Assessment report on *Syzygium aromaticum* (L.) Merill et L.M. Perry, flos and *Syzygium aromaticum* (L.) Merill et L.M. Perry, floris aetheroleum

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)	Syzygium aromaticum, flos
Herbal preparation(s)	Syzygium aromaticum, floris aetheroleum
Pharmaceutical forms	Liquid dosage forms for oromucosal and dental use
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Assessor(s)	R. Länger, B. Hochenegg

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1. Introduction

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

- Herbal substance(s)

According to the European Pharmacopoeia (1/2008: 1091), Caryophylli flos consists of the whole flower buds of *Syzygium aromaticum* (L.) Merill et L.M. Perry (Syn. Eugenia caryophyllus (C. Spreng.) Bull. et Harr.) which were dried until they become reddish-brown. They contain not less than 150 ml/kg essential oil.

According to the International Plant Names Index, which is the electronic version of the Index Kewensis, the correct spelling of the author should be 'Merrill' (abbr. Merr.) after Elmer Drew Merrill (1876–1956).

Constituents (according to Blaschek et al 2008):

Essential oil: 15–17%, three main components account for nearly 99% of the essential oil: eugenol (75–88%), acetyl eugenol (4–15%), β-caryophyllene (5–14%).
Further components: chavicol, (Z)- and (E)-isoeugenol, benzyl acetate, α- and β-pinene, limonene; α-ylangene, γ- and α-caryophyllene (= humulene), caryophyllene epoxide, caryophyllenoxide, caryophylla-3(12),7(13)-dien-6α-ol and caryophylla-3(12),6-dien-4-ol [39] as well as 4,4-dimethyltricyclo[6.3.2.02.5]trideca-8-en-1-ol, caryophylla-4(12),8(13)-dien-5β-ol, caryophylla-3,8(13)-dien-5α-ol and caryophylla-3,8(13)-dien-5β-ol, α-copaen, α-cubebene, farnesol.

Aldehydes: benzaldehyde, m-methoxybenzaldehyde.

Alcohols: benzyl alcohol.

Ketones: heptan-2-one (= methyl-n-amylketone) and octan-2-one (= methylheptylketone).

Hydrocarbons: naphthalene.

Acetophenonderivates: 2,6-dihydroxy-4-methoxyacetophenone, methylxanthoxylin.

Flavones: quercetin, kaempferol, kaempferid, rhamnetin, kaempferol-3-O-β-D-glucoside, quercetin-3-O-β-D-glucoside, quercetin-3-O-β-D-galactoside, quercetin-3,4'-O-β-D-diglucoside.

Tannins: ellagitannins, including eugeniine.

Phenolic acids: gallic- and ellagic acid, 3- and 4-caffeoyl-, 3-p-cumaroyl- and 3-feruloylquinic acid, ferulic acid, p-hydroxybenzoic acid, caffeic acid, salicylic acid, syringic acid, vanillic acid, gentisic acid, protocatechuic acid and p-coumaric acid.

Triterpenes: oleanolic acid, crataegolic acid.

Phytosterols: β-sitosterol, stigmasterol, campesterol.

Sugars: glucose, xylose, arabinose.

- Herbal preparation(s)

According to the European Pharmacopoeia (1/2008: 1091) Caryophylli floris aetheroleum is obtained by steam distillation from the dried flower buds of *Syzygium aromaticum* (L.) Merill et L.M. Perry.

Composition (according to Blaschek et al 2008, Chaieb et al 2007):

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Three main components account for nearly 99% of the essential oil: eugenol (75–88%), acetyl eugenol (4-15%), β-caryophyllene (5–14%).

Further components: chavicol, (Z)- and (E)-isoeugenol, benzyl acetate, α- and β-pinene, limonene; α-ylangene, γ- and α-caryophyllene (= humulene), caryophyllene epoxid, caryophyllenoxide, caryophylla-3(12),7(13)-dien-6α-ol and caryophylla-3(12),6-dien-4-ol [39] as well as 4,4-dimethyltricyclo[6.3.2.02.5]trideca-8-en-1-ol, caryophylla-4(12),8(13)-dien-5β-ol, caryophylla-3,8(13)-dien-5α-ol and caryophylla-3,8(13)-dien-5β-ol, α-copaen, α-cubebene, farnesol.
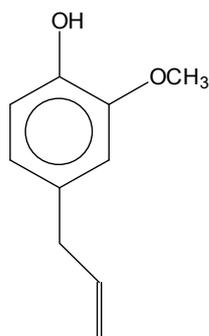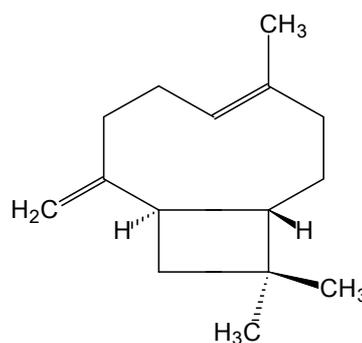
eugenol   β-caryophyllene

Methyleugenol is not reported for clove oil (De Vincenzi et al 2000).

- 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.

This assessment refers only to Caryophylli flos and Caryophylli floris aetheroleum.

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

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Combinations only
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Combinations only
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Combinations only
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

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This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

Search terms: Syzygium aromaticum, Gewürznelke, Caryophylli flos, eugenol.

Databases: Pubmed and Toxnet.

Libraries: University Vienna, centre of pharmacy; Medical University Vienna, central library.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

The medicinal use of Caryophylli flos can be traced in literature back to the 13th century (cited in Benedum et al 2006), it is also mentioned by Matthiolus and Lonicerus in the 17th century (cited in Benedum et al 2006).
Caryophylli flos has been in therapeutic use for many decades. However, there are no reports of any medicinal product containing cloves. Other information available on the medicinal use is considered insufficient to establish a Community herbal monograph.

The medicinal use of Caryophylli floris aetheroleum can be traced in literature back to the 15th century (according to Gildemeister & Hoffmann 1899). It is also mentioned by Schröder and Vietz in the 17th and 18th century (cited in Benedum et al 2006). The essential oil is the only active substance of several authorised medicinal products in UK. Although the SmPCs of these medicinal products state authorisation dates back to 1988, most of them were in medicinal use prior to 1980, according to information provided by the UK national authority MHRA. Moreover, the evidence on traditional medicinal use is supported by a large number of publications providing consistent information.

Therefore for Caryophylli floris aetheroleum, a period of at least 30 years in medicinal use as requested by Directive 2004/24 EC for qualification as a traditional herbal medicinal product is fulfilled.

Type of tradition: European.

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

Caryophylli flos is traditionally used as spice such as for gingerbread flavouring. Many spice blends, including curry contain powdered cloves, most herb liqueurs and bitter liqueurs contain clove macerates (Blaschek et al 2008).
Clove has been traditionally used in dyspeptic complaints, flatulence and diarrhoea as a decoction (Blaschek et al 2008).

Caryophylli floris aetheroleum has been traditionally used externally or locally for the treatment of toothache and minor infections of the mouth and skin, dressing of minor wounds, sore throats and coughs associated with the common cold, myalgia, rheumatic complaints, insect bites, flatulent colic or nausea (Blaschek et al 2008, WHO Monographs 2002, Koch 1953, Dingermann et al 2004, Barnes et al 2002, Frerichs et al 1938).
The German commission E proposes the use of the essential oil for treatment of inflammations of the oral and pharyngeal mucosa and in dentistry for topical anaesthesia (German commission E in Blumenthal et al 1998).

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Caryophylli floris aetheroleum or eugenol alone is widely used in dentistry mixed with zinc oxide as temporary filling material (Blaschek et al 2008).

The high content of eugenol makes the medicinal use in the proposed indication plausible.

Indication

	Herbal substance (Caryophylli flos)	**Indication**
A	Herbal substance	Traditionally as decoction for treatment of dyspeptic complaints, flatulence or diarrhoea (Blaschek et al 2008)

	Herbal preparation (Caryophylli floris aetheroleum)	**Indication**
A	Mouth washes	Inflammations of the oral and pharyngeal mucosa (German commission E in Blumenthal et al 1998)
B	Undiluted essential oil or solutions in a strength of minimum 50% or gels in a strength of 20%	Authorised products in UK: temporary relief of toothache due to dental cavity

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

Posology/Strength

	Herbal substance (Caryophylli flos)	**Posology**
A	Herbal substance	One piece when necessary in case of toothache (Blaschek et al 2008) Decoction: no information (Blaschek et al 2008)

	Herbal preparation (Caryophylli floris aetheroleum)	**Posology**
A	Inflammations of the oral and pharyngeal mucosa	Mouth washes corresponding to 1–5% essential oil (German commission E in Blumenthal et al 1998)
B	Temporary relief of toothache due to dental cavity	Authorised products in UK: undiluted essential oil or solutions in a strength of minimum 50% or gels in a strength of 20% Apply directly to the tooth cavities

Traditional use in children and adolescents:

Oromucosal use:
Children between 1 and 4 years of age: a strength of 1–2% of the essential oil is proposed (Dorsch et al 2002).
Children between 4 and 12 years of age and adolescents: a strength of 1–5% of the essential oil is proposed (Dorsch et al 2002).

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Duration of use

Dental use:
According to the SmPCs of UK medicinal products: The relief of toothache by clove essential oil is only a provisional measure. Dental attention should be sought as soon as possible. Repeat administration after 20 minutes, then every 2 hours thereafter if necessary.

Method of administration

Dental use:
According to the SmPCs of UK medicinal products: A small piece of cotton wool should be soaked in the undiluted oil or in a diluted solution; semisolid dosage forms should be placed on a cotton bud. Cotton bud or cotton wool should be accurately directed to the decayed part of the tooth. Avoid contact with gums.

Oromucosal use.

Assessor's comment on data on traditional use

Traditional use of Caryophylli flos:
Although there are consistent data on a traditional use of cloves for the short term suppression of toothache, the development of a community monograph does not seem appropriate because clove for such use will be taken from food and not from medicinal products. Moreover, there are no reports on medicinal products containing cloves as an only active ingredient.

Traditional use of Caryophylli aetheroleum:
The analgesic and antimicrobial properties of clove oil, as described below, make the dental and oromucosal use plausible. Traditional use for more than 30 years is documented. Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity of clove oil have not been performed, however experimental data on genotoxicity suggest that the main constituent of clove oil, eugenol, might be harmful.

Use of eugenol in dentistry:
Eugenol can be part of temporary pulp fillings in dentistry. Eugenol is mixed with zinc oxide, giving a paste which hardens quickly when coming into contact with saliva. This special application is not within the scope of a Community herbal monograph and will therefore not be further discussed.

3. Non-Clinical Data

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

Effects of Caryophylli flos

Antiseptic, antibacterial, antifungal, antiviral, local anaesthetic and spasmolytic effects are attributed to the drug. This information is only partially covered by experimental work (Blaschek et al 2008).

Antimicrobial effects:
The addition of 1 g clove powder to 9 ml culture medium inhibited the growth of *Aspergillus flavus*, *A. ocharceus* and *A. versicolor* totally (Hikoto et al 1980 cited in Blaschek et al 2008). A methanolic extract from cloves demonstrated preferential antimicrobial activity against the periodontal pathogens *Prevotella intermedia* and *Porphyromonas gingivalis* with MICs of 156 and 625 μg/ml (Cai & Wu 1996). An extract prepared with methanol 70% showed antibacterial activity against 32 strains of *S. aureus* (Betoni et al 2006).

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Taguchi et al (2005) studied the effect of a suspension of clove powder in water on oral and intestinal candidiasis in mice. When the preparation was administered into the oral cavity, the oral symptoms improved and the number of viable Candida cells in the cavity was reduced. After intragastric administration, the oral symptoms did not improve, but viable Candida cells in the stomach and faeces were decreased.

Antiviral effect:
Eugeniine isolated from cloves by solvent distribution and multiple column chromatography (yield 82 mg/50g drug) inhibits *in vitro* in FL-cell cultures at a concentration of 10 μg/ml the replication of Herpes simplex virus (HSV). When eugeniine was added one hour after virus seeding, no giant cells could be observed after 24 hours of incubation (Takechi & Tanaka 1981).

Kurokawa et al (1998) studied the effects of eugeniine on Herpes simplex virus-1. The effective concentration for 50% plaque reduction for HSV-1 on Vero cells was 5 μg/ml, which is approximately 14 fold lower than the 50% cytotoxic concentration. The viral DNA synthesis was found to be one of the major target sites of the inhibitory action.

The combination of hot water clove extract with acyclovir had a stronger anti HSV-1 activity compared to the sum of the single compounds (Kurokawa et al 1995). When acyclovir and/or the herbal extract were orally administered at doses corresponding to human use (250 mg/kg extract, 5 mg/kg acyclovir), the combination significantly limited the development of skin lesions and/or prolonged the mean survival times of infected mice compared to acyclovir or the extract alone. The combination was not toxic to the mice.

Anti-Hepatitis C Virus Protease-activity was caused by *Syzygium aromaticum*. The methanol extract exhibited significant inhibitory activity (≥ 90% inhibition). The IC_{50} was 33 μg/ml (Hussein et al 2000).

Anticarcinogenic effects:
Dimethylbenzanthracen-croton oil treated mice had a visible rough granular surface on the shaved skin with varying degrees of erythema and sometimes with white plaque like lesion. Treatment with an aqueous infusion of clove delayed the onset of papillomas in the treated groups. Treatment was most effective in the group which received the clove infusion orally. At the dose of 100 μl the onset of papillomas was delayed by two weeks. 200 μl clove infusion also delayed the onset of papillomas but not to the extent seen with 100 μl. 50 μl of clove infusion were not effective (Banerjee & Das 2005).

The effect of clove aqueus infusion was very pronounced (p < 0.01) on the incidence of Carcinoma *in situ* (CIS). The infusion was administered at a dose of 100 μl/mouse/day. While 70% of benzopyrene-exposed animals (Newborn Strain A mice) had CIS, after treatment with clove infusion, only 10% animals showed CIS, indicating 85.71% inhibition after such treatment. Incidence of hyperplasia and dysplasia evident in the carcinogen control group were effectively reduced after treatment with clove infusion. Significant reduction in the number of proliferating cells and an increased number of apoptotic cells was also noted in these benzopyrene-induced lung lesions following clove treatment (Banerjee et al 2006).

Molluscicidal effect:
The toxicity of flower-bud powder of *Syzygium aromaticum* and its organic solvent extracted fractions against the snail *Lymnaea acuminata* were time and concentration dependent. The LC_{50} of the flower-bud powder was at 24 hours 172.75 mg/l and at 96 hours 51.98 mg/l, respectively. The ethanol extract was more toxic than other organic extracts. The 24 hours LC_{50} of ethanol extract of the flower-bud powder against *L. acuminata* was 83.53 mg/l. The 24 hours LC_{50} of the column purified fractions of *S. aromaticum* flower-bud powder was 20.73 mg/l; 96 hours LC_{50} 7.87 mg/l; 24 hours LC_{50} of eugenol was 11.03 mg/l (Kumar & Singh 2006).

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Antithrombotic effects:
Two different polysaccharides with rhamnogalacturan backbone and arabinan side chain were isolated which exhibit antithrombotic activity. After intravenous application of the low molecular weight polysaccharide (MW 34.000) in doses up to 1000 mg/kg body weight in mice, no signs of acute toxicity were observed, while the high molecular weight polysaccharide (MW 103.000) exhibited approximately half the toxicity of heparin (LD_{50} 322 mg/kg) (Lee et al 2001).

Antiallergic effects:
Kim et al (1998) investigated the effect of a hot water extract (DER app. 14:1) of clove on the immediate hypersensitivity in rats. The extract inhibited the compound 48/80-induced systemic anaphylaxis in rats with an IC_{50} of 31.25 mg/kg when administered intraperitoneally. The extract also inhibited the local immunoglobulin E-mediated passive cutaneous anaphylactic reaction (IC_{50} = 17.78 mg/kg, i.v., IC_{50} = 19.81 mg/kg, p.o.). The extract also inhibited dose-dependently the induced histamine release from rat peritoneal mast cells.

Effect on NO-formation:
A decoction (0.1%) of clove reduced NO levels by 57.2%, in comparison with the control value at a concentration of 250 µg/ml. The scavenging effects were concentration-dependent (Yokozawa et al 2000).

Antioxidative effects:
A decoction (10%) of clove exhibited antioxidative effects on the lipid peroxidation and protein oxidative modification of mice brain homogenate produced by copper *in vitro* (Toda 2001, Toda 2003).

Effects on the gastro-intestinal tract:
Agbaje (2008) investigated a hot aqueous extract using selected doses in the various study models. The effect of the decoction on intestinal propulsion was studied by administering 300 and 700 mg/kg extract to groups of overnight fasted mice, while using charcoal meal as a marker. The effect of the herbal drug was compared with other standard drugs and antagonists. In an identical design the same doses of the extract were administered orally to groups of overnight fasted rats prior to challenge with different necrotizing agents-absolute ethanol (1 ml/rat), indomethacin (30 mg/kg) and 70% ethanol in 150 mM HCl (1 ml/rat). Both negative and positive controls were similarly treated simultaneously with distilled water (10 ml/kg) and standard antiulcer drugs (omeprazole 20 mg/kg, cimetidine 100 mg/kg and misoprostol 0.2 mg/kg), respectively. Lastly, the effect of the clove decoction was investigated on a segment of isolated rabbit ileum and subsequently compared with acetylcholine 5.5 x 10(-5) M. The extract was found to increase the gut muscle propulsion similar to the standard drugs, carbachol and metoclopramide. When used together with atropine, the herbal preparation produced a reduction in intestinal propulsion which suggested the involvement of cholinergic mechanisms in the action of the extract. In the ulcer models, the decoction reduced the ulcer number and ulcer area in the ethanol and HCl-ethanol models, with significant respective ulcer indices of 2.80 +/- 3.51 and 11.4 +/- 3.79 compared with controls ($p < 0.05$). In the indomethacin model, the extract, 700 mg/kg, compared favourably with misoprostol with an index of 0.20 +/- 0.11 which was also found to be significant compared with the control. In the *in vitro* investigation on the rabbit ileum, the decoction (200-6400 µg/ml) contracted the tissue in a dose-dependent fashion, but it was found to be less effective than acetylcholine (5.5 x 10(-5) M). Atropine sulphate 3.4 x 10(-6) M and 3.4 x 10(-5) M reduced gut contractility induced by clove decoction, similar to the *in vivo* observation. The author concludes that the herbal drug exerts its effect via a cholinergic mechanism.

Aphrodisiac activity:
Tajuddin et al (2003, 2004) studied the effect of an extract of clove prepared with ethanol 50% on the sexual behaviour of male rats. After oral administration of 100, 250 and 500 mg/kg extract, a

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significant and sustained increase in sexual activity was observed. The highest effect was achieved with the dose of 500 mg/kg.

Other effects:

Male 7 to 8 weeks old Swiss-albino mice were given over 10, 20 or 30 days a feed containing 0.5%, 1% and 2% (m/m) clove powder. The content of acid-soluble, free SH-groups in the liver homogenates increased significantly dose- and time-dependent compared to untreated controls. After 30 days 27.79 or 30.01 or 33.10 µmol/g tissue (control 19.89 µg/g; $p < 0.005$) were found. The formation of malondialdehyde was reduced after γ–irradiation: 30 days, 1.90 ($p < 0.01$), 1.01 or 0.89 ($p < 0.0005$) nmol/mg protein; control 2.29 nmol/mg protein. The glutathion-S-transferase activity and the cytochrome-*b5*-content increased significantly in all groups (with the exception 0.5%, 10 days) compared with controls ($p < 0.0005$ to $p < 0.01$). The cytochrome P450-content fell significantly in all dose groups after 30 days. The activity of arylhydrocarbonhydroxylase was unaffected. A possible protective effect of the drug against chemical pollutants is discussed (Kumari 1991 cited in Blaschek et al 2008).

A methanol extract from cloves is said to induce the differentiation of M1-cells (myelotic leukemia of mouse) in macrophage *in vitro*. The fractionation of the extract yielded oleanolic acid and crategolic acid as active ingredients, which induce at a concentration of 5×10^{-5} or 2×10^{-5} M a differentiation. Oleanolic acid resulted at a concentration of 5×10^{-6} M also in a differentiation of HL-60 cells (promyelotic human leukemia) while the effect of crategolic acid was overlaid by cytotoxic effects (Umehara et al 1992).

The toxic effect of cloves on *Culex pipiens larvae*, the common European mosquito, was investigated by El Hag et al (1999). The LC_{50} for the methanol extract was 824.7 ppm (assay time: 6 days) and the LC_{50} for the ethanol extract was 921.3 ppm (assay time: 6 days). The highest mortality (70%) was obtained in the 1000 ppm concentration after 10 days.

Effects of Caryophylli floris aetheroleum

Due to a high content of eugenol in Caryophylli floris aetheroleum, the effects of eugenol are often extrapolated to the entire essential oil.

Analgesic effects:

Patch-clamp experiments showed that eugenol reversibly activates calcium ion channels and chloride ion channels in dorsal root ganglion cells from rats. The applied eugenol concentrations ranged from 0.125 to 1 mmol/l. These effects may be responsible for the analgesic activity (Gruenwald et al 2004). Sodium and calcium channels act as targets for eugenol for its analgesic effect. Eugenol inhibits ATP-induced P2X currents in trigeminal ganglion neurons, which contributes to the analgesic effect (Li et al 2008).

Intrathecal treatment of mice with eugenol (12.5 to 50 µg) for 24 hours, dose-dependently inhibited the formalin-induced nociceptive response. Capsazepine shifted the dose-response curves in parallel to the right. Eugenol may exert its antinociceptive effect via the capsaicin receptor located on sensory terminals in the spinal cord. These results indicate that eugenol acts as a capsaicin-like substance (Ohkubo & Shibata 1997)

Anti-inflammatory effects:

Eugenol inhibited the NO production in a dose-dependent manner in RAW264.7 cells treated with 1 µg/ml lipopolysaccharide for 24 hours. Isoeugenol was more effective. LPS-dependent expression of COX-2 was also inhibited by isoeugenol and less effectively by eugenol (Li et al 2006).

Anticarcinogenic effects:

Mice received 20 mg of isolated sesquiterpenes once every 2 days. The sesquiterpenes ß-

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caryophyllene, ß-caryophyllene oxide, alpha-humulene, alpha-humulene epoxide and eugenol induced the detoxifying enzyme glutathione S-transferase in the mouse liver and intestine (Zheng et al 1992). Eugenol showed chemopreventive effects. Eugenol, but not its isomer isoeugenol, was found to be a potent inhibitor of melanoma cell proliferation. It inhibits the growth of melanoma cells in culture (50% inhibition by 0.5 µM). Eugenol causes significant tumour growth delay, decrease of tumour size and prevents tumour metastasis in mice (125 mg/kg) (Ghosh et al 2005).

Antimicrobial effects:
The majority of publications on pharmacological effects of clove, clove essential oil and eugenol deal with the antimicrobial effects. Only some selected references are cited below:

Eugenol (1 mg/ml) showed pronounced antibacterial properties against Gram+ as well as against Gram- microorganisms comparable with 500 µg/ml neomycin (Laekeman et al 1990). Growth inhibition was even more pronounced for *Candida* species in comparison with nystatin (5000 U/ml).

0.4% clove oil in 63% sugar syrup inactivated after 2–7 min *Candida albicans, Clostridium perfringens, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The effect was not affected by addition of serum. Added sugar is not needed for an effect; however, it stabilizes the dispersion of the essential oil (Briozzo et al 1989).

The effect of 0–300 ppm clove oil on the growth and synthesis of aflatoxins from *Aspergillus parasiticus* was studied in submerged culture. 0–250 ppm led to a slower growth, but had no influence on the weight of the mycelium after 21 days. 300 ppm completely inhibited the growth. The production of aflatoxins was dose-dependently delayed (Bullerman et al 1977).

Clove oil was superior to rosemary oil when tested against several Gram-positive and Gram-negative bacteria as well as against two fungi. A synergistic effect was observed against *Candida albicans*, an antagonism for *Aspergillus niger* (Fu et al 2007).

In an investigation of various aromatic waters, clove oil–water had no sustainable growth-inhibiting effect on *Pseudomonas*. Clove oil-water exhibited in the serial dilution test and agar diffusion test only a weak antimicrobial effect. The minimum inhibitory concentration in the serial dilution test was in the case of *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* for each 1:20, for *Mycobacterium phlei* 1:640 and for *Staphylococcus aureus* 1:160 or 1:320. The minimal fungicidal dilution was 1:320 in case of *Aspergillus niger*, for *Penicillium chrysogenum* 1:40 and for *Mucor*, *Rhizopus* and *Candida albicans* for each 1:20. The results in the agar diffusion test differ in part from that in the serial dilution test (Yousef & Tawil 1980).

Ali et al (2005) found that eugenol inhibits the growth of 30 *Helicobacter pylori* strains tested, at a concentration of 2µ/ml after 9 and 12 h of incubation. A lower pH-value increased the activity. The bacteria did not develop any resistance even after 10 passages grown at sub-inhibitory concentrations. The authors conclude that eugenol may prevent the growth of *H. pylori*.
Dorman & Deans (2000) tested the antibacterial activity of the essential oil of *S. aromaticum* in 25 bacteria. The results suggest that the essential oil is equally effective against both Gram-positive and Gram-negative microorganisms. A different sensitivity of the bacteria tested was observed.

Saini et al (2009) investigated the effect of orally administered essential oil on respiratory tract infections with *Klebsiella pneumoniae* in rats. The daily oral supplementation was 0.5 ml of a 1% w/v solution. The comparison of short term (15 days) and long term (30 days) treatment resulted in a significantly lower bacterial load in the lungs of mice fed clove oil for 30 days. The authors stated also a significant decrease of bacterial colonization already after 15 days.

Khan et al (2009) studied the influence of clove oil and of eugenol on quorum sensing (QS = mechanism by which bacterial populations coordinate the expression according to the density of the

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local population) regulated functions in bacteria. The production of violacein by *Chromobacterium violaceum* is QS-controlled. Clove oil reduces at sub-MICs this production up to 78% compared to control.

The swarming motility in *Pseudomonas aeruginosa* which is also QS-controlled was reduced up to 78%. Eugenol was not responsible for these effects.

The antibacterial activity of eugenol may be due to an interaction of eugenol with the bacterial cell membrane (Devi et al 2010). The membrane is disrupted and macromolecules of the membrane are deformed.

The antifungal activity of clove essential oil against *Aspergillus* section *Flavi* was evaluated in sterile maize grain. The effect of the essential oil added to maize grains on growth rate, lag phase and aflatoxin B1 (AFB1) accumulation of *Aspergillus* section *Flavi* were evaluated at different water activity conditions (a measure for water content; 0.982; 0.955; and 0.90). The essential oil had an inhibitory effect on *Aspergillus* section *Flavi* growth rate; the efficacy depended mainly on the water activity and concentration. Clove essential oil showed a considerable inhibitory effect on the AFB1 accumulation. When the water activity was 0.982, the AFB1 inhibition percentage for all aflatoxigenic strains exceeded 98% at all clove essential oil concentrations (Bluma & Etcheverry 2008).

The antifungal effect of clove oil was tested against several dermatophytes using the agar diffusion method (Park et al 2007). Hyphal growth was completely inhibited in Trichophyton mentagrophytes, T. rubrum and Microsporum gypseum in concentrations of 0.2 mg clove oil per ml. Eugenol was found to be the most effective compound against T mentagrophytes and M. canis.

The composition and antifungal activity of clove essential oil were tested by Pinto et al (2009). MICs, determined according to Clinical and Laboratory Standards Institute protocols, and minimum fungicidal concentration were used to evaluate the antifungal activity of the clove oil and its main component eugenol, against *Candida*, *Aspergillus* clinical isolates (e.g. American Type Culture Collection strains). The essential oil and eugenol showed inhibitory activity against all the tested strains. Propidium iodide rapidly penetrated the majority of the yeast cells when the cells were treated with concentrations just over the MICs. Therefore the fungicidal effect may result from extensive lesions of the cell membrane. Clove oil and eugenol also caused a considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component. Germ tube formation by *Candida albicans* was completely or almost completely inhibited by the essential oil and eugenol concentrations below the MIC values. The authors conclude that the results indicate that clove oil and eugenol have considerable antifungal activity against clinically relevant fungi, including fluconazole-resistant strains.

Eugenol significantly reduced the number of colony forming units sampled from the oral cavity of immunosuppressed rats treated for 8 days. Eugenol was used in a concentration of 24 mM (= double MIC) in agar solution. Nystatin was used as a poisitive control in a concentration of 58 µM (= tenfold MIC). Eugenol and nystatin gave similar results. Only few zones were occupied by hyphae with eugenol, while under nystatin hyphae were found in the folds of the tongue mucosa (Chami et al 2004).

There was a significant reduction of colony counts in a prophylactic approach and a treatment approach in cases of vaginal candidiasis in an immunosuppressed rat model. The rats received 10 mg/kg/day eugenol via an intravaginal route (Chami et al 2004a).

Lee et al (2007) evaluated the antifungal effect of eugenol against skin lesions in guinea pigs infected with *Microsporum gypseum*. Eugenol was adjusted to 10% concentration with a base of vaseline petroleum jelly and was applied topically to the skin lesions daily for 3 weeks. Eugenol was clinically active.

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Antiviral effect:
For eugenol no significant antiviral activity against herpes simplex virus type 1 was found in vitro using the plaque reduction assay (Astani et al 2009).

Spasmolytic effect:
A saturated aqueous solution of clove oil was active *in vitro* on isolated organs against various spasmogenes: rat/duodenum/acetylcholine: 20 to 40% inhibition; rat/duodenum/barium chloride: 40 to 60% inhibition; guinea-pig/ileum/histamine: >60% inhibition; rabbit/jejunum/nicotine: >60% inhibition. No further details on the methodology are available (Debelmas & Rochat 1967 cited in Blaschek et al 2008).

Clove oil antagonized *in vitro* the carbachol-induced spasm of guinea pig trachea muscles and the electrically stimulated contraction of longitudinal muscles of guinea pig ileum. The EC_{50} was 3.8 mg/ml (trachea; isoprenaline EC_{50} = 3.9 nmol/l) or 6.8 mg/ml (ileum; papaverine EC_{50} = 3.7 µmol/l) (Reiter & Brandt 1985).
Eugenol relaxes the rabbit thoracic aorta while suppressing the Ca^{2+} sensitivity and both the uptake and extrusion mechanisms for Ca^{2+} (Nishijima et al 1999).

Effect on coagulation:
Clove oil inhibited *in vitro* the platelet aggregation which was induced by arachidonic acid, epinephrine and collagen. The formation of thromboxane B_2 induced by arachidonic acid was inhibited in intact and in lysed platelet preparations. The effect, which exceeds the *in vitro* effect of acetylsalicylic acid, might be attributed to eugenol and eugenyl acetate. The combination of these compounds inhibits the platelet aggregation in a superadditive manner (Srivastava 1987, 1993).

The IC_{50} of eugenol (3.0 10^{-7} M) and isoeugenol (7.2 10^{-7}M) were comparable with indomethacin (2.2 10^{-7} M) on platelet aggregation (Laekeman et al 1990).

Eugenol and isoeugenol inhibit the arachidonic acid (1 x 10^{-4} g/ml) induced platelet aggregation, the IC_{50} values were 4.5 x 10^{-8} g/ml and 1 x 10^{-7} g/ml respectively (Rasheed et al 1984, 1984a).

Effect on prostaglandin synthesis:
The addition of 37 µM clove oil to *in vitro* preparations from sheep seminal vesicles inhibits (based on average molecular weight of 200) the prostaglandin synthesis from [1-14C] arachidonic acid by 84.1% compared to a control without the essential oil. The IC_{50} of eugenol was 11 µM; the IC_{50} of indomethacin was 1.2 µM (Wagner et al 1986).

Eugenol and its derivatives are inhibitors of LOX-5 and COX-2 (Hübner 2008).

Sedative effect:
Wagner & Sprinkmeyer (1973 cited in Blaschek et al 2008) investigated the sedative effect of clove essential oil. Mice received 1 to 100 mg/kg p. o. The motility in the photocell cage was compared with the results of the day before (without treatment). The authors observed a non dose-dependent reduction of motility.

Antiprotozoal effects:
Clove oil inactivated *in vitro Trichomonas vaginalis* in a dose- and time-depending manner. After addition of 4, 2, 1, 0.5 and 0.25 mg/ml to the culture medium no surviving Trichomonads were detectable after an incubation period of 5 min to 8 hours. Concentrations of 0.01 and 0.05 mg/ml were not effective. With eugenol comparable effects were achieved. With 4–0.05 mg/ml of the reference substance metronidazol the effect was achieved after 30 min to 2 hours (Salem 1980 cited in Blaschek et al 2008).

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Treatment of epimastigotes of *Trypanosoma cruzi* with different concentrations of clove essential oil resulted in a dose-dependent growth inhibition with IC_{50}/24 hours of about 99.5 µg/ml; IC_{50}/24 hours values obtained after treatment of bloodstream trypomastigotes were about 57.5 µg/ml. The values obtained for epimastigotes treated with eugenol were 246 µg/ml, while treatment of bloodstream trypomastigotes resulted in IC_{50}/24 hours values of 76 µg/ml for eugenol (Santoro et al 2007).

Effect as repellent:
In a study by Eamsobhana et al (2009) commercially produced essential oils of 13 plant species and ethanol (control) were tested for repellent activity against host-seeking larvae of *Leptotrombidium imphalum*. Dilutions of each essential oil were prepared in absolute ethanol. Clove essential oil exhibited 100% repellent activity at 5% concentration.

Antimutagenic activity:
Miyazawa & Hisama (2003) identified dehydrodieugenol and trans-coniferyl aldehyde as the antimutagenic compounds in clove. These compounds showed suppressive effects on umu gene expression of the SOS response in *S. typhimurium* TA1535/pSK10002 against furylfuramide, 4NQO, and MNNG, which do not require liver-metabolizing enzymes, and AFB1 and Trp-P-1, which require liver-metabolizing enzymes and UV irradiation. Dehydrodieugenol had stronger suppressive potencies.

Antigenotoxicity:
Antigenotoxic effects of eugenol were assessed in the mouse bone marrow micronucleus test by Abraham (2001). The test doses of eugenol were administered to mice by gavage 2 and 20 hours before exposure to the genotoxic agent. A pre-treatment with 50-500 mg/kg body weight eugenol resulted in significant reductions with cyclophosphamide, procarbazine, methylnitronitrosoguanidine and urethane. The administration of eugenol alone did not exert genotoxicity.

Cytotoxicity:
An *in-vitro* study demonstrates cytotoxic properties of both the essential oil and eugenol towards human fibroblasts and endothelial cells. Clove oil was found to be highly cytotoxic at concentrations as low as 0.03% (v/v) with up to 73% of this effect attributable to eugenol. ß-Caryophyllene did not exhibit any cytotoxic activity, indicating that other cytotoxic components may also exist within the essential oil. The viability of all cell types dropped by 60–90% when the concentration of the oil was increased from 0.01% to 0.03% (Prashar et al 2006).
High doses (0.05% clove oil; 2.50 mM eugenol) of the essential oil and its components into culture media already markedly increased the percentage of both necrotic and apoptotic cells after 1 hour (clove oil: 18.04%; eugenol: 21.64%). The medium doses (0.01% clove oil; 0.52 mM eugenol) did not cause significant damage to the Caco-2 population after 1 hour culture when compared with the control (Fabian et al 2006).

Other effects:
Hepatoprotective effects: eugenol may protect the liver from damage by certain chemicals, including iron overload. The mechanism may involve eugenol acting both as an antioxidant to prevent lipid peroxidation and by scavenging free radicals. In an animal study, eugenol reduced the hepatic injury caused by iron overload. Eugenol lowered liver lipid peroxidation by 38% and serum lipid peroxidation by 30% in iron-treated rats (Gruenwald et al 2004).

Clove essential oil increased the total white blood cell count and enhanced the delayed-type hypersensitivity response in mice. Moreover, it restored cellular and humoral immune responses in cyclophosphamide-immunosuppressed mice in a dose-dependent manner. The immunostimulatory activity found in mice treated with clove essential oil is due to improvement in humor- and cell-mediated immune response mechanisms (Carrasco et al 2009).

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Eugenol attenuates the reduction of dopamine. Eugenol administration 3 days before and 7 days after one intracerebroventricular injection of 6-hydroxydopamine prevented the reduction of striatal dopamine and its metabolites (Kabuto et al 2007).

Eugenol depressed cell respiration in homogenates of human dental pulp and in mouse fibroblast monolayers. The authors conclude that the irritant effect of zinc oxide eugenol when applied directly to soft tissue is due to the fact that concentrations of eugenol are achieved which are sufficient to inhibit respiration and thus kills cells (Hume 1984).

Molluscicidal activity: treatment with 20% and 60% of the LC_{50}-96 hours of eugenol caused in the test animals of the snail *Lymnaea acuminata* significant inhibition of the alkaline phosphatase and acetylcholinesterase activities (Kumar et al 2009).

Allen & Cornforth (2009) demonstrated the iron chelating ability of eugenol. In presence of iron, type I antioxidants like eugenol had a significant prooxidant effect.

The aim of the study by Khan et al (2009) was to evaluate quorum sensing (QS) inhibitory activity of plant essential oils using strains of *Chromobacterium violaceum* (CV12472 and CV026) and *Pseudomonas aeroginosa* (PAO1). Significant inhibition of pigment production was detected in clove oil with 19 and 17 mm zone of pigment inhibition against CV12472 and CV026 strains. Clove oil, at lower concentration (2 µl) showed no activity, but at higher concentration (20 µl) antibacterial activity was observed along with anti-QS activity (zone of inhibition 21 mm). Eugenol does not seem to be responsible for these effects.

Clove oil is active against the eggs and second-stage juveniles of the parasitic nematode *Meloidogyne incognita* (Meyer et al 2008).

Mild hypertension has resulted in dogs after receiving 0.05 ml of eugenol (Gruenwald et al 2004).

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

No specific data are available on Caryophylli flos, Caryophylli floris aetheroleum.

After oral administration of 40 mg/kg in rats, eugenol reaches maximal concentrations in the plasma and blood within 0.25 hour and 2.13 hours, respectively. The terminal elimination half-life was determined as 18.3 hours (blood) and 14 hours (plasma) which indicates that after repeated oral administration accumulation may occur (Guenette et al 2007).
Glucuronide and sulphate conjugates of eugenol were identified in the urine (Guenette et al 2006).

Some allylbenzenes like methyleugenol are metabolised in the liver by several CYP 450 enzymes at least partially into reactive 1' hydroxy-derivatives or 2', 3'-(allylic) epoxide derivatives (Jeurissen et al 2006, Guenthner & Luo 2001). The metabolism of eugenol via the same bioactivation pathway has been alluded to, but not directly demonstrated. It seems probable that the formation of the ultimate genotoxicant and carcinogen from eugenol is insignificant as compared to methyleugenol.

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

Toxicity of Caryophylli flos

Acute toxicity:
The acute toxicity of a decoction of clove was studied in 30 overnight fasted mice. Doses of 100-

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520 mg/kg body weight were administered intraperitoneally, larger doses of 500-5000 mg/kg body weight by oral gavage. The animals were observed for respiratory, GIT, CNS symptoms, behavioural patterns and mortality. The only toxic manifestation was abdominal cramps.
The LD_{50} was interpolated as 263 mg/kg (i.p.) and 2500 mg/kg (oral) (Agbaje et al 2009).

Tajuddin et al (2003) studied the acute toxicity of an ethanolic extract (DER app. 10:1, ethanol 50%). 6 mice received 500 mg/kg extract p.o. No signs of mortality or gross behavioural changes were observed.

Subchronic toxicity:
Swiss albino mice received for 10, 20 and 30 days 0.5%, 1% and 2% w/w clove powder in the diet. Enhanced glutathione-S-transferase, cytochrome *b5* and sulphydryl enzyme levels were observed in all the treatment groups, except those maintained on a 0.5% diet for 10 days. A significant reduction in P450 and malondialdehyde levels was observed in all groups at 30 days duration (Kumari (1991).

After 90 days of administration of a decoction of clove at doses of 300 mg/kg and 700 mg/kg in rats significant alterations in liver enzymes and haematological parameters were observed. Even in the lower dose histopathological modifications could be found in body organs. The authors conclude that a prolonged use of decoctions of clove should be avoided (Agbaje et al 2009).

Mutagenicity:
The dry residue of aqueous and methanolic extracts showed mutagenic effects in the rec assay in *Bacillus subtilis*. The mutagenic activity in the Ames test on *Salmonella typhimurium* TA98 and TA100 was not assessable due to the antimicrobial action (Morimoto et al 1982).

After administration of a decoction (1:100) of cloves to *Drosophila melanogaster* no genotoxic effects were observed (Schulz & Herrmann 1980).

An *in vivo* bone marrow micronucleus assay demonstrated that the administration of 0.5% and 2% of cloves in the diet of mice for 10 days neither significantly induced micronuclei nor could effectively modulate the 7, 12-dimethylbenz[a]anthracene genotoxicity in mice (Kumari 1991).

Reproduction toxicity:
Data from Caryophylli flos are not available. However, the herbal substance contains up to 2% oleanolic acid. For oleanolic acid isolated from *Syzygium jambos* flowers a possible anti-estrogenic effect is documented (Rajasekaran et al 1988). Rats received orally 15 mg/kg body weight or 30 mg/kg body weight olenolic acid daily over a period of 60 days. This dosage is equivalent to approximately 0.8 to 3 g clove per kg body weight. A histological examination of the testes showed a dose-dependent reduction in the number of spermatides and spermatocytes from 2.69 (control) to 1.73 and 0.93. The number of fertilized females was reduced from 20/20 (control) to 7/20 or 2/20. The number of implants also decreased from 8.80 to 5.43 and 2.55.
Mishra & Singh (2008) investigated a hexane extract of cloves in doses of 15-60 mg/kg body weight *per os* in mice over 35 days. The treatment did not induce systemic toxicity at the doses tested. At 15 mg/kg body weight the activities of testicular enzymes and serum levels of testosterone were increased. At doses of 30 mg and 60 mg/kg body weight these parameters were inhibited. Additionally non-uniform degenerative changes in the seminiferous tubules associated with a decrease in daily sperm production were observed.

Toxicity of Caryophylli floris aetheroleum and of eugenol

Toxicity:
Essential oil (Blaschek et al 2008):

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Rat: p.o., LD$_{50}$ 1.8–3.72 g/kg
Rabbit: cutaneous application, LD$_{50}$ 5 g/kg

Eugenol (Blaschek et al 2008):
Rat: p.o., LD$_{50}$ 2.68 g/kg; i.p. LD$_{50}$ 800 mg/kg
Mouse: p.o., LD$_{50}$ 3 g/kg; i.p. LD$_{50}$ 500 mg/kg

Acute toxicity (essential oil):
After oral administration of 5000 mg/kg of the essential oil in rats, all animals died within 24 hours. The autopsy showed bleeding in the stomach and intestines, and pleural effusion (Blaschek et al 2008).

A single oral dose of 140 mg/animal killed rats within a short time. Undiluted clove oil applied on the dorsal skin of hairless mice did not cause irritation. On intact or shaved rabbit skin clove essential oil acted under occlusive conditions as a weak irritant. Phototoxic effects were not observed with undiluted clove oil on hairless mice and pigs (Opdyke 1975 cited in Blaschek et al 2008).

Acute toxicity (eugenol):
On the isolated rabbit lung, the addition of 1 mM eugenol resulted in oedema, as measured by the increase in lung weight and wet/dry weight of the lung. The addition of catalase (1000 U/ml) or dimethylthiourea (30 mM) decreased the response. Dimethylurea, superoxide dismutase or heat inactivated catalase had no influence (McDonald & Heffner 1991).

Chronic toxicity (essential oil):
Clove essential oil in oral dosages of 35 or 70 mg per animal (rat) over 8 weeks was tolerated without signs of toxicity. Higher doses led to inactivity and weight loss. 105 mg/animal p.o. daily for 2 to 3 weeks led to serious liver and kidney damage and death of the animals (Opdyke 1975 cited in Blaschek et al 2008).

Clove oil is allowed as food additive and therefore an administration to food-producing animals is possible. The allowed daily intake for the United States is reported with 2.5 mg/kg (Oetinger 2003).

Chronic toxicity (eugenol):
Within the US National Toxicology Program (National Toxicology Program 1983) eugenol was tested over a period of 13 weeks in F344 rats and B6C3F1 mice. In concentrations up to 12,500 ppm (rats) and 6,000 ppm (mice) of eugenol in the diet no compound-related histopathological effects were observed.

Genotoxicity (entire clove oil):
No signs of a mutagenic effect could be observed in the *in vitro* chromosomal aberration test in fibroblasts from Chinese hamsters at concentrations up to 0.04 mg/ml of clove oil (Ishidate et al 1984).

No evidence of a mutagenic activity could be detected in clove oil (10 to 250 µl) *in vitro* in *Salmonella typhimurium* TA1530 and G46 without metabolic activation (Blaschek et al 2008).

Genotoxicity (eugenol):
The National Toxicology Program (NTP) performed a mutagenic study on eugenol. The study was finished in 1980. Outcome was the following:
Ames test (TA1535, TA100, TA98, TA1537 strains with metabolic activation): negative; Mouse lymphoma: positive, Sister chromatide exchange: positive; Chromosome aberrations: positive; Micronucleus: negative; *Drosophila*: negative; *in vivo* sister chromatid exchange: positive; *in vivo* chromosome aberrations: equivocal (National Toxicology Program 1983).

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Eugenol was tested for mutagenic activity in the AMES-test using *S. typhimurium* TA 1535, TA 100 and TA 98. For TA 100 and TA 98 strains no mutagenicity was detected, but in case of TA 1535 dose-dependent mutagenicity was observed (Swanson et al 1979).

Eugenol induces chromosome aberrations in Chinese hamster lung cells and exerts a cell-transforming activity in Syrian hamster embryo cells (Hikiba et al 2005).

Maralhas et al (2006) demonstrated that eugenol induces chromosome aberrations, including exchanges in V79 cells, in particular in the presence of rat liver S9 mix, which suggests biotransformation to reactive metabolites. Eugenol induced chromosomal aberrations significantly (3.5% aberrant cells) at 2500 µM, demonstrating cytotoxicity in higher doses. S9 increases the number of aberrant cells to 15% with a high frequency of chromatid exchanges. Additionally an increase in endoreduplicated cells was observed. The authors suggest that eugenol exhibits topoisomerase II inhibiting activity.

Eugenol was also tested by Ellahuene et al (1994) in the mouse bone marrow micronucleus assay. Single doses of 400 and 600 mg/kg eugenol i.p. induced a statistically significant increase in the induction of micronucleus-polychromatic erythrocytes compared to the negative control. No signs of genotoxicity were observed at a dose of 100 mg/kg.

Munerato et al (2005) investigated the phenolic compounds eugenol, isoeugenol and safrole for genotoxicity in the wing spot test of *Drosophila melanogaster*. The assay was applied in its standard version with normal bioactivation and in its variant with increased cytochrome P450-dependent biotransformation capacity. Eugenol produced in concentrations up to 15 mM a positive recombinagenic response only in the improved assay, which was related to a high CYP 450-dependent activation capacity. This suggests the involvement of this family of enzymes in the activation of eugenol rather than in its detoxification. On the contrary, isoeugenol was clearly non-genotoxic at the same millimolar concentrations as used for eugenol in both the crosses. The responsiveness of SMART assays to recombinagenic compounds, as well as the reactive metabolites from eugenol and safrole were considered responsible for the genotoxicity observed.

Burkey et al (2000) investigated the cytotoxicity and genotoxicity of several allylbenzenes. Cytotoxicity was determined by measuring lactate dehydrogenase release, while genotoxicity was determined by using the unscheduled DNA-synthesis (UDS) assay. Isoeugenol and eugenol were more cytotoxic to the hepatocytes than methyleugenol or safrole. Isoeugenol and eugenol had LC_{50} values of 300 mM for rat hepatocytes and 200 mM for mice, with both compounds showing relatively steep dose–response curves.

The results of this study correspond with the opinion of the authors that methyleugenol and safrole cause UDS in rat and mouse hepatocytes over a range of concentrations, while isoeugenol and eugenol do not. The difference in the genotoxicity of the two groups of compounds may be related to the biotransformation of the compounds. Both safrole and methyleugenol lack free hydroxy groups on their rings that are present on isoeugenol and eugenol. The lack of these freely available hydroxyl groups may allow both methyleugenol and safrole to avoid immediate conjugation and elimination, providing a greater opportunity for metabolism to take place on the allyl side chain.

The authors conclude that methyleugenol is minimally cytotoxic to hepatocytes isolated from rats and mice while causing UDS in both species. Safrole showed similar patterns of toxicities. In contrast, isoeugenol and eugenol showed significant cytotoxicity at extremely high concentrations in rodent-derived hepatocytes but did not cause UDS. It seems likely that extensive glucuronidation and sulfation of the para-hydroxy groups of isoeugenol and eugenol greatly reduce bioactivation on the alkenyl side chain. Safrole and methyleugenol, which lack this structural feature, undergo hydroxylation at C-1, and thus produce the precursor of a genotoxic metabolite in greater amounts.

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Guenthner & Luo (2001) demonstrated that potentially genotoxic 2', 3' epoxide metabolites occur readily *in vivo* using the isolated perfused rat liver, but these metabolites are rapidly further metabolised to less toxic dihydrodiol or glutathione conjugates. The authors conclude that the epoxide formation at the allylic double bond represents, therefore, a potentially genotoxic bioactivation pathway for allylbenzene analogs. However, comparison of the relative kinetics of epoxide metabolism and epoxide formation suggests that a wide margin of protection from DNA covalent adduct formation exists in the rat liver, thus preventing genotoxicity resulting from this pathway to any significant degree. The authors also observed that the general rate of epoxide hydrolysis is much greater in human liver than in rat liver. It is therefore suggested that while the epoxidation pathway poses a potential genotoxic threat to humans, no actual genotoxicity occurs as a result of this metabolic pathway.

Anti-genotoxic effects of eugenol were assessed in the mouse bone marrow micronucleus test by Abraham (2001). The test doses of eugenol were administered to mice by gavage 2 and 20 hours before exposure to the genotoxic agent. A pre-treatment with 50-500 mg/kg body weight eugenol resulted in significant antigenotoxic effects against cyclophosphamide, procarbazine, methylnitronitrosoguanidine and urethane. The administration of eugenol (50-500 mg(kg BW) alone did not exert genotoxicity.

Rompelberg et al (1996) however found only limited support for the suspected antigenotoxic potential of eugenol *in vivo*. The effects of eugenol in rats were investigated in the unscheduled DNA synthesis (UDS) assay with established mutagens and the *Salmonella typhimurium* mutagenicity assay. In addition, the effect of *in vivo* treatment with eugenol on benzo[a]pyrene (B[a]P)-induced genotoxicity in human hepatoma cell line Hep G2 was investigated in the single-cell gel electrophoresis assay. The mutagenicity of B[a]P in the *S. typhimurium* assay was lower in liver S-9 fractions from control rats. Incubation of liver S-9 fractions from eugenol-treated rats with dimethylbenzanthracene (DMBA) had no antimutagenic effect. Eugenol did not modify UDS activity in hepatocytes isolated from rats pretreated with eugenol orally, after exposure of these cells *in vitro* to DMBA and aflatoxin B1. Four different treatment schemes of combinations of B[a]P and eugenol were examined in Hep G2 cells: pre-treatment with eugenol; simultaneous treatment with eugenol and B[a]P; a combination of these (pretreatment/simultaneous treatment); and post-treatment with eugenol. An increase in the genotoxicity of B[a]P was found in Hep G2 cells. No effect of eugenol on the genotoxicity of B[a]P was found with the pre- and post-treatments. It is concluded that the effect of eugenol on genotoxicity induced by established mutagens is equivocal; *in vivo* treatment of rats with eugenol resulted in a reduction of the mutagenicity of B[a]P in the *S. typhimurium* mutagenicity assay, while in the UDS assay no effect of eugenol was found. *In vitro* treatment of cultured cells with eugenol resulted in an increase in genotoxicity of B[a]P.

Reproductive and developmental toxicity:
Domaracky et al (2007) investigated the effects of clove essential oil on the growth and development of mouse pre-implantation embryos *in vivo*. The animals received 0.25% essential oil in the commercial diet (= 375 mg/kg per day). Treatment with clove essential oil induced a significantly higher percentage of dead cells compared to the control group.

Female CD-1 mice were given clove oil from day 6 to day 15 of gestation p. o. 2.2 to 215 mg/kg body weight daily. The foetuses were obtained on day 17. The use of clove oil had no apparent toxic effect on the implantation and survival of the mother and foetus. The number of malformations of the soft tissues and the skeletal system was not different from that in the control group spontaneously occurring. Similar results were obtained in female Wistar rats following daily p. o. administration of 2.8 to 280 mg/kg body weight (6 to 15 of gestation, foetuses at day 20), to golden hamsters after p.o. 1.8

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to 177 mg/kg body weight (6 to 10 of gestation, foetuses at day 14) and in rabbits by 1.72 to 172 mg/kg body weight (6 to 18 of gestation, foetuses at day 29) (Blaschek et al 2008).

Carcinogenicity (eugenol):

Within the US National Toxicology Program (National Toxicology Program 1983) eugenol was tested over a period of two years in F344 rats and B6C3F1 mice.

Rats: eugenol was administered in the diet in a concentration of up to 6000 ppm. Considering the mean diet consumption per day the daily uptake of eugenol was for male rats about 1100 mg/kg body weight at the beginning to 245 mg/kg body weight at the end of the study; for female rats about 790 mg/kg body weight at the beginning to 237 mg/kg body weight at the end of the study. The study outcome was negative in both sexes.

Mice: Considering the mean diet consumption per day, the daily uptake of eugenol was for male mice about 1160 mg/kg body weight at the beginning to 564 mg/kg body weight at the end of the study; for female mice about 1440 mg/kg body weight at the beginning to 718 mg/kg body weight at the end of the study. Eugenol caused increased incidences of both carcinomas and adenomas of the liver in male mice and eugenol was associated with an increase in the combined incidences of hepatocellular carcinomas or adenomas in female mice. These findings were judged to be statistically significant at concentrations from 3000 ppm upwards in the diet. However, the study outcome was considered to be equivocal.

Recently, Auerbach et al (2010) classified eugenol as a non-hepatocarcinogen allylbenzene, while methyleugenol, safrole and estragol are classified as hepatocarcinogens. This classification was supported by several vector machine classification models.

Local toxicity:

Undiluted eugenol (no data on amount of eugenol) was applied to a circumscribed area 3 mm in diameter of rat labial mucosa for one minute. Reaction periods of 15 minutes, 1, 2, 4 and 6 hours were then permitted. Using routine histological procedures for processing the experimental tissues it was observed that eugenol caused denaturation of cytoplasmatic proteins and loss of staining capacity of epithelium, loss of cell boundaries, swelling and cell necrosis. In addition, vesicle formation, oedema in the corium, and striated muscle dissolution were observed (Kozam & Mantell 1978).

3.4. Overall conclusions on non-clinical data

The published data concerning the special indications and preparations is very limited, but on the basis of existing data the pharmacological activities support the traditional use of Caryophylli aetheroleum and preparations thereof in the proposed indication: For the temporary relief of toothache due to a dental cavity and for the symptomatic treatment of minor inflammations in the mouth or the throat.

The efficacy of traditional herbal medicinal products is only plausible but not based on clinical data. Nevertheless, the safety must be guaranteed. In the case of Caryophylli aetheroleum the main component eugenol gives reason for safety concerns. Natural compounds with a similar allylbenzene structure like safrole and methyleugenol are known as genotoxic carcinogens. Available data regarding genotoxicity and carcinogenicity of eugenol are inconsistent and equivocal. In general the toxicity of eugenol is estimated to be considerably lower compared to methyleugenol. In human liver the rate of detoxification reactions of the 2', 3' epoxidemetabolites appears to be considerably higher compared to rat liver. Due to the presence of a free para-hydroxyl group, eugenol is much more easily conjugated with sulphate and glucuronide resulting in a reduced probability of the formation of reactive metabolites like in methyleugenol. Additionally, when considering the relatively high concentrations of eugenol used in the carcinogenicity studies the actual risk for short term use seems to be relatively low.

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Therefore, from the potential toxicity point of view, the short term local use of clove oil for the temporary relief of toothache due to a dental cavity and for the symptomatic treatment of minor inflammations in the mouth or the throat in traditional herbal medicinal products can be supported.

Because available data regarding genotoxicity and carcinogenicity are inconsistent and equivocal the establishment of a Community list entry is not recommended.

4. Clinical Data

4.1. Clinical Pharmacology

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

Eugenol caused a 'comfortable feeling' in the 13 female subjects. Alpha 1 of EEG significantly decreased after inhalation. Suppression of alpha 1 indicates the neural activity around the brain regions. There is a possible positive correlation between alpha 1 activity and subjective evaluation (Masago et al 2000).

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

No data available for the entire essential oil.

Eugenol:
The metabolism of eugenol was investigated in male and female healthy volunteers by Fischer et al (1990). Eugenol was rapidly absorbed and metabolized after oral administration and was almost completely excreted in the urine within 24 hours. Unmetabolized eugenol was found in the urine less than 0.1% of the dose. The urine contained conjugates of eugenol and of nine metabolites. The authors could identify 4-hydroxy-3-methoxyphenyl-propane, cis- and trans-isoeugenol, 3-(4-hydroxy-3-methoxyphenyl)-propylene-1,2-oxide, 3-(4-hydroxy-3-methoxyphenyl)-propane-1,2-diol, and 3-(4-hydroxy-3-methoxyphenyl)-propionic acid. 95% of the dose was recovered in the urine, most of which (greater than 99%) consisted of phenolic conjugates; 50% of the conjugated metabolites were eugenol-glucuronide and sulphate.

4.2. Clinical Efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

Caryophylli flos:

Alqareer (2006) compared the anesthetic properties of cloves, benzocaine and placebo in 73 adult volunteers. The volunteers received either 2 g of a gel containing 40% clove powder and 60% glycerine or 2 g of a gel containing 20% benzocaine on one side of the canine buccal mucosa and placebo on the other side. Five minutes after the administration each participant received two needle sticks. The pain response was registered using a visual analogue pain scale. Both clove and benzocaine lowered the pain score significantly (p = 0.005), no difference was found between benzocaine and the clove preparation.

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Caryophylli aetheroleum:

Clinical studies in the proposed indication: no data available.

Other clinical studies:
Central effects: The influence of clove oil on psychometric parameters such as mood, affective reaction, memory and cognitive abilities in 21 male and 51 female probands was studied in a cross-over trial. The concentration in the room air conditioning was corresponding to 0.0057–0.0167 g/m^3. No differences in the examined parameters could be observed (Wagner & Sprinkmeyer).

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 data are available for Caryophylli flos and Caryophylli aetheroleum in order to support well-established use. The traditional use in the proposed indications is made plausible by pharmacological data.

Therefore the medicinal use has to be regarded as traditional.

5. Clinical Safety/Pharmacovigilance

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

No data available.

5.2. Patient exposure

No data available.

5.3. Adverse events and serious adverse events and deaths

Skin and mucosal irritations:
In concentrated form, oil of clove may be irritating to mucosal tissues (Gruenwald et al 2004).
In contrast to this Anton et al (2001) report that there is no skin irritation (undiluted oil) on hairless mice. Under occlusion the undiluted clove oil was moderately irritating in rabbits.

Allergic effects:
In patients sensitized to Peru balsam, a hexane extract of clove, caused, in concentrations higher than 0.12% in petrolatum, local reactions. In a concentration of 1% in petrolatum, in two of four patients, a moderate reaction was observed, in the other two, an intense reaction occurred (large, infiltrated, dark spots with numerous vesicles) (Bouhlal et al 1989).
In an epicutaneous test with clove powder (on filter paper moistened with water), out of 78 patients with allergy against Peru balsam, 36 reacted positive. In a control group of 156 probands lacking Peru balsam allergy, nobody responded positively (Niinimäki).
A 22 years old patient with eczema on the hands reacted to a p.o. stress test 2 times 100 mg clove powder in gelatine capsules) with blisters on palms and fingers (Niinimäki 1984).
Clove cigarettes have been reported to cause acute respiratory problems in humans that rapidly progress to hemorrhagic pulmonary oedema or pneumonia (Blaschek et al 2008, Gruenwald et al 2004).

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In a patch test study a 10% ethanol extract of Caryophylli flos was investigated among other herbal preparations used in the Traditional Chinese Medicine. Out of 30 patients 8 reacted positively to clove extract (Chen et al 2003).

Clove oil, 20% incorporated in petrolatum, produced in 2 of 25 healthy subjects an erythema. In concentrations of 2% and 0.2% in petrolatum, no reactions were observed (Opdyke 1975).

When trying to administer clove essential oil onto an aching tooth, a 24 year old woman disposed accidentally the oil on the upper lip and cheek. Although she tried to remove the essential oil, a sensation of burning and inflammation occurred, which disappeared within a few hours. Subsequently, local anaesthesia and reduced sweat production in the affected areas were observed. The medical examination after 11 months revealed a dry, slightly erythematous skin with reduced pressure sensitivity. During the following 9 months the situation remained unchanged (Isaacs 1983).

Data from root canal fillings with eugenol cement:

When eugenol cement is applied near the pulpa (intact dentin layer) no toxicity is observed. However, when applied directly to the exposed pulp, pulp necrosis and inflammation appeared (Reichl et al 2007).

A root canal filling with eugenol cement resulted in a patient with a generalized urticaria. In the skin test, the patient responded positively to Peru balsam and cloves. A distributed oral provocation test with 0.1 to 0.5 ml of eugenol, in water, resulted in urticaria which persisted for several weeks (Grade & Martens 1989).

Serious events:
A 17-year-old male high-school student died of rapidly progressive inflammatory lung disease that developed hours after smoking a clove cigarette. The student was recovering from a lower respiratory infection at the time. The California Department of Health Service and Centers for Disease Control collected 110 cases of clove cigarette toxicity by 1984, two of which were fatal (Gruenwald et al 2004).

During 1984 and 1985, the Centers for Disease Control received 11 case reports of clove cigarette smoking-associated acute respiratory system injury in adolescents and young adults; two deaths were also reported. The reported respiratory adverse effects included hemoptysis, bronchospasm, hemorrhagic and nonhemorrhagic pulmonary oedema, pleural effusion, respiratory insufficiency, respiratory infection and aspiration of foreign material (Gruenwald et al 2004).

Contraindications:
The medicinal use of clove essential oil should be contraindicated in cases of hypersensitivity to clove essential oil as well hypersensitivity to Peru balsam (Blaschek et al 2008).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Interactions:
The antiplatelet effect of clove oil may increase the risk of bleeding if taken with these medications. Clove may result in a false increase in phenytoin levels (Gruenwald et al 2004).

Assessor's comment: The proposed routes of administration are the oromucosal and dental use; the duration of use is limited. Therefore these mentioned theoretical drug interactions are not relevant for

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the traditional use of clove oil for the short term treatment of toothache or as an antiseptic mouthwash.

Pregnancy and lactation:

No data are available. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

Overdose:

Case reports:

A 7-month-old boy received 1 teaspoon of clove oil. The dose corresponds to about 500 mg/kg eugenol. On admission to hospital, an attenuation of the CNS (awake, but without a direct response to environment), leukocytosis, proteinuria and ketonuria were observed. The also observed metabolic acidosis was attributed to the already existing diarrhoea. 3 hours after ingestion, gastric lavage was performed with the addition of activated carbon. An endoscopy the next morning showed no evidence of mucosal damage in the stomach or oesophagus. After 48 hours acidosis and leukocytosis were no longer detectable. The patient recovered completely and was released from hospital after 4 days (Lane et al 1991).

A 2 year old boy drank 5 to 10 ml of clove oil. After 1 hour only mild drowsiness was observed. Within the next 3 hours, a drastic deterioration occurred with deep coma and severe acidosis. 8.5 hours after the ingestion, generalized cramps occurred which were treated with diazepam. The patient had an unrecordable blood glucose level which was treated with intravenous dextrose. 24 hours after ingestion, the patient was unconscious. A severely impaired liver function and disseminated intravascular coagulopathy (therapy with plasma, heparin, antithrombin III, protein C, factor VII) was observed. The liver function deteriorated further in the following days. During the 5th day, the patient awoke and on day 6, he was fully conscious. From this time point, the symptoms gradually disappeared, the patient fully recovered (Hartnoll et al 1993).

A very similar case - ingestion of about 10 ml of clove oil by a 2 year old boy resulting in convulsions, unconsciousness and severe coagulation - is described by Brown et al (1992). The patient was treated with heparin and fresh frozen plasma, and, following specific haemostasis assays, with appropriate coagulation factor and inhibitor concentrates.

A 3 month old girl developed a fulminant hepatic failure after ingestion of less than 8 ml of clove oil (exact amount not documented). She was successfully treated with N-acetylcystein infusion and recovered completely (Eisen et al 2004).

A similar case is reported by Janes et al (2005): a 15 month old boy developed a fulminant hepatic failure after ingestion of 10 ml of clove oil. After 24 h, the ALT level was in excess of 13,000 U/l, with blood urea and creatinine of 11.8 mmol and 134 µmol/l respectively. The hepatic impairment resolved after intravenous administration of N-acetylcysteine so that 6 h later, the ALT level was approximately 10,000 U/l. The liver function and clinical status improved over the next 4 days.

5.6. Overall conclusions on clinical safety

Clove essential oil acts in high concentrations as local irritant, allergic reactions may also be possible. However, when applied in diluted form, no reports on severe adverse events are published. When applied correctly in the proposed routes of administration, clove essential oil can be considered as clinically safe.

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6. Overall conclusions

The positive effects of Caryophylli flos and Caryophylli floris aetheroleum and preparations thereof on inflammatory changes of the oral and pharyngeal mucosa and for topical anaesthesia have long been recognised empirically. The use is made plausible by pharmacological data. There is a lack of controlled clinical studies, using herbal preparations, containing the herbal substance Caryophylli flos or Caryophylli floris aetheroleum.

In conclusion, Caryopyhlli flos and Caryophylli floris aetheroleum and its preparations can be regarded as traditionally used, in the following indications: temporary relief of toothache due to a dental cavity and symptomatic treatment of minor inflammations in the mouth or the throat.

There is no documented use of medicinal products containing Caryophylli flos as the only active ingredient. Therefore no monograph for Caryophylli flos has been developed, which is communicated in a public statement.

In Caryophylli aetheroleum, the main component eugenol gives reason for safety concerns. Natural compounds with a similar allylbenzene structure, like safrole and methyleugenol, are known as genotoxic carcinogens. Available data regarding genotoxicity of eugenol are inconsistent and equivocal. In general the toxicity of eugenol is estimated to be considerably lower compared to methyleugenol. In the human liver, the rate of detoxification reactions of the 2', 3' epoxide metabolites appears to be considerably higher compared to rat liver. Due to the presence of a free para-hydroxyl group, eugenol is much more easily conjugated with sulphate and glucuronide resulting in a reduced probability of the formation of reactive metabolites compared to methyleugenol. Additionally, when considering the relatively high concentrations of eugenol used in the carcinogenicity studies the actual risk for short term use seems to be relatively low.

Therefore, from the potential toxicity point of view, the short term local use of clove oil for the temporary relief of toothache due to a dental cavity and for the symptomatic treatment of minor inflammations in the mouth or the throat in traditional herbal medicinal products can be supported.

The safety concerns do not allow the establishment of a Community list entry.

Annex

List of references

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