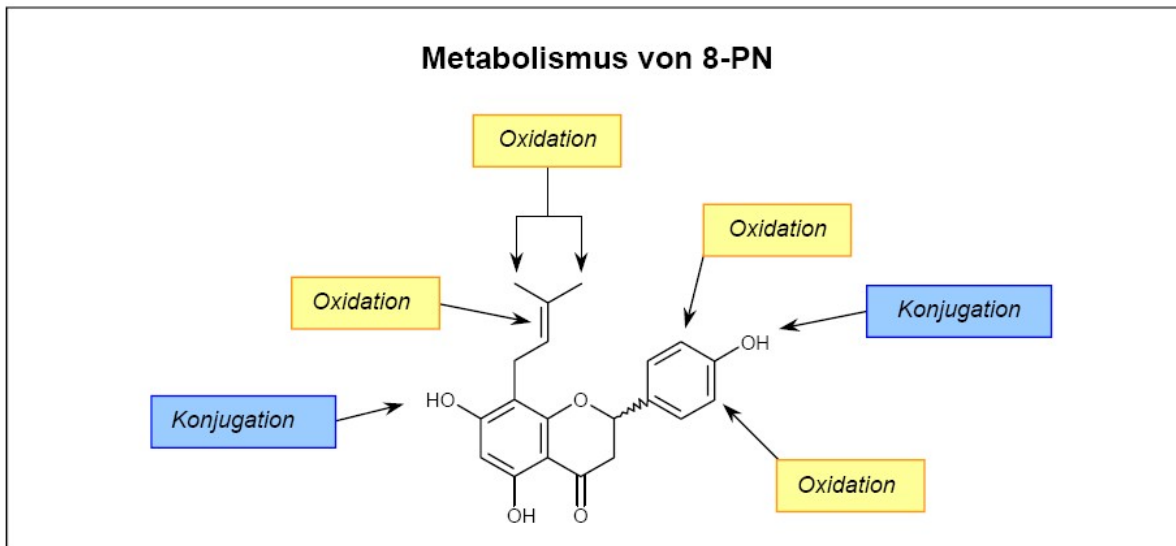
Fig. 1 Metabolism of 8-PN

Guo *et al.* (2006) performed a study on the identification of human hepatic cytochrome P450 enzymes involved in the metabolism of 8-PN and IX from hops. CYP2C19 was found to catalyze the formation of both cis- and trans-alcohols (phase I metabolisation) of the prenyl side chain of 8-PN. CYP2C8 converted 8-PN regioselectively to the trans-alcohol of the prenyl group. Finally, CYP1A2 was found to catalyze the O-demethylation of IX to generate 8-PN. The enzymes CYP1A2, CYP2C19 and CYP2C8 exhibit polymorphism in humans. The results obtained by Guo *et al.* suggest that the oestrogenicity of hop constitutents *in vivo* will depend in part on metabolic conversion that may show individual variation.

The possibility that IX would act as a pro-oestrogen was considered by Coldham *et al.* (2002). The assumption was based on the extensive biotransformation capacity of the liver, which includes demethylation. However, the exposure of IX to liver microsomes did not lead to an increase in oestrogenic activity, from which it was concluded that no 8-PN was produced. In contrast, Nikolic *et al.* (2005) described how liver microsomes can demethylate IX, but not X. However, it was shown that, besides demethylation, microsomes also modify the prenyl side-chain, finally resulting in a large variation of minor degradation products. Schaefer *et al.* (2004) identified low levels of 8-PN in urine after oral intake of IX by two test persons and attributed this to demethylation by the liver.

Besides the liver, the colon is also an important transformation site in the human body. The human colon contains ~ 10^{12} micro-organisms/cm^3 (about 400 different species), with an enormous catalytic and hydrolytic potential. The importance of this microbial population in the metabolism of phyto-oestrogens in general has been clearly established. Decroos *et al.* (2005) recently isolated a microbial consortium capable of transforming the soy phyto-oestrogen daidzein into equol. Moreover, several intestinal bacteria were shown to enhance the bioavailability of phyto-oestrogens as they possess β-glucosidases, which are necessary for the hydrolysis of phyto-oestrogen glycosides (Rowland *et al.*, 2003). Thus, the gut microbiota are considered to be a factor of utmost importance for phyto-oestrogen bioavailability (Turner *et al.*, 2003). In view of the importance of the microbial microflora observed with other well-known phyto-oestrogens, the group of Verstraete (University of Ghent, Belgium) has launched a study to investigate the most important substances in the hops extract (IsoX, 8-PN and X) in a SHIME reactor (SHIME or 'Simulator of the Human Intestinal Microbial Ecosystem'). Parts of the results have already been published by Possemiers *et al.* (2005). The results obtained showed that certain bacteria in the gut are able to O-demethylate IX, resulting in 8-PN formation. In an experiment with faecal cultures, this conversion was observed in one-third of the samples,

indicating the importance of interindividual variability in the intestinal microbial community. These results stress the importance of the gut microbial community toward the final biological activity of phyto-oestrogens, a factor that should not be neglected when determining the final active dose inside the body (Possemiers *et al.*, 2006).

3.2.2. Constituents : 8-PN

The pharmacokinetic data which can be found in literature are mostly on xanthohumol (X). They show that the bioavailability of X is rather low (Nookandeh *et al.*, 2004). X and its metabolites are excreted mainly in faeces within 24 h of administration.

Schaefer investigated the pharmacokinetics of 8-PN in rats and dogs (2004). The full ADME profile and bioavailability profile (using synthetic 8-PN) that he proposes can be found in the thesis. Briefly, it was found that the ingested 8-PN is almost completely absorbed after ingestion. Systemically circulating 8-PN concentrations were only 5% of the ingested amount, indicating a strong action (presystemic elimination) before the 8-PN could go into the systemic circulation. The 8-PN in the systemic circulation is also bound to serum proteins (sex hormone binding globulins in dogs). This binding would be less likely for X, in view of its non-oestrogenic characteristics. Also Nikolic *et al.* (2004, 2006) concluded that the bioavailability of 8-PN was reduced significantly by intestinal and hepatic metabolism.

The comparatively high metabolic stability of 8-PN and its pronounced presystemic elimination via the bile resulted in enterohepatic recirculation in both rats and dogs. The concentration profile in the serum after oral uptake showed two maxima: one after 1 h and another (higher concentration) after 2 to 4 h, which is an indication of the enterohepatic concentration. About 50% of the ingested amount was recovered unchanged in the faeces. The excretion mainly goes via the biliary route, as renal excretion (unchanged and conjugated 8-PN) is limited, especially after oral administration.

The pharmacokinetic results obtained in rats and dogs were more or less confirmed in a human study performed by Rad *et al.* (2006). The study was performed using a randomized, double-blind, placebo-controlled, dose-escalation design with three groups of eight healthy postmenopausal women. In each group six subjects received 8-PN and two subjects received placebo. 8-PN was given orally in doses of 50, 250 or 750 mg. Drug concentrations in serum, urine and faeces were measured up to 48 h. Serum concentrations of free 8-PN showed rapid drug absorption and secondary peaks suggestive of marked enterohepatic recirculation. Independent of the treatment group, approximately 30% of the dose was recovered in excreta as free compound or conjugates over the 48 h observation period. The first C_{max} and AUCO-48 h showed dose linearity with ratios of 1:4.5:13.6 (C_{max}) and 1:5.2:17.1 (AUC).

Conclusions

The *in vitro* studies indicated extensive liver biotransformation of X, IX and 8-PN upon absorption. However, the extent of dietary polyphenol absorption in the small intestine is rather limited (10-20%), thereby implying that a large proportion reaches the colon. Naringenin, the non-prenylated analogue of 8-PN, showed extensive biotransformation in the intestine, including ring cleavage and dehydroxylation, followed by absorption and urinary excretion. The extent of degradation strongly depended on compound concentration and individual composition of the gut microflora of the different human subjects. On the other hand, when X was fed to rats, it was mainly recovered in unchanged form from the faeces (89%).

The finding that IX may be 0-demethylated by human hepatic cytochromes P450 to form the potent phyto-oestrogen 8-PN is of considerable importance. In addition, under the acidic conditions of the stomach X can be cyclized to form IX and provide yet another route for the formation of 8-PN. Based

on these results, IX and X should be included among the compounds used for standardisation of hop extracts to be evaluated for oestrogenicity *in vitro* and *in vivo.*

The pharmacokinetic profile of 8-PN was studied in rats and dogs and later in human females. It was shown that 8-PN was rapidly and almost completely absorbed after oral administration. The free substance is then taken up in the jejunum, enters the portal circulation and goes to the liver. Thereafter, besides unchanged 8-PN which goes into the systemic circulation, 8-PN undergoes mainly phase II metabolisation and the hydrophilic conjugates formed are mainly eliminated in the bile.

Bacteria in the gut can deconjugate the phase II metabolites, and lipophilic substances can be taken up again and go to the enterohepatic circulation. Free substance is mainly excreted via the faeces, but also conjugates are excreted from the blood with urine.

In conclusion, the pharmacokinetic profile of 8-PN is characterized by rapid and probably complete enteral absorption, a pronounced presystemic elimination with subsequent enterohepatic recirculation, dose linear pharmacokinetics and competitively little phase I metabolism. Conjugation to β-glucuronides or sulphates seems to constitute the main metabolic pathway. Intestinal and hepatic phase I metabolism plays a minor role in the inactivation of 8-PN. This is in complete contrast to other natural or synthetic oestrogens, whose decreased oral bioavailability is due to intestinal conjugation and hepatic metabolic inactivation.

The studies also indicate that 8-PN is transformed into a wide array of metabolites, and some of these products may have different pharmacological activity than their precursors. It would, therefore, be of interest to determine the biological activities and potential toxic effects of these metabolites, particularly since it is known that hydroxylated metabolites of 17-β-oestradiol (mainly the 16-OH metabolite) are much more active, thereby increasing the risks for oestrogen-dependent cancers.

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

Hops have been used in the brewing industry for centuries without any known adverse effect to the health of consumers. Thus, given the history of long-term and present use in humans with no significant adverse effects, it is considered that hops are safe (Chadwick *et al.*, 2006).

3.3.1. Single dose toxicity

The following toxicological data are given in Hagers Handbuch (1993).

Ethanolic hop extract: LD_{50} 3,500 mg/kg/b.w. p.o., 1,200 mg/kg/b.w. s.c. (mice): LD_{50} 2,700 mg/kg/b.w. p.o. (rats).

Methylisobutylketone-hop extract: LD_{50} 2,700 mg/kg/b.w. p.o. (mice); LD_{50} 415 mg/kg/b.w. p.o. (rats).

Lupulone: LD_{50} 525 mg/kg/b.w. p.o.; LD_{50} 1,200 mg/kg/b.w. s.c.; 600 mg/kg/b.w. i.m. (mice); LD_{50} 1,800 mg/kg/b.w. p.o.; LD_{50} 330 mg/kg/b.w. i.m. (rats).

Humulone: LD_{50} 1,500 mg/kg/b.w. p.o.; 600 mg/kg/b.w. i.m. (rats).

A hydro-alcoholic hop extract enriched in 8-PN, and consisting of 0.22% 8-PN, 0.2% 6-PN, 5.5% xanthohumol and 1,7% isoxanthohumol (marketed in Belgium as food supplement) was tested for acute toxicity and mutagenicity in rats (Personal communication, according to information by the Rapporteur).

The starting dose used in the acute toxicity studies was based upon the dose which was used in the clinical trial *viz*. 2.6 mg/kg/day and calculated via a multiplication factor of 6.66 from man to rats (Notox BV. Safety and Environmental Research, Hertogenbosch, The Netherlands, Toxicological Report 2005, unpublished, available to the Rapporteur). Three female Wistar rats in four groups each were treated with escalating doses of 25, 250, 1000 and 2500 mg hop extract/kg body weight (b.w.) per day by oral gavage during 2 consecutive days in order to determine the maximum tolerated signs dose (MTD). Clinical, body weight and food consumption were determined, as well as clinical pathology prior to microscopy and macroscopy at termination.

Based on the data from this dose escalation phase the dose level for MTD was selected to be 2500 mg/kg/day. Although a real MTD was not reached, this dose level represents a 100-fold expected human dose, so that the safety margin is expected to be sufficiently high. During the MTD-phase 10 rats (5 males and 5 females) were given one dose of 2500 mg/kg daily for 5 consecutive days by oral gavage. No mortality or clinical signs were noted during the whole MTD-phase and finally no treatment related findings in haematology and clinical biochemistry parameters were observed and no findings were noted after macroscopic and microscopic examination. It was concluded that repeated oral administration of 2500 mg/kg/b.w. in male and female Wistar rats on 5 consecutive days were well tolerated and that no clear signs indicative of test substance induced toxicity were found.

These results obtained in rats were confirmed in humans by Rad *et al.* (2006), who investigated the kinetics and systematic endocrine effects of 8-PN after single oral doses. It was found that single oral doses of up 50-75.0 mg 8-PN (ca. 3500-fold the intended amount of a product in clinical studies) were well tolerated by postmenopausal women and that no drug-related adverse events were recorded.

Toxicity and cell cycle effects of synthetic racemic 8-PN in human cells were studied in comparison to quercetin and related flavonoids. These investigations were done on different cell lines *viz*. the promyeloid leukemia cell line HL 60 and the adherent breast cancer cell line MCF 7. A low toxicity and weak cytostatic properties of 8-PN and related naringenin derivatives were found (Tokalov *et al.*, 2004).

Besides the oestrogenic effects, Effenberger *et al.* (2005) also studied the cytostatic effects of the most prominent prenylflavonoids present in hops. It was shown that only at high concentration ($> 10^{-4}$M) prenylflavonoids such as 8-PN, 6-PN, X and IX displayed cytostatic effects on V79 cells (Chinese hamster fibroblasts).

3.3.2. Sub-acute and chronic toxicity

To date, no data on sub-acute and chronic toxicity studies on hop strobiles or its preparations are available.

In their investigations on the oestrogenic effects of 8-PN on bone metabolism, Miyamoto *et al.* (1998) found no overt signs of toxicity after administration of 8-PN at a dose of 30 mg/kg/drug subcutaneously for 2 weeks.

Christoffel *et al.* (2006) have published a study on the effects of 8-PN on the hypothalamo-pituitary-uterine axis in rats after a 3-month treatment. Therefore, a number of oestrogen-related parameters, in the hypothalamus, pituitary and uterus were chosen to compare the putative oestrogenic effects of 8-PN with those of 17β-oestradiol. Two doses of 8-PN and 17β-oestradiol-3-benzoate (E_2B) respectively 6,8 and 68,4 mg/kg/b.w. of 8-PN and 0,17 and 0,7 mg/kg/b.w. of E_2B and their effects were compared on uterine weight, pituitary hormones (LH, FSH and prolactin) and the expression of oestrogen-regulated genes and of oestrogen receptor $(ER)_\alpha$ and ER_β in the hypothalamus, pituitary and uterus.

Both doses of E_2B and the high dose of 8-PN suppressed serum LH and FSH, and stimulated serum prolactin levels, uterine weight, and progesterone receptor, insulin-like growth factor I and complement protein C_3 mRNA transcripts.

In the preoptic and the mediobased areas of the hypothalamus, all treatments had negligible effects on ER_α and ER_β and gonadotrophin-releasing hormone (GnRH) receptor gene-expression, while $ER\beta$ and GnRH receptor transcripts in the anterior pituitary were reduced under both E_2B doses and the high 8-PN dose. The mRNA concentrations of the LH_α and LH_β subunits in the pituitary were suppressed by E_2B and 8-PN.

In summary, 8-PN had very similar though milder effects than E_2B on all tested parameters. The authors concluded that inhibition of climacteric complaints by E_2B takes place in the hypothalamus, where it inhibits the overactive GnRH pulse generator. 8-PN may be used to inhibit climacteric symptoms effectively. They proposed to carry out human pharmacological studies in order to show whether the stimulatory effect of 8-PN on the uterus would require the concomitant administration of progesterone to prevent endometrial over stimulation.

To date, no data are available on the long-term toxicology of hop extracts when used by menopausal women. It should, however, be noted that hop extracts are rich in xanthohumol, which is considered to possess antiproliferative effects on both cancer cell lines of human breast and ovaries by inhibitory mechanisms at the initiation, promotion and progression stages (Gerhauser *et al.*, 2002; Gerhauser and Frank, 2005 ; Van Hoecke *et al.*, 2005).

It is being questioned whether dietary and/or environmental exposure to phyto-oestrogens could impose health risks such as endocrine disruption. In case of hop prenylflavonoids, beer is the main dietary source. The average beer consumption in the United States was calculated at about 225 ml of beer per capita per day in 2001 (USDA, 2003). When assumed that this amount was consumed as US major brand lager/pilsner beers (500-1000 µg prenylflavnoids/l beer), the daily intake of prenylflavonoids would be about 0.14 mg (Stevens *et al.*, 1999b). However, the concentrations detected in beer (and therefore average intake) strongly depend on the brewing process, as strong ales contain up to 4 mg prenylflavonoids/l. Although xanthohumol (X) is the predominant prenylflavonoid present in hops (0.1-1% of dry weight), most of it is transformed into isoxanthohumol (IX) by thermal isomerisation during wort boiling. Therefore IX is the major prenylflavonoid found in beer and is present in concentrations from 500 µg/l (lager/pilsner) up to 4 mg/l (strong ale (Stevens *et al.*, 1999b; Rong *et al.*, 2000). Similarly, desmethylxanthohumol (DMX) is converted into 8-PN resulting in final concentrations in beer of up to 100 µg 8-PN/l. But despite the high activity of 8-PN, the total oestrogenic activity in beer is still 500 to 1000 times lower than the concentration needed for harmful *in vivo* activity (~ 100 mg/l) (Milligan *et al.*, 2002). Moreover, many beers are now made using hop extracts instead of whole hops, giving lower concentrations of 8-PN or no 8-PN at all. Therefore, it is generally agreed that based on the current knowledge, no detrimental health effects can be attributed to phyto-oestrogens upon moderate beer consumption (Milligan *et al.*, 1999, 2002; Stevens *et al.*, 2004).

Hop extracts have also been used for many years in food supplements without any known problems, also in combination with other plants such as valerian. The Belgian Health Authorities have set upper-limits for phyto-oestrogens in dietary supplements. For hop extract, the threshold was set at 400 µg 8-PN/day (for soy isoflavones at 40 mg/day) (Belgian legislation on herbals: MB, 25/02/2005).

The potential side-effects of soy preparations such as the effects on the thyroid have not been raised for hops, so that every phyto-oestrogen should be considered differently.

3.3.3. Genotoxicity

To date, no data on genotoxicity studies on hop strobiles or its preparations are available.

An overview on the genotoxicity of 8-PN was found in the "Comparative toxicogenomics database" on the internet: http://ctd.mdibl.org/voc.go?voc=chem&acc=C119737&view=ixn

8-PN has been mentioned therein for interactions with alkaline phosphatase, CYP1A2, ESR1 and GSTA1, IL6, PGR, TFF1 and VWF.

In the Ames mutagenicity test, a hydroethanolic extract of hop strobile showed weakly mutagenic potential in *Salmonella typhimurium* strains TA98 and TA100 with or without activation (Göggelmann *& Schimmer*, 1986). These data were confirmed for TA98 strains only for a hop extract enriched in 8-PN (unpublished data).

A hop extract enriched in 8-PN has been tested in the *Salmonella typhimurium* reverse mutation assay with four histidine-requiring strains (TA1535, TA1537, TA100 and TA98) and in the *Escherichia coli* reverse mutation assay with a tryptophan requiring strain of *Escherichia coli* WP2vvrA. The tests were performed in two independent experiments in the presence and absence of S9-mix (Aroclor-1254 induced rat liver S9-mix). The concentration range varied from 100 to 5000 µg/plate. The hop extract showed up to 3.6 and 2.8-fold, dose-related increases in the number of revertant colonies in tester strain TA98 compared to the solvent control, both in the absence and presence of S9-mix (2 experiments). All other bacterial strains showed negative responses over the entire dose range (unpublished data available to the Rapporteur). Since hop extracts contain quercetin glycosides (McMurrough 1981; De Cooman *et al.*, 1998), the slight mutagenic effect in the *Salmonella typhimurium* TA98 strain which was not observed in the other strains, might be a false positive result, as it is well-known that quercetin gives positive results in the Ames test but negative results in other genotoxicity tests. Therefore, it was decided to carry out a mouse lymphoma test in order to show that the hop extracts are not mutagenic.

A hop extract enriched in 8-PN was tested up to concentrations of 160 and 100 µg/ml in the absence and presence of 8% and 12% (V/V), S9 mix respectively in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells. In this test the effects on the induction of forward mutation at the thymidine-kinase locus (TK-locus) in L5178Y mouse lymphoma cells were determined. It was shown that the hop extract was not mutagenic in the TK mutation test system under the experimental conditions and consequently does not cause a point mutation or chromosomal aberrations (unpublished data available to the Rapporteur). Since the suppressed mutagenic effect in the Ames test was not confirmed in the same mutation test in mammalian cells, an overall conclusion can be drawn that this hop extract is not mutagenic and that therefore carcinogenicity studies would not be needed according to Guideline EMEA/HMPC/32116/2005.

Conclusions

To date, adequate genotoxity studies have not been carried out for the aqueous or hydroalcoholic extracts of hops included in the monograph on traditional use.

3.3.4. Carcinogenicity

To date, no data on carcinogenicity studies on hop strobiles or its preparations are available.

3.3.5. Reproduction and developmental toxicity

No preclinical data on reproductive and developmental toxicity of hop strobiles or its preparations are available.

Kumai and Okamoto (1984) investigated purified fractions of hop strobile extract for reproductive toxicity in female rats primed with 25 IU of pregnant mare's serum gonadotrophin.

Fractions F_1 (20 mg/rat) and F_2 (50 mg/rat), administered subcutaneously twice daily for 3 days, did not induce any change in uterine weights but ovarian weights decreased significantly ($p<0,05$) by 25,7% and 24,9% respectively. Under the same conditions, two further fractions (4 mg/rat) purified from F_1 produced significant decreases ($p<0.01$) of 42.0% and 33.1% in ovarian weight.

Caujolle *et al.* (1969) showed that an alcoholic extract of hop strobile (1 g of dried drug in 10 ml of 70% ethanol) inhibited concentrations of rat uterus with an ED_{50} equivalent to $31x10^{-6}$ of hop strobile per ml.

In view of this *in vitro* antispasmodic effect on the uterus and lack of toxicity data, the use of hops and its preparations should be avoided during pregnancy and lactation. It is clear that the extracts enriched in 8-PN are intended for menopausal complaints and consequently should not be used by fertile women. This is also the case for the indications of hop preparations as sedative.

3.3.6. Local tolerance

Data on local tolerance for hop strobiles and its preparations are not available. Allergic reactions have been reported for hops following external contact with the herb and the oil.

3.4. *Overall conclusions on non-clinical data*

The many *in vitro* and *in vivo* experiments clearly demonstrate that the indications for traditional use of hops and its preparations are justified.

Studies in animals have been carried out with the aim of identifying constituents responsible for the sedative effects of hop preparations. A number of constituents, including the alpha-acids (humulones), beta-acids (lupulones) and constituents of hop oil are considered to contribute to the sedative activity. However, studies confirming that these constituents are responsible for the therapeutic activity of hop preparations in humans are currently lacking.

On the basis of the available information, alpha-acids (humulones) and beta-acids (lupulones), if present, may be contributing to the activity of the hop herbal preparations and therefore they might be appropriate to serve as active markers for quantified herbal preparations. However, information on quantified hop preparations was not available for the HMPC assessment and evidence to support the use of such products may not exist.

Toxicological tests on hops or its preparations are limited. However, there is a history of long-term and present human use of hops and hop preparations in beer and food supplements and no safety concerns have arisen. In view of the absence of sufficient data, the use during pregnancy and lactation is not recommended. In addition, adequate tests on genotoxicity are lacking as well as tests on reproductive toxicity, fertility and carcinogenicity.

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 constituent(s)

No data available

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

No data available

4.2. Clinical Efficacy

4.2.1. Dose response studies

No data available

4.2.2. Clinical studies (case studies and clinical trials)

4.2.2.1. Sedative activity

No clinical studies have been conducted to date with hops or hop preparations as single component products for the treatment of restlessness or insomnia.

Four non-controlled and three placebo or reference-controlled, double-blind clinical studies in patients suffering from non-organic sleep disorders have been conducted with a fixed extract combination (Ze 91019) of valerian root and hop strobile extracts. Both extracts were prepared with 45% methanol m/m with a dry extract ratio of 5-3:1 (valerian) and 6.6:1 (hops), respectively. (Brattström, 1996, Lataster and Brattström, 1996, Flesch, 1997, Füssel *et al.*, 2000, Notter *et al.*, 2003, Rodenbeck and Hajak, 1998, Schellenberg *et al.*, 2004 and Koetter *et al.*, 2007)

In addition, one non-controlled and four placebo or reference-controlled double-blind clinical studies are reported using a related fixed combination product consisting of dry extracts of valerian root and hops (Wegener *et al.*, 2003, Schmitz and Jäckel, 1998, Morin *et al.*, 2005, Leathwood *et al.*, 1982, Müller-Limmroth and Ehrenstein, 1977).

A randomized placebo controlled trial has been carried out on a dietary supplement containing polyunsaturated fatty acids in association with a hop extract aiming to improve the quality of sleep. The dietary supplement had no effect on the perceived quality of sleep, nor on the melatonin metabolism and sleep-wake cycle. It should be noted that no information on the composition of the mixture was given and that specifications for the hop extract were not provided (Cornu *et al.*, 2010).

Following their study on quails Franco *et al.* (2012) investigated the sedative effect of non-alcoholic beer on healthy female nurses, which were considered as a work-stressed population. The experiment was conducted on 17 healthy female nurses working rotating and/or night shifts. Overnight sleep and chronobiological parameters were assessed by actigraphy (Actiwatch R) after moderate ingestion of non-alcoholic beer with hops (333 ml with 0.0% alcohol, San Miguel R) with supper for 14 days (treatment). Data were obtained in comparison with a control group without consumption of beer with supper. Actigraphy demonstrated improvement of night sleep quality as regards the most important

parameters: Sleep Latency diminished (p= or less than 0.05) in the treatment group (12.01 +/- 1.19 min) when compared to the control group (20.50+/-4.21 min) as also did Total Activity (p=/<0.05; treatment group =5284.78 +/- 836.99 activity pulses versus control group =7258,78 +/- 898,89 activity pulses). In addition, anxiety as indexed by the State Trait Anxiety Inventory (STAI) decreased in the treatment group (state anxiety 18.09+/-3.8 versus control 20.69+/-2.14).

This study should be considered as anectodal as it was assumed that the non-alcoholic beer contained 0.3% hops, which has not been demonstrated in the publication.

Conclusions

Preclinical and clinical studies are available for combinations of hops with other sedative plants such as valerian, passion flower and lemon balm.

The only clinical study on hops was carried out with non-alcoholic beer for which the hops content was assumed, but not demonstrated.

4.2.2.2. Oestrogenic activity

As described in previous sections, preclinical *in vitro* and *in vivo* tests suggested that hop extracts exert an oestrogenic activity.

Before the publication in 1999 by Milligan *et al.*, only one small clinical trial specifically related to the oestrogenic properties of hops had been identified. In this placebo controlled study, 20 patients experiencing hot flushes due to ovarian insufficiency (15 in menopausal phase and 5 following ovariectomy), were treated with a dry aqueous extract of hop strobile (5:1), initially at 1.6-2.6 g/day, later in some cases reduced to 1.2-1.6 g/day. Five other patients received placebo. Assessment was based on scores calculated by multiplying the intensity of hot flushes (scale 1 to 3) by their frequency (scale 1 to 9). In *verum* patients, the initial average score of 22.7 decreased to 8.2 after 30 days of treatment, whereas in placebo group, the initial score of 20 decreased only to 18. Compared to the placebo group, 76% of the *verum* patients achieved a statistically significant improvement in scores and 7 out of 20 patients achieved a reduction of at least 15 points (Goetz, 1990). While the author reported that the formulation was effective in treating hot flushes, it was neither chemically nor biologically standardized according to modern standards (Chadwick *et al.*, 2006).

A first prospective, randomized, double-blind placebo-controlled study on the use of a hydro-alcoholic standardized hop extract to alleviate menopausal discomforts was published (Heyerick *et al.*, 2006). The hop extract was standardized on 8-PN (100 or 250 µg), which may seem a small amount, but comparison of 8-PN with established phyto-oestrogens indicated that 8-PN had several 100-fold more potent oestrogenic activity than e.g. soy isoflavones such as genistein and dadzein, which have been administered in daily doses of 50 to 100 mg (Milligan *et al.*, 1999). It was therefore deduced that amounts of 100 µg of 8-PN might have considerable efficacy. The hop-based capsules contained hop extract that was obtained by an aqueous ethanolic extraction of spent hops following extraction of hops using SC-C0$_2$.

In this three-armed clinical trial 67 patients experiencing mild to severe menopausal discomforts (more specifically at least 2-5 hot flushes per day corresponding to a score of at least 2 for the item "hot flushes" on the modified Kupferman-index (KI) during several weeks), were treated with the hop extract standardized on 100 µg 8-PN (n:20), the hop extract standardized on 250 µg 8-PN (n=20) or placebo (n=26) over 12 weeks. Assessment was based on the responses obtained using a modified KI and a patients questionnaire. All groups, including placebo, showed a significant reduction of the KI both after 6 and after 12 weeks. The hop extract at 100 µg 8-PN was significantly superior to placebo after 6 weeks (p=0.023) but not after 12 weeks (p=0.086). No dose-response relationship could be

established, as the higher dose (250 μg) was less active than the lower dose both after 6 weeks and after 12 weeks. Still, a trend for a more rapid decrease of KI was noticed for both active groups as compared to placebo. In particular, the decrease in hot flushes score (isolated from KI) was found significant for both treatment groups after 6 weeks (p<0.01) with respect to placebo. Results of the patient's questionnaire were consistent with those of the KI, with the most pronounced effects being observed for the 100 μg treatment. The authors concluded that a daily intake of a hop extract, standardized on 8-PN, exerted favourable effects on vasomotor symptoms and other menopausal discomforts. Since, however, no dose-response relationship could be established, further clinical studies are needed to confirm the present observation.

A 16-week randomized, double-blind, placebo-controlled, cross-over pilot study on the use of a hop extract (standardized at 100 microgram 8-prenylnaringenin per day) to alleviate menopausal discomforts has been published in 2010 (Erkolla *et al.*, 2010). The participants (36 healthy postmenopausal women) were randomly allocated to either placebo or active treatment (Belgian food supplement containing a hop extract standardized on 8-PN, see above) for a period of 8 weeks after which treatments were switched for another 8 weeks. The Kuppermann Index (KI), the Menopause Rating Scale (MRS) and a multifactorial Visual Analogue Scale (VAS) were assessed at baseline, and after 8 and 16 weeks. After 8 weeks, both active treatment and placebo significantly improved all outcome measures when compared to baseline with somewhat higher average reductions for placebo than for the active treatment After 16 weeks only the active treatment after placebo further reduced all outcome measures, whereas placebo after active treatment resulted in an increase for all outcome measures. Although the overall estimates of treatment efficacy (active treatment-placebo) based on linear mixed models do not show a significant effect, time-specific estimates of treatment efficacy indicate significant reductions for KI (P=0.02) and VAS (P=0.03) and a marginally significant reduction (P=0.06) for MRS after 16 weeks. The authors concluded that , although the first treatment period resulted in similar reductions in menopausal discomforts in both treatment groups , results from the second treatment period suggests superiority of the standardized hop extract over placebo. Moreover, no adverse effects have been recorded and the hop extract has shown good tolerability as there were no dropouts.

These results should be interpreted with caution as the study is subject to a number of limitations. The populations in the two different treatment regimens were imbalanced (12 vs 14) as block randomization was not used during the preparation of this study. The significant effects observed in the second treatment period are therefore, mostly based on the results of the smaller group and may thus be more prone to statistical error. Furthermore, the active treatment regimen was only given for 8 weeks, whereas it is customary to use a 12 week study time for vasomotor studies. In addition, no wash out phase between the cross-over strategy has been used. It is therefore clear that the observed effects still need to be confirmed in further studies.

Earlier a study has been published in which a Menopausal Rating Scale (medical device in form of gel was tested intra-vaginally in women with genital atrophy. This study was designed as an open non-controlled clinical study to assess the efficacy and safety of the gel, containing 1% of a mother tincture hop extract (according to the French Pharmacopoeia X), hyaluronic acid, liposomes formed by cholesterol and lecithin, all considered to be active ingredients. The authors conclude that the results showed a marked effect of the tested product on the vaginal dryness and other menopausal symptoms and that no treatment related adverse events were reported by the patients (Morali *et al.*, 2006).

Although the results of this study are interesting, it is difficult to draw conclusions, as the hop extract was not standardized on 8-PN and the relative contribution of the other ingredients cannot be deduced from the results obtained. Therefore, the efficacy of the medical device's respective components should be investigated separately in the future.

Conclusions

To date only three small placebo-controlled clinical studies have been published using hop extracts, on 20, 67 and 36 patients, respectively. A standardized hop extract (on 8-PN) was used in two of these studies. The fact that in the three-armed clinical trial no clear dose-response relationship was found is of concern. The relatively small numbers of patients may have contributed to the absence of a clear dose–response relationship; a larger follow-up study is required to demonstrate clinical efficacy. Thus studies to date have limitations and fail to show clinical efficacy. Consequently, the clinical data available are not sufficient to support a well-established use indication for hops in the treatment of menopausal symptoms.

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

No data available.

4.3. Overall conclusions on clinical pharmacology and efficacy

No clinical studies with single hop preparations have been published. Three small clinical studies with hop extracts on postmenopausal women with discomforts have not shown a clear efficacy. The extracts seemed to be well tolerated.

5. Clinical Safety/Pharmacovigilance

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

In all clinical trials carried out with hop preparations in combination with other sedative plants no serious side effects have been found. The hop preparations tested for oestrogenic effects in humans have been shown to be well tolerated without serious side effects.

5.2. Patient exposure

Products containing hops and/or its preparations are available in most Member States. Many of these products are commercially available in combinations with other sedative plants with marketing authorizations or registrations or as food supplements. Hops are listed by the Council of Europe as a natural source of food flavouring (Category N2). This category indicates that hops can be added to foodstuffs in small quantities, with a possible limitation of an active principle in the final product. Previously, hop has been listed as GRAS (Generally Recognised As Safe) (Barnes *et al.*, 2007). Consequently a considerable patient or consumer exposure might be assumed.

5.3. Adverse events and serious adverse events and deaths

5.3.1. Side effects

Based on traditional use and available studies, there have been no serious side effects reported with hops. Drowsiness or sedation may occur. Caution should be exercised if driving or operating heavy machinery. In animal studies, hops have increased stomach acid, but there is no available research in humans in that area. Based upon animal studies, hops may increase blood sugar levels in diabetic patients, but may lower blood sugar in non-diabetic patients. Consequently, the effects of hops on blood sugar levels are unclear (Anonymous, 2003a). It should be noted that oral intake of isohumulones, present in hops and its preparations, is reported to improve insulin sensitivity in

patients suffering from diabetes type 2. Consequently, it might be expected that the blood sugar level in such patients would decrease after oral intake of hops.

Allergic reactions from hops have been reported, particularly in hop harvesters. The contact dermatitis that has occurred in hop-pickers is attributed to myrcene, present in fresh oil but readily oxidized (Mitchell and Rook, 1979). Additionally, a mechanical dermatosis has been attributed to the rough hairs on the stem and secretions of the yellow glandular hairs on hops (Estrada *et al.*, 2002).

Respiratory allergy caused by handling of hop cones have been documented (Newark, 1978); a subsequent patch test using dried, crushed flowerheads proved negative. Positive patch test reactions have been documented for fresh hop oil, humulone and lupulone. Both of these allergic reactions are not likely to occur when using the hop extract, since allergens are supposed to be removed (Estrada *et al.*, 2002). Moreover, no clinical cases of allergy or anaphylaxis resulting from the therapeutic use of hops have been published (Anonymous, 2003a).

Since hop extracts might contain small amounts of oestrogens, it is not known as yet what the effects would be of use for *viz.* more than three months, on conditions such as breast, uterine, cervical or prostate cancer or endometriosis. However, it should be noted that oestrogens such as 8-PN are only present in hop preparations in significant amounts when old hops are used as starting material and special enrichment procedures are employed for the hop preparations. It should also be noted that hop preparations may contain antiproliferative compounds such as humulones, xanthohumol and isoxanthohumol. Finally, some hop preparations contain high levels of alcohol and should be avoided during pregnancy.

5.3.2. Contra-indications, warnings

It has been suggested that hops should not be taken by individuals suffering from depressive illness, as the sedative effect may accentuate symptoms (Anonymous, Harvard Medical School, 2003b). The sedative action may potentiate the effects of existing sedative therapy and alcohol.

Allergic reactions have been reported for hops, although only following external contact with the herb and oil has been documented. In view of this and lack of toxicity data, the use of hops during pregnancy and lactation should be avoided.

5.4. Interactions

Interactions with drugs, dietary supplements and other herbs have not been thoroughly studied. From the many publications on hops and its constituents, it is theoretically possible that hops may increase the drowsiness caused by sedative drugs and alcohol. Hops may also affect blood sugar levels but no clinical data are available to support this observation.

5.5. Overdoses

Not known.

5.6. Laboratory findings

No data available

5.7. Safety in special populations and situations

No data available

5.8. Overall conclusions on clinical safety

In view of its long term use and present use in humans hops is considered to be non-toxic and safe with no significant adverse effects in the condition of use proposed for the monograph.

The experimental toxicological data on hop preparations are rather limited and incomplete but as a whole show low toxicity. Substantial toxicological experiments have been carried out on a standardized hydro-alcoholic extract, enriched in 8-PN, and consisting of 0.22% 8-PN, 0.2% 6-PN, 5.5% xanthohumol and 1.7% isoxanthohumol (marketed as food supplement in Belgium). The single-dose toxicity studies as well as the genotoxicity studies showed the extract to be safe in all concentrations tested i.e. no adverse effects have been seen in doses up 1000-fold the intended dose for clinical studies (unpublished data, available to the Rapporteur).

Investigations on the toxicity of the oestrogenic active ingredient of hop extracts *viz*. 8-PN were carried out during 2 weeks and also three months, indicating that 8-PN had very similar but milder effects than 17β-oestradiol on all tested oestrogenic parameters. Although, today no data are available on the long-term toxicology of hop extract when used by menopausal women, it should nevertheless, be stressed that hop extracts also contain xanthohumol, which is considered to possess antiproliferative effects in several cancer cell lines.

Hop extracts have been used for several years in food supplements without any known adverse effects. The Belgian authorities have set upper-limits for phyto-oestrogens in dietary supplements. For hop extracts and soy-isoflavones the threshold was set at 400 µg 8-PN/day and 40 mg/day isoflavonoids, expressed as the glycoside of the main isoflavonoid respectively. It can also be concluded from the many publications that no detrimental effects can be attributed to phyto-oestrogens upon moderate beer consumption.

In conclusion, the safety assessment of hops and hop preparations is mainly based on many years of experience from the extensive medicinal use in humans, which indicate hop preparations to be safe pharmaceutical agents.

6. Overall conclusions

The sedative effect of hops and preparations thereof has long been recognised empirically so that a period of at least 30 years of traditional medicinal use is easily fulfilled for this indication.

Its use has been made plausible by many *in vitro* and *in vivo* pharmacological studies. Recently, it has been suggested that besides the acids and the essential oil other constituents, such as xanthohumol, may play an important role in the sedative effect of hop preparations.

No clinical studies have been conducted to date with hop extracts alone as active drugs for insomnia, but at least seven placebo- or reference-controlled clinical studies have been carried out with fixed combinations of hop extracts and valerian root extracts. Whether hop extracts act as mild sedative independently, as a synergist, or not at all remains to be determined.

Although several hop-containing cosmetic preparations have been patented since 1961 for the external treatment of various gynaecological disorders, almost no traditional oestrogenic formulations of hops have been found in literature until the discovery of the oestrogenic principle in hops in 1988.

Consequently, a traditional use of hops as a phyto-oestrogenic preparation does not fulfil the requirement for at least 30 years of documented safe and plausible long-standing medicinal use. 8-prenylnaringenin (8-PN) is recognized as the phyto-oestrogenic principle in hops and several modern hop extracts enriched and standardised on this active constituent have been prepared and

pharmacologically investigated. It has been shown that 8-PN behaves like 17β-oestradiol, but has much milder effects on all oestrogenic parameters tested than the parent compound.

To date, only three small placebo-controlled clinical studies have been conducted with extracts standardised on 8-PN to investigate menopausal discomforts. These studies, should, however, be considered only as pilot studies with results, encouraging enough to justify a full-scale trial. The experimental toxicological data obtained with these extracts, have shown those extracts to be devoid of genotoxicity and adverse effects in concentrations up 1000-fold the dose used for the clinical studies. However, the available data are not sufficient to support the well-established use of hop extracts as phyto-estrogenic substances.

In conclusion, the available data do not support hop strobiles as active substance of well-established medicinal products with recognised efficacy and acceptable safety. However, the traditional uses of single hop preparations are accepted within the Member States for their sedative effects and are well documented in literature, including standard herbal reference books.

The efficacy of hops and preparations thereof is considered plausible on the basis of long-standing use and experience for the administration to adolescents, adults and the elderly for indications considered within the scope of Directive 2004/24/EC. Fixed combination products consisting of dry extracts of hop strobiles with other sedative plants may be considered in separate monographs.

The requirements for medicinal use for at least 30 years, including 15 years within the Community, according to Directive 2004/24/EC are considered fulfilled for the following herbal preparations in the indication "Traditional herbal medicinal product for relief of mild symptoms of mental stress and to aid sleep":

a) Comminuted herbal substance

b) Powdered herbal substance

c) Liquid extract (DER 1:1), extraction solvent ethanol 45% V/V

d) Liquid extract (DER 1:10), extraction solvent sweet wine (only for relief of mild symptoms of mental stress)

e) Tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent ethanol 60% V/V

f) Dry extract (DER 4-5:1), extraction solvent methanol 50% V/V

On the basis of the available information *(see section 3.4 Overall conclusions on non-clinical data)* the HMPC has concluded that:

- no constituent with known therapeutic activity could be defined for the hop preparations listed in the Monograph.

- Alpha-acids (humulones) and beta-acids (lupulones) can serve as analytical markers and are used as characteristic constituents for TLC identification of the herbal substance (Ph Eur).

An HMPC monograph can therefore be adopted based on traditional use only.

However, a Community list entry is not supported due to lack of data on genotoxicity (Ames test) for the herbal preparations of hops covered by the monograph.

Annex

List of references